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Research Article

Stocking Density Induced Stress on Plasma Cortisol and Whole Blood Glucose Concentration in Nile Tilapia Fish (*Oreochromis niloticus*) of Lake Victoria, Kenya

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Effects of high stocking densities (HSDs) were evaluated for Nile tilapia fish (*Oreochromis niloticus*) under culture to determine its influence on plasma cortisol and whole blood glucose concentration. Plasma cortisol levels (ng/ml) were assayed by *E*nzyme-*L*inked *I*mmunosorbent *A*ssay (ELISA). Whole blood glucose levels were determined using a hand-held one touch ultraglucose meter (MD-300) and test strips. Plasma cortisol and whole blood glucose level determinations were replicated three times for *O. niloticus* reared under both low stocking densities (LSD) and HSD. One way Analysis of Variance (ANOVA) was performed on the data collected, and comparison of significant differences in means was carried out between LSD and HSD at 0.01%. Plasma cortisol levels revealed statistically ($P \leq 0.01$) significant values of HSD at 6.32 ± 1.06 ng/ml than in LSD at 4.62 ± 1.58 ng/ml for the *O. niloticus* groups studied. Whole blood glucose analysis revealed a statistical ($P < 0.05$) difference in the means in HSD and LSD *O. niloticus* groups ($F_{(df,1;\ 8)} = 7.946 > F_{crit} = 4.414$; $P = 0.01$). Mean plasma glucose concentration was statistically ($P \leq 0.01$) higher for HSD than LSD *O. niloticus* groups at mean ± SD, 96.84 ± 5.28 and 76.82 ± 5.92, respectively. The findings of this study demonstrate that high stocking densities increase both cortisol and whole blood glucose concentration in tilapia fish, indicating a marked increase in stress levels. Elevated plasma cortisol and whole blood glucose concentration can be used as biomarkers for acute stress in *O. niloticus* produced under aquaculture systems. The findings of this study can help inform policy on the management of stress caused by overstocking of *O. niloticus* and other related Cichlids under industrial aquaculture production.

1. Introduction

Fish and fisheries play an important role in addressing nutritional and livelihood food security, especially of the poor in the developing countries [1]. Globally over 2 billion people get at least 20% of their animal protein intake from fish [2, 3]. Nile tilapia (*Oreochromis niloticus*)are among the most important warm water fishes used for aquaculture production [4] and only the second most popular farmed fishes after Carps [3, 5, 6]. They are recognized as one of the most important species in tropical and subtropical aquaculture [2, 5, 7]. Nile tilapia have a mild white flesh that appeals to customers, making them economically important fish [8] and the most desired by Lake Victoria Community [9]. It is also an important fish model for studies on social stress due to overcrowding [10].

Stocking density is considered a key factor in determining the productivity of fish aquaculture systems [11]. Crowding is judged as one of the most influential stressors affecting fish physiology and, thus, the status of well-being in aquaculture [12], and it is a common husbandry practice in aquaculture [13, 14]. High stocking density negatively affects both fish growth and feed utilization [1, 15]. High stocking densities have been shown to reduce feeding activity and growth rates in Coho Salmon (*Oncorhynchus kisutch*) and rainbow trout (*Oncorhynchus mykiss*) but have a positive

effect on these parameters in Arctic charr (*Salvelinus alpinus*) [16]. It also increases the level of metabolites such as urine and faeces in cultured *Chrysichthys nigrodigitatus* [17]. This stress response changes water quality [16], thus further subjecting fish to chemical stressors [18]. High stocking density subjects fish to chronic stress [19, 20]. It is reported by [21] that stocking density and social interactions between specific fish have a significant influence on stress. Stress is considered to be a generated response, but it can be modulated by specific stressor conditions. One of the most frequent causes of chronic stress is social interaction among members of the same species.

