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Consequences of Environmental Stressors on Hematological Parameters, Blood Glucose, Cortisol and Phagocytic Activity of Nile Tilapia Fish

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Authors' contributions

This work was carried out in collaboration between all authors. Author MMZ proposed the experiment and wrote the final form of the manuscript. Author KMEA executed the treatments and analyzed the data. Author MAZ supervised the nutritional statuses and helped in writing the manuscript. Author BRNA determined the phagocytic analysis. Author EEM helped in writing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Under intensive fish culture of Tilapia, there appears to be susceptible various environmental stressors. Stress factors normally reduce growth and damage the biological system of the fish resulting in a great economic loss.
Aims: The study aimed at elucidating effects of lack of feedstuffs, overcrowding and protein deficiency on Tilapia physiology and production.
Study Design: Ninety Tilapia fish, half males and half females were randomly allocated into four treatments [control (C), protein deficiency (P), fasting (F) and overcrowding (O)].

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Materials and Methods: Three aquaria were used for each treatment. Each aquarium has 6 fishes except in case of overcrowding each aquarium contained 12 fishes. Treatment lapsed for 30 days in C and P, 21 days for F and 14 days for O. Blood was collected for hematological traits and for biochemical attributes.

Results: There exists significant increase ($p<0.05$) in MCV values in fishes (males & females) exposed to deprivation of food. Moreover, males exhibited significant high MCV value when they were overcrowded. Mean values for MCV in males were 112.93, 123.45, 118.41 and 121.68 μm^3 for C, F, P and O, respectively. Males didn’t express changes in MCH due to treatment. The values for MCH (pg) were; 35.15, 35.45, 34.15 and 35.74 pg for C, F, P and O, respectively. On the other hand, there exist significant ($p<0.05$) decreases in MCH values in overcrowded and protein deficiency-group. The respective MCH values in females were; 37, 35.05, 34.35 and 34.46 for C, F, P and O, respectively. Percentage of MCHC showed significant decreases ($p<0.05$) in treated males and females compared with control fish. Values of mean corpuscular hemoglobin concentration in males were 31.11, 28.92, 28.98 and 29.42 mg/dl for C, F, P and O, respectively. Similarly in females the respective values were; 30.99, 27.84, 28.16 and 29.21 mg/dl, respectively.

Conclusions: Adverse environmental stressors had negative impacts on different physiological parameters of Tilapia fish causing severe reduction in the productivity and consequent severe economic losses.

Keywords: Environmental stressors; impact; freshwater fish; physiological parameters.