Stressors cause distinct stress responses in *O. niloticus* [22] leading to elevated plasma cortisol [16, 23], the main hormone that activates glucose [24], therefore elevating glucose levels [19, 23, 25]. Fish respond to stress by increasing circulating cortisol [16, 23] and glucose [23]. Cortisol is released in response to chronic stressors. Elevated plasma cortisol [19, 26] and glucose levels [16, 21, 26, 27] are used as indicators of stress. This study investigated the effect of high stocking density-induced stress on the concentration of cortisol and whole blood glucose in *Oreochromis niloticus* of Lake Victoria under culture.

2. Materials and Methods

2.1. Study Area. Nile tilapia fingerlings were obtained from the Kenyan part of Lake Victoria (4, 100 km^2), a fresh water lake. They were then transferred to the fish rearing facility at Maseno University (0° 22′ 11.0″ S, 35° 55′ 58.0″ E/Latitude; 0.369734, Longitude; 35.932779) located 400 km west of Nairobi where this study was conducted. Tropical climatic conditions characterize this region.

2.2. Fish and Experimental Procedures. The study protocol was reviewed and optimised by experts from the Department of Zoology, Maseno University, Kenya, and the Department of Biological Sciences, Masinde Muliro University of Science and Technology, Kenya. Healthy Nile tilapia of Lake Victoria were obtained from the fish rearing facility of the Department of Zoology, Maseno University. They were first acclimatised to the laboratory conditions (temperature, dissolved oxygen, water quality, water pH, nutritional status, photoperiod, size, weight, colour, and shape of experimental aquaria) and progenesis (i.e., fish obtained from the same place) as reported in [13, 26, 28]. The fish were then reared under natural environmental conditions (natural photoperiod 12L : 12D, tropical temperature, and standard water quality) by the seining technique [29]. Fish of mixed sex [30] were randomly distributed into 2 indoor glass aquaria (0.050 m^3) in triplicate at low and high stocking densities of 150 g and 300 g, respectively. All fish were matched for body weight (standard mass, mean ± SD 15 ± 1 g) [13] and age [28].

The aquaria were fitted with aerator pumps (Lp Low Noise Air pumps) manufactured by Resun®, China, and mercury thermometers. A sand-fine gravel filter system was also put into each aquarium. The aquaria were filled with equal volumes (40 litres) of rain water. The fish in each aquarium were fed on a carbohydrate-based feed of chick mash (18% protein) (Sigma Feeds Ltd., Nairobi, Kenya) supplemented with crushed silver cyprinid (*Rastrineobola argentea*) to 25% protein at a feed portion of 10 g/kg of life body weight, and the feeding was terminated 24 hours to sampling [13].

2.3. Sample Collection and Storage. 21-day-old fish were anaesthetized with 2-phenoxyethanol (chemical formula: $C_8H_{10}O_2$; active substance: ethylene glycol monophenyl ether) obtained from BDH Laboratory Reagents, England at a concentration of 0.30 ml l^{-1} of water for 10 min before samples were extracted [31] to minimize suffering. A large plastic sieve was then used to draw fish ($n = 5$) samples from each of the six aquaria. Blood was then drawn through cardiac puncture using EDTA- (ethylene diamine tetraacetic acid-) coated (obtained from BDH Laboratory Reagents, England) hypodermic syringes [32] as an anticoagulant. This took not more than 1 min for each fish so as to avoid rise in blood cortisol levels due to handling stress [13, 22]. Some of the drawn blood samples ($n = 20$, i.e., three samples from each of the six aquaria and a further one sample from each of the second aquaria of the LSD and HSD groups) were then transferred into labelled Eppendorf tubes, each containing 1 ml EDTA solution, placed onto crushed ice (0°C) in an icebox, and transported to the laboratory for storage under refrigerated conditions.

2.4. Sample Analyses

2.4.1. Plasma Cortisol. Plasma cortisol levels (ng/ml) were assayed for by *E*nzyme-*L*inked *I*mmuno*s*orbent *A*ssay (ELISA) using a Neogen Corporation ELISA kit (Lansing, Ml, USA, 2010) [33] and the company's Cortisol ELISA protocol. This quantitative analysis of cortisol levels in the biological fluid (*O. niloticus* blood plasma) was performed at the Kenya Medical Research Institute (KEMRI), Kisumu, Kenya. An antibody-coated 96-well microplate was used. The standard solutions and the diluted samples were first added to the microplate in duplicates. Diluted enzyme conjugate was then added, and the mixture was shaken and incubated at room temperature for 1 hour to allow competition to take place between the enzyme conjugate and cortisol in the samples for the limited number of binding sites on the antibody-coated plate. The plate was then washed with a wash buffer to remove all the unbound material. The bound enzyme conjugate was detected by the action of substrate which generated an optimal colour after 30 minutes. Quantitative test results were obtained by measuring and comparing the absorbance reading of wells of the samples against the standards with a microplate reader set at 650 nm using SoftMax Pro Microplate Data Acquisition & Analysis Software. The samples were each diluted ten (10) times before being assayed.

2.4.2. Whole Blood Glucose. Blood glucose levels were determined from whole blood using a hand-held one touch

ultraglucose meter (MD-300) and test strips manufactured by TaiDoc. Technologies Corporation and supplied by MD instruments Inc., as was established by [30, 34, 35] at the sampling site. Whole blood was applied onto the test strips fixed in the hand-held glucose meter. Glucose concentrations were read in mmol.$^{-1}$

2.5. Statistical Analyses. Means, standard deviations (SD), and Standard Error of Means (SEM) have been used to describe the data. They were determined using One-Way ANOVA. Microsoft Office Professional plus Excel 2013 software was used to present the data graphically for easy interpretation and understanding. The accuracy with which the distributions of the extracted blood samples for LSD and HSD groups represents the expression of stress related to stocking densities in *O. niloticus* under aquaculture systems is measured using SD at 2 standard deviation of the means at 95% level of confidence [36]. The SEM shows the precision of the sample means of LSD and HSD groups to the true control and experimental groups' means. One-Way ANOVA was used to test the hypotheses; $H0$: $\bar{x}_{LSD1} = \bar{x}$ $LSD2 = \bar{x}_{LSD3}$, $\bar{x}_{HSD1} = \bar{x}_{SHD2} = \bar{x}_{HSD3}$, H_A: not H_0 at F_{crit} ($df_{\text{AMONG}} = 2$, $df_{\text{WITHIN}} = 6$, $\alpha = 0.05$) = 5.14 for individual samples and $H0$: $\bar{x}_{LSD1,2,3} = \bar{x}_{HSD1,2,3}$, H_A: not H_0 for the true sample means at F_{crit} ($df_{\text{AMONG}} = 1$, $df_{\text{WITHIN}} = 4$, $\alpha = 0.05$) = 7.71 for cortisol. Similar statistical analyses were conducted on whole blood glucose concentrations at F_{crit} ($df_{\text{AMONG}} = 2$, $df_{\text{WITHIN}} = 12$, $\alpha = 0.05$) = 3.18 for LSD_1, LSD_2, LSD_3, HSD_1, HSD_2, and HSD_3 individual sample groups and at F_{crit} ($df_{\text{AMONG}} = 1$, $df_{\text{WITHIN}} = 8$, $\alpha = 0.05$) = 5.32 for $\text{LSD}_{1,2,3}$; $\text{HSD}_{1,2,3}$ true sample means.

3. Results

3.1. Plasma Cortisol. The percent of maximal binding (%B/B_0 value) was determined by dividing the averages of each standard absorbance value ($B_1 \ldots B_7$) by the B_0 absorbance value and, then, multiplied by 100 to achieve the percentages (Table 1).

The data from Table 1 was used to graph the standard curve (Figure 1) by plotting the $\%B/B_O$ for each standard concentration on the ordinate (y) axis against concentration on the abscissa (x) axis a using a curve-fitting routine.