1. INTRODUCTION

Several studies are focusing on the effects of fasting and restricted fish diets. These studies concluded that the majority of fish did experience severe depletion for a part of time of their lives. The metabolic changes associated with starvation differ between species. In non-fatty species, fasting mainly affected glycogen levels in both liver and muscles, together with the insoluble myofibril proteins. Murat et al. [1] suggested that during fasting blood glucose levels in fish were largely maintained by gluconeogenesis, where glucocorticoids might play an important role in migrating fish especially in promoting gluconeogenesis during starvation. Mahajan and Dheer [2] observed that fasting negatively affected normal physiology of fish as in *Channa punctatus* showing hematological changes in erythrocytes and neutrophils. These changes appeared to be secondary to the stress induced by the starvation or any other factors as well as some biochemical values such as liver and muscle glycogen in addition to the blood glucose level as it might be good indicators of food deprivation stress in fish. Rainbow trout subjected to prolonged starvation for 30 days produced no significant changes in the MCV, MCH and MCHC, whereas erythrocyte counts (RBCs), hemoglobin concentration (Hb) and hematocrit value significantly declined [3]. In contrast, Mahajan and Daheer found that in the air-breathing fish (*Channa punctatus*) fasted for 8 weeks, the MCV, MCH, MCHC, leukocytes, erythrocytes counts, hemoglobin content and hematocrit value progressively increased for 5 weeks [2]. However, after that time a sharp decline in the above values were recorded until the end of fasting period. Shalabi found no significant changes in the hematocrit value, hemoglobin content, erythrocyte count, MCV, MCH and MCHC of the Nile tilapia (*Oreochromis niloticus*) after starved for 3 days [4]. However, after 6 days he found a significant reduction in the hematocrit value and highly significant reduction in total leukocyte count and hemoglobin content. Moreover, in channel catfish, erythrocyte count, leukocyte count and hematocrit didn't differ after 4 weeks of food deprivation; however hemoglobin content significantly increased after this period [5]. Pirhonen et al. [6] studied the effects of fasting during *A. salmonicida* infection in Chinook salmon for 32 days. They noticed that fasting reduced feed intake, decreased mortality rate and increased the survival rate in fish that exposed to *A. salmonicida* infection, compared to the well-nourished fish. Plasma cortisol concentration has widely been used as an indicator of stress in fish. Decreased cortisol concentration was observed when fish exposed to lack of feed. Benhaïm et al. [7] studied effects of fasting on self-feeding activity in Juvenile Sea bass. Self-feeding system has been primarily developed to allow fish to obtain food according to their energy and nutritional requirements. Several fish species live through natural fasting periods and can survive for months without feed showing compensatory growth after fasting. Under fasting changes in the behavior responses were also noticed. Fasting also caused endocrine alterations such as modification in plasma cortisol levels in Senegalese sole fish and metabolic changes in the used carbohydrates, lipid and proteins of different parts of the body. Cortisol concentrations were reduced in response to fasting [8,9]. El-Khaldi [10] studied the effects of different stress factors (i.e. hypoxia, overcrowding and starvation) on some physiological measurements of Nile tilapia, and the author found that the cortisol levels were 134.15, 144.27 and 154.12 ng/ml in control, overcrowded and starved fish, respectively. Moreover, significant reduction in the activity of lactate dehydrogenase (LDH) in overcrowded and fasting fish. Whereas, pyruvate kinase (PK) activity increased in overcrowded and in starved fish.

High stocking density imposes increased energy demands that require fish to cope with metabolic adjustments such as changes of gluconeogenic and glycolytic enzyme activities. As under such conditions feed consumption is reduced, the extra expenditure of energy has to be met by the body reserves, resulting in reduced growth [11]. Montero et al. [12] studied the hematological parameters in the blood of gilthead sea bream (*Sparus aurata*) juveniles held at different stocking densities. They found significant increases in hemoglobin content, hematocrit value and red blood cell count in fish held at high stocking density. On the contrary, no significant changes were observed between groups in MCV, MCH and MCHC when fish subjected to low density (30 fish/250 l) and high density (120 fish/250 l) for 15 weeks. Also, a linear or curvilinear relationship is usually found between stocking density and growth and survival rate of fish [13]. Loss of feeding behavior, hypoglycemia and hypochloremia were the more prominent features recorded in both Juvenile Coho salmon and Rainbow trout exposed to bad handling and overcrowding in intensive fish breeding farms [14]. Ajani et al. [15] evaluated some hematological parameters of juvenile African catfish fed two levels of crude protein (40, 45%) under varying stocking densities (10 fish/m^3 control, and 15 and 20 fish/m^3) for 12 weeks. They noticed significant increases in plasma glucose and protein in relation to stocking densities. They concluded that enhancement of feed quality especially protein level in the fish diet may hasten growth, reduce stressor's effects and improve health status of the fish. Under the intensive fish culturing, the dangerous stress level are of great importance or has a bad hazardous on fish health status and welfare as it increased the susceptibility of fish to diseases [16].

Protein is a major component in fish feeds because it provides the essential and nonessential amino acids to synthesize body protein and in part provides energy for maintenance. It is well known that protein content of diets should be maintained at a suitable level to minimize feed costs. Besides, when dietary protein level exceeds the requirement, the fish excretes more ammonia nitrogen into the surrounding environment, there by polluting the culture water. Growth of the fish also might be affected since energy that otherwise could have been used for growth is directed to deaminate the excess amino acids absorbed. On the contrary, a protein-deficient diet results in reduced growth of the fish [17-19]. Therefore, the objective of this study aimed at elucidating effects of such common stressors on Tilapia fish (male and female) hematogram and immunological responses.

2. MATERIALS AND METHODS

2.1 Location and Experimental Design

This study was conducted at the Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University during September 2010-April 2011. In the present study 90 Nile Tilapia (*Oreochromis niloticus*) were used; 45 males and 45 females. Fish weighed 50 ± 5 g on average. Fish were fed at 3% of mean body weight per day, three times a day, as adaptation period and the unhealthy fish were removed from experimental aquaria and replaced with other healthy fish. Feed ingredients in case of 30% crude protein (CP) composed of fish meal (24%), soybean meal (17%), wheat bran (25%), yellow corn (29%), corn oil (3%) and vitamins and minerals (2%). However, feed ingredients in case of 15% CP composed of fish meal (5%), soybean meal (5%), wheat bran (30%), yellow corn (55%), corn oil (3%) and vitamins and minerals (2%). The fish were acclimatized for at least 20 days before the beginning of the experiments. According to the previous literature, twelve glass aquaria were used (3 per treatment) for rearing the males and females fish tilapia in the experiment. Each aquarium measured 70 (L) ×40 (D) ×30 (W) cm with a capacity of 84 liters and each was allowed to maximally contain 60 liters of de-chlorinated tap water. Also the aquaria were supplied with a good aeration throughout the period of adaptation and experimentation. Water temperature was thermostatically adjusted at 27 ± 2℃ as optimum temperature for *O. nilotica*, hydrogen ion concentration (pH) was monitored using pH meter with a pH range of 7-8. As shown in Table 1, four treatments were designed and duration of treatment varied depending on the nature of treatment. Also, schedule of blood sampling varied upon the duration of each treatment. In fasting group, the fish were deprived of feed during 21 days of the experiment.

2.2 Calculation of Erythrocyte Indices

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae [20].

MCV (μm^3) = (Hematocrit value % ×10/ RBCs×10^6 (per mm^3))

MCH (pg) = (Hemoglobin (g/dl) ×10/ RBCs×10^6 (per mm^3))

MCHC (g/dl) = (Hemoglobin (g/dl) ×100/ Hematocrit value %)

2.3 Phagocytic Activity and Phagocytic Index

Phagocytic activity was determined according to Kawahara et al. [21] and Soliman [22]. Fifty µg *Candida albicans* culture (previously adjusted to be 100 mg/ml W/V) was added to 1 ml of heparinized blood and shaken in a water bath at 25℃ for 5 hours. Air dried blood smears were then stained with Gimsa stain. Phagocytosis was estimated by determining the proportion of macrophages, which contained intracellular yeast cells in a random count of 300 macrophages and expressed as percentage of phagocytic activity (PA). The number of phagocytized organisms was counted in the phagocytic cells and called phagocytic index (PI).

Phagocytic activity (PA) = (Percentage of phagocytic cells containing yeast cells/ Total number of phagocytic cells)

Phagocytic index (PI) = (Number of yeast cells phagocytized/ Number of phagocytic cells containing yeast cells)

2.4 Cortisol and Glucose Concentration Determination

Cortisol concentration in blood serum was determined by a commercial ELISA kit (Dima, Germany) using the method of [23]. Intra-assay CV was 4.3%. Serum glucose was determined according to Trinder (GOD-PAP method) [24] by colorimetric kits (Diamond, Egypt).

2.5 Statistical Analysis

In a factorial arrangement (4 treatments × 2 sexes) data were analyzed using least square analysis of variances between treatments and between sexes [25]. Differences between means were tested by the Duncan Multiple Range Test (DMRT) [26]. Significance level was considered at P<0.05. The statistical model used was as follow;

$$Y_{ijk} = \mu + T_i + S_j + (TS)_{ij} + e_{ijk}$$

Where:

Y_{ijk}: An observation on individual k,
µ: Overall mean,
T_i: Fixed effect of the i^{th} treatment,
S_j: Fixed effect of j^{th} sex,
$(TS)_{ij}$: The interaction between treatment and sex,
e_{ijk}: A random error assumed to be independent normally distributed with mean= 0 and variance= σ^2.