The standard curve was used to determine the concentration of the samples from their respective $\%B/B_O$ and, then, multiplied with a dilution factor of 10 (Table 2).

One-way ANOVA analysis revealed a statistical ($P < 0.05$) difference in the means of plasma cortisol concentrations in HSD and LSD *O. niloticus* groups ($F_{(\text{df},1;18)} = 7.946 > F_{\text{crit}.} = 4.414$; $P = 0.01$). Mean plasma cortisol concentration was statistically ($P \leq 0.01$) higher for HSD than LSD *O. niloticus* groups at mean ± SD, 6.32 ± 1.06 ng/ml and 4.62 ± 1.58 ng/ml, respectively (Table 2). It is evident from the small values of standard error, SE, (0.33_{LSD}; 0.50_{HSD}) that the sample means (6.32_{LSD}; 4.62_{HSD}) are reliable indications of cortisol levels in *O. niloticus* reared under the two stocking densities in aquaculture systems.

The data on cortisol concentration levels (Table 2) were then organised in an a ascending order for both HSD and LSD and, then, used to establish a graph of cortisol concentration in ng/ml against individual samples of *O. niloticus* assayed and plotted so as to show trends (Figure 2). Note that the data marked with an asterisk (*) were obtained from samples extracted from the second aquaria for HSD and LSD groups to bring the data to 10 a piece for better graphing. This was due to the fact that the second aquaria positions in between the first and third aquaria ensured homogeneity in experimental conditions for the HSD and LSD groups.

Note that the sample number marked with an asterisk did not have its cortisol values used in computing the LSD_2 and HSD_2 group means.

One-way ANOVA analysis of the triplicate *O. niloticus* control groups gave no statistical ($P > 0.05$) difference in their means ($F_{(\text{df},2;\ 6)} = 0.26 < F_{\text{crit}.} = 5.14$; $P = 0.78$) at 4.80 ± 0.53, 4.80 ± 1.12, and 3.87 ± 2.83 mg/dl for LSD_1, LSD_2, and LSD_3, respectively. Similarly, no statistical ($P > 0.05$) difference ($F_{(\text{df},\ 2;\ 6)} = 0.43 < F_{\text{crit}.} = 5.14$; $P = 0.67$) was revealed between the means of the triplicate *O. niloticus* experimental groups at 4.8 ± 0.53, 4.77 ± 1.12, and 3.87 ± 2.83 mg/dl for HSD_1, HSD_2, and HSD_3, respectively, using one-way ANOVA analysis. A comparison of the statistical ($P < 0.05$) difference ($F_{(\text{df},2;\ 6)} = 20.32 > F_{\text{crit}.} = 5.14$; $P = 0.01$) between the means 4.49 ± 0.54 and 6.33 ± 0.46 for $\text{LSD}_{1,2,3}$ and $\text{HSD}_{1,2,3}$ groups, respectively, revealed a significant difference between the two means. The small values of SEM (0.31_{LSD}; 0.27_{HSD}) for the true means (4.49_{LSD}; 6.33_{HSD}) confirm the fact that the true means vary negligibly from the control and experimental groups' means and are, therefore, reliable (Table 3).

3.2. Whole Blood Glucose. One-way ANOVA analysis of the triplicate *O. niloticus* control groups gave no statistical ($P < 0.05$) difference in their means ($F_{(\text{df},2;12)} = 0.161 < F_{\text{crit}.} = 3.885$; $P \geq 0.01$) at 77.76 ± 12.03, 74.52 ± 11.64, and 78.12 ± 9.23 mg/dl for LSD_1, LSD_2, and LSD_3, respectively. Similarly, no statistical ($P < 0.05$) difference ($F_{(\text{df},2;\ 12)} = 0.674 < F_{\text{crit}.} = 3.885$; $P \geq 0.01$) was revealed between the means of the triplicate *O. niloticus* experimental groups at 100.8 ± 14.40, 94.68 ± 5.78, and 95.04 ± 5.28 mg/dl for HSD_1, HSD_2, and HSD_3, respectively, using one-way ANOVA analysis (Table 3). One-way ANOVA analysis revealed a statistical ($P < 0.05$) difference in the means of whole blood glucose concentrations in HSD and LSD *O. niloticus* groups ($F_{\ (\text{df},1;8)} = 31.845 > F_{\text{crit}.} = 5.318$; $P = 0.01$). Mean plasma glucose concentration was statistically ($P \leq 0.01$) higher for HSD than LSD *O. niloticus* groups at mean ± SD, 96.84 ± 5.28 mg/dl and 76.80 ± 5.92 mg/dl, respectively. Like in cortisol, the small values of SEM (2.65_{LSD}; 2.36_{HSD}) for the true means ($76.80 \pm 5.92_{\text{LSD}}$; $96.84 \pm 5.28_{\text{HSD}}$) similarly confirm the fact that the true means vary negligibly from the control and experimental groups' means and are, therefore, reliable (Table 4).

Finally, whole blood glucose concentration levels were organised in an ascending order for both LSD and HSD and,

Table 1: Standard concentration (ng/ml), optical density (absorbance value), and $\%B/B_o$.

Standard	Standard Concentration (ng/ml)	Optical density (Absorbance value)	$\%B/B_o$
S_o (B_o)	0.0	0.764	100
S_1 (B_1)	0.04	0.655	86
S_2 (B_2)	0.10	0.627	82
S_3 (B_3)	0.2	0.439	57
S_4 (B_4)	0.4	0.427	56
S_5 (B_5)	1.0	0.290	38
S_6 (B_6)	2.0	0.252	33
S_7 (B_7)	10.0	0.226	30

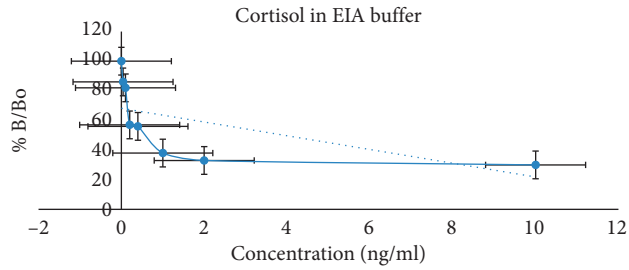

Figure 1: Standard curve.

Table 2: Plasma cortisol levels and means ($P < 0.05$) for HSD and LSD *O. niloticus*.

HSD		LSD	
Samples	Cortisol, ×10 ng/ml	Sample	Cortisol, ×10 ng/ml
Un_1	7.2	Un_{11}	0.6
Un_2	6.0	Un_{12}	5.0
Un_3	6.7	Un_{13}	5.4
Un_4	4.28	Un_{14}	3.5
Un_5	6.5	Un_{15}	5.2
Un_6	5.9	Un_{16}	5.6
Un_7	6.7	Un_{17}	5.2
Un_8	5.6	Un_{18}	5.6
Un_9	8.3	Un_{19}	4.2
$^*Un_{10}$	6.0	$^*Un_{20}$	5.9
Mean	6.32	Mean	4.62
SD	1.06	SD	1.58
SE	0.33	SE	0.50

Un_1–Un_{10} represent HSD, while Un_{11}–Un_{20} represent LSD. *Un: extra samples drawn from the second aquaria from both HSD and LSD groups.

then, used to establish a graph of glucose concentration in $mgdl^{-1}$ against individual samples of *O. niloticus*, as shown in (Figure 3).