Table 1. Experimental design, duration and schedule of blood sampling within treatments*

Group	Treatment	Duration (days)	Blood sampling interval (days)
I- Control (C)	6 fish /aquarium, protein level in the diet was 30%.	30	0, 7, 14, 21, 30
II- Protein deficiency (P)	The same conditions as in group 1, except the dietary protein level was 15%.	30	0, 14, 30
III- Fasting (F)	The same conditions in as group 1 except these fish were fasted.	21	0, 14, 21
IV- Overcrowding (O)	The same conditions as in group 1 except the number of fish/ aquarium was 12.	14	0, 7, 14

**Three aquaria were used per a group*

3. RESULTS AND DISCUSSION

3.1 Changes in the Erythrocyte Indices (MCV, MCH and MCHC)

Red blood corpuscles count significantly ($P<0.05$) decreased among treatments by 11-16% below the control. The response of males and females to stressors was similar in the reduction of RBCs count. However, no difference was found between the two sexes in the RBCs value. Contrariwise, there were significant increases ($P<0.05$) by 6-12% in WBCs count due to protein deficiency and overcrowding, but not due to fasting in both sexes. Overall, hematocrit value (PCV) decreased by 9-11% and hemoglobin decreased by 14-18% in stressed compared with control fish. There exists a significant increase ($P<0.05$) in MCV values (Table 2) in fishes (males & females) exposed to deprivation of food, however protein deficiency in the diet and overcrowding didn't alter this value. Moreover, males exhibited significant ($P<0.05$) higher MCV value when they were overcrowded, whereas the female values were higher in other treatments. Mean values for MCV in males were 112.93, 123.45, 118.41 and 121.68 μm^3 for control, fasting, protein deficiency and overcrowding, respectively. The respective values for MCV in females were 119.39, 126.41, 122.18 and 118.74 μm^3.

The increase in the mean volume of the blood corpuscle in treated as compared to the control fish ranges between 6-7% in females and 7-16% in males.

Table 2. Mean (LSM) corpuscular volume (MCV, μm^3) in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	112.93^{d}	119.39^{cd}	116.16^{C}
Fasting	123.45^{bc}	126.41^{b}	124.94^{B}
Protein deficiency	118.41^{cd}	122.18^{bc}	120.93^{BC}
Overcrowding	121.68^{c}	118.74^{cd}	120.21^{BC}
SEM	0.810	0.810	0.810

$^{a, b, c, d}$ Means in the same column with different superscripts significantly differ (P< 0.05).
$^{A, B, C}$ Means in the same column with different superscripts significantly differ (P< 0.05)

As shown in Table 3 males didn't express changes in MCH due to treatment. The values for MCH (pg) were; 35.15, 35.45, 34.15 and 35.74 pg for C, F, P and O, respectively. On the other hand, females exhibited significant ($p<0.05$) decreases in MCH values in overcrowded and protein deficiency-group. The respective MCH values in females were; 37, 35.05, 34.35 and 34.46 for C, F, P and O, respectively. The range of the decrease in MCH in treated females relative to the control was 7-8%.

Table 3. Mean (LSM) corpuscular hemoglobin (MCH, pg) in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	35.15^{ab}	37.00^{a}	36.07^{AB}
Fasting	35.45^{ab}	35.05^{ab}	35.25^{BC}
Protein deficiency	34.15^{b}	34.35^{b}	34.24^{C}
Overcrowding	35.74^{ab}	34.46^{b}	35.09^{BC}
SEM	0.237	0.237	0.237

$^{a, b}$ Means in the same column with different superscripts significantly differ (P< 0.05).
$^{A, B, C}$ Means in the same column with different superscripts significantly differ (P< 0.05)

Similar findings to the current study were reported on GIFT tilapia when they undergone a stress of feed imbalance [27]. Percentage of MCHC (Table 4) showed significant decreases ($p<0.05$) in stressed males and females as compared with control fish. Values of mean corpuscular hemoglobin concentration in males were 31.11, 28.92, 28.98 and 29.42 mg/dl for C, F, P and O, respectively. However, in females the respective values were; 30.99, 27.84, 28.16 and 29.21 mg/dl. The decreases in this parameter range between 5-7% in males and 6-9% in females. Overall, there were non-significant differences between males and females in the three indices (Table 5).