4. Discussion

4.1. Plasma Cortisol. The plasma cortisol concentration level was significantly high in HSD than in LSD for the *O. niloticus* groups. The mean ± SD (i.e., 6.32 ± 1.06 ng/ml and 4.62 ± 1.58 ng/ml) plasma cortisol values obtained from HSD and LSD groups (Table 2) compared favourably with the reported normal mean basal cortisol range of 5–60 ng/ml for *O. niloticus* [22,37]. This range also accommodates the mean ± SD basal value range of 31.08 ± 4.94 to 47.97 ± 9.37 ng/ml ($n = 6$) obtained on day 0 in an experiment on conditioning of stress in Nile tilapia [28]. Other studies cited in [37] show the mean basal plasma cortisol ranges of 5–15 ng/ml, 16.43–39.22 ng/ml, and 5–50 ng/ml for *O. niloticus* and 20–60 ng/ml for a related Cichlid, *O. mossambicus*. Studies involving other related fish families such as Cyprinid *Cyprinus carpio*, Salmonids *Oncorhynchus clarkii*, and *Oncorhynchus mykiss* showed similar ranges of basal plasma cortisol levels.

The slightly lower than normal basal corticosteroid stress response mean ± SD value in LSD in this study is not a universal phenomenon in this fish group [12]. However, the relatively low intensities of cortisol in both the experimental and control groups *O. niloticus* may

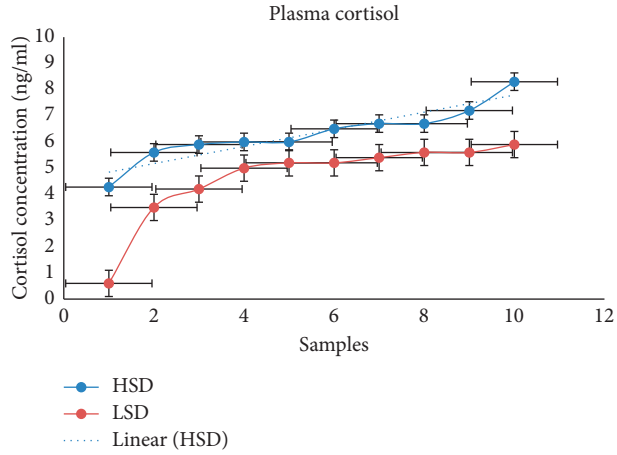

Figure 2: Plasma cortisol concentrations in High Stocking Density- (HSD-) and Low Stocking Density- (LSD-) reared *O. niloticus* after 21 days.

Table 3: Cortisol concentrations and group means for LSD and HSD triplicates, at $P < 0.05$.

Sample number	Cortisol concentrations, ×10 in ngml^{-1}							
	LSD_1	LSD_2	LSD_3	Mean	HSD_1	HSD_2	HSD_3	Mean
1	5.2	3.5	5.6	4.8	5.6	6.7	4.28	5.5
2	4.2	5.6	0.6	3.5	8.3	7.2	6.7	7.4
3	5.0	5.2	5.4	5.2	6.0	5.9	6.5	6.1
*4	—	5.9	—	—	—	6.0	—	—
Mean	4.80	4.80	3.87	4.50	6.60	6.60	5.80	6.30
SD	0.53	1.12	2.44	0.89	2.12	0.64	1.34	1.08
SE	0.31	0.65	1.41	—	1.22	0.37	0.77	—
SEM	—	—	—	0.51	—	—	—	0.62

Table 4: Whole blood glucose concentrations and group means for LSD and HSD triplicates, at $P < 0.05$.

Sample number	Concentrations (mmol·l^{-1} ×18) in mgdl^{-1}							
	LSD_1	LSD_2	LSD_3	Mean	HSD_1	HSD_2	HSD_3	Mean
1	84.6	79.2	66.6	76.8	99.0	90.0	99.0	96.0
2	57.6	77.4	88.2	74.4	126.0	95.4	93.6	105.0
3	84.6	79.2	72.0	78.6	91.8	102.6	90.0	94.8
4	86.4	82.8	86.4	85.2	91.8	88.2	91.8	90.6
5	75.6	54.0	77.4	69.0	95.4	97.2	100.8	97.8
Mean	77.76	74.52	78.12	76.80	100.80	94.68	95.04	96.84
SD	12.03	11.64	9.23	5.92	14.40	5.78	4.67	5.28
SE	5.38	5.21	4.13	—	6.44	2.58	2.09	—
SEM	—	—	—	2.65	—	—	—	2.36

have been as a result of extrinsic nature where response is affected by external factors, i.e., season, time of the day, and from the intrinsic nature dependent on the genotype or phenotype of the fish such as rapid conversion of cortisol into less immunoreactive cortisone [26]. It should also be noted that differences in corticosteroid stress responses exist among stocks of the same fish, hence the low cortisol levels recorded [12].

Effects of extrinsic stress factor(s) or prolonged sub-optimal conditions unknown to the researcher may also have led to the relatively low cortisol intensities in both control and experimental *O. niloticus* groups because the interrenal tissues may have become less sensitive to the action of ACTH or other pituitary hormones leading to low cortisol secretion [26]. Different hormones such as alpha-melanocyte-stimulating hormone (MSH), endorphin from the pars intermedia (PI), and some sympathetic nerve fibres [38] have been implicated in cortisol release during the chronic phase in fishes, functioning as an emergency system. However, if the suboptimal condition persists, this system may be deleted [16], leading to impaired cortisol release in fish subjected to stressors. It should, however, be noted that the net effect of these apparent unknown stress factors had no bearing on cortisol levels of the control and experimental fish because of the standardized experimental conditions. However, if the effect was there, then it must have been uniform due to randomization and replication of the experiments.

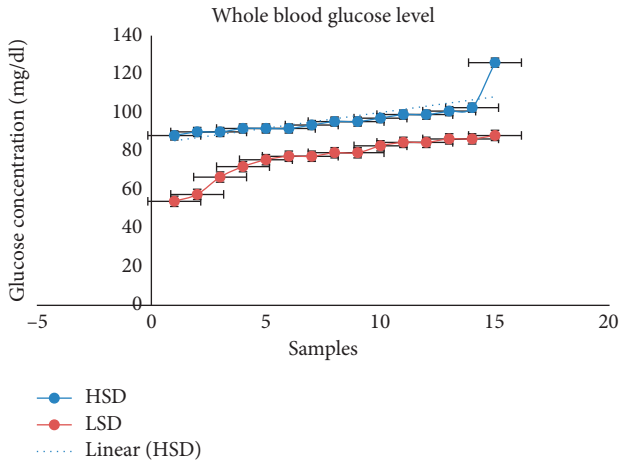

Figure 3: Glucose concentrations in Low Stocking Density- (LSD-) and High Stocking Density- (HSD-) reared *O. niloticus*.

Both physiological and biological status of the fish used in this study were standardized prior to the experiment leading to a basal cortisol mean value of 4.62 ± 1.58 ng/ml in LSD-reared fish against which the rise in plasma cortisol in HSD-reared *O. niloticus* is compared.

In the current study, the high plasma cortisol concentration level in the HSD group than in the LSD group was stress-induced [39], with chronic stress (i.e., high stocking density), producing a sustained elevation of cortisol in fish [40]. Elevated plasma cortisol, therefore, indicates that chronic stress occurred in HSD-reared *O. niloticus* [16, 26]. Plasma cortisol is actually a good acute stress marker [41], with adrenaline considered as the stress hormone and cortisol, the adaptive hormone [42].

Figure 2 clearly demonstrates variation in the trend of the cortisol concentrations with subsequent HSD samples against lower but increasing cortisol concentrations in subsequent LSD samples. The linear (HSD) trend line shows that all but one sample had cortisol concentration below the highest cortisol concentration recorded from LSD samples. This reinforces the fact that high stocking density induced stress in *O. niloticus*.

4.2. Whole Blood Glucose. Since glucose is an innate immune parameter mediated by stress, an increase in glucose concentration is a secondary response to stress, and the level of the increase is a measure of stress [21]. Glucose concentration has been widely used in a variety of fish species as an indicator of stress in genetic studies [26, 27]. Glucose is an indicator of sympathetic activation during stress [38].

The high blood glucose concentration recorded for HSD in this study can be associated with the single independent variable introduced, high stocking density, thus indicating the occurrence of chronic stress in the experimental *O. niloticus* group. This is consistent with previous studies which have reported statistically increased blood glucose levels in stressed fish. A three- (3-) week period of crowding stress elevated glucose in gilthead sea bream (*Sparus auratus*) [26]. During stress episodes, catecholamine acts directly on the liver to stimulate glycogenolysis, which results in the mobilization of glucose [43]. Catecholamines promote the phosphorylation of the enzyme glycogen phosphorylase which results in increased glycogenolysis. Enhanced glycogenolysis or a decreased clearance of glucose from the blood is the source for increased plasma glucose concentrations in stressed tilapia [44].

Barreto and Volpato [22] report a mean basal glucose concentration of 39.6 and 34.2 mg/dl for electroshock and social stressors, respectively, both of which induced acute stress in *O. niloticus*. These concentrations compare favourably with a whole blood glucose mean concentration of 76.82 ± 5.92 mg/dl obtained from the triplicate LSD control *O. niloticus* groups. In addition, the triplicate *O. niloticus* groups' whole blood concentration means for LSD_1, LSD_2, and LSD_3, respectively, showed no statistical difference. Similarly, no statistical difference was established between the means of the triplicate *O. niloticus* experimental groups, HSD_1, HSD_2, and HSD_3, respectively (Table 4). This could be attributed to the standardized experimental conditions. In the present study, mean ± SD for the blood glucose concentration level was statistically high in HSD at 96.84 ± 5.28 mg/dl than in LSD at 76.80 ± 5.92 mg/dl for the *O. niloticus* groups. This is supported by strong evidence that the blood glucose concentration means for both experimental and control groups were statistically ($P < 0.05$) different. Stress hormones, adrenaline, and noradrenaline in conjunction with cortisol mobilized and elevated glucose to cope with the energy demand in response to high stocking density-induced stress and, hence, an increase in the blood plasma glucose level in HSD *O. niloticus*.

Like in cortisol, Figure 3, a clear demonstration of variation in the trend of whole blood concentrations with subsequent HSD samples against lower but also increasing whole blood glucose concentrations in subsequent LSD samples (Figure 3) is observed. The linear (HSD) trend line shows that only the highest whole blood concentration (88.2 mg/dl) in LSD matched the lowest whole blood concentration recorded in HSD samples. The linear trend line

further shows a steady rise in whole blood concentrations in HSD samples compared to LSD samples. This proves that high stocking density induced stress in *O. niloticus*.

5. Conclusions

The results of this study demonstrate the fact that high stocking densities have a significant effect on plasma cortisol and whole blood glucose concentration in tilapia fish in aquaculture systems. The elevated level of plasma cortisol and whole blood glucose indicated that chronic stress induced by high stocking density occurred in the experimental fish. It can, therefore, be argued that the fish suffered increased glycogenolysis or a decreased clearance of glucose from the blood, thus raising plasma glucose concentration in the tilapia.

It was evident that cortisol concentration in plasma elevated in response to stress and aided in carbohydrate metabolism and promoted glucogenesis. Stress can eventually affect physiological activities such as feeding and the immunity of the fish leading to exposure to pathogenic microbes that may lead to impaired growth thus a reduction in yields in tilapia reared under aquaculture systems.

The findings of this study can help inform the policy on the management of stress caused by overpopulation of *O. niloticus* and other related Cichlids under industrial aquaculture production.

Data Availability

The primary data used to support the findings of this study are available from the corresponding author upon request. Table 1 contains part of the primary data.

Conflicts of Interest

The authors declare no conflicts of interest.

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