Table 4. Mean (LSM) corpuscular hemoglobin concentration (MCHC, %) in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	31.11^{a}	30.99^{a}	31.05^{A}
Fasting	28.93^{b}	27.84^{b}	28.38^{B}
Protein deficiency	28.98^{b}	28.16^{b}	28.57^{B}
Overcrowding	29.42^{b}	29.21^{b}	29.31^{B}
SEM	0.163	0.163	0.163

$^{a, b}$ Means in the same column with different superscripts significantly differ (P< 0.05).
$^{A, B}$ Means in the same column with different superscripts significantly differ (P< 0.05)

The study of Johansson et al. [28] on European eels exposed to starvation for 47 days exhibited similar trend in the above parameters. On the contrary, Lane et al. [3] found no significant changes in MCV, MCH and MCHC in his study on feed-deprived rainbow trout for 30 days. Similar trend was found with Shalabi [4] on feed-deprived Nile tilapia for 6 days. Montero et al. [12] reared the gilthead sea bream juveniles at high stocking density (120 fish/ 250 l) and reported no changes in such parameters. There appears that there exist large variability between fish spices as far as the tolerance against stress parameters. Additionally, the differences in treatment duration (i.e. 6 days in Shalabi [4] study and 21 days in the current study) could explain such differences. The increase in corpuscular volume (MCV) might be ascribed to the increased water content in red blood cells resulting of chloride shift and decreased in plasma chloride at the same state of starvation and high ammonia in water. Moreover, the decrease of MCHC resulting from stress factors might be attributed to the hemodilution and/or the lack of production of hemoglobin in circulation.

Table 5. Differences in overall mean of erythrocyte indices between males and females of Nile tilapia

Sex	MCV (μm^3)	MCH (pg)	MCHC (%)
Male	121.59^{a}	35.59^{a}	29.47^{a}
Female	122.91^{a}	35.50^{a}	29.03^{a}
SEM	0.812	0.2366	0.163

^{a,b}Means in the same column with different superscripts significantly differ (P< 0.05)

3.2 Changes in the Leucocytes Differential Count

Percentage of neutrophil (Table 6) increased in the stressed tilapia fish (males and females). Overall, the degree of elevation in neutrophil was statistically high (P<0.05) in case of starvation and protein deficiency. Significant (P<0.05) increase was found only in case of protein deficiency in males, however in females elevation was found in fasting and protein deficiency. There existed significant differences between males and females as the values in females tended to be higher than in males. Percentage of neutrophil was higher in females (30.63%) than in males (27.76%).

Percentages of eosinophil are shown in Table 7. Overall, values of eosinophil were not significant than in control fish, although significant low levels were observed at fasting and protein deficiency in males, but not in females.

Table 8 presents data of basophil which didn't show a change in stressed as compared with control fish. Mean values were; 8.87, 8.48, 8.63 and 8.39 in males and 8.24, 8.17, 7.97 and 8.35 in females for C, F, P and O, respectively. The sole difference found in basophil was in case of protein deficiency in female Tilapia.

Table 6. Mean (LSM) of neutrophil percentage in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	26.22^{c}	28.13^{bc}	27.18^{B}
Fasting	27.48^{bc}	31.70^{a}	29.59^{A}
Protein deficiency	28.85^{b}	32.04^{a}	30.44^{A}
Overcrowding	27.21^{bc}	28.23^{bc}	27.72^{B}
SEM	0.234	0.234	0.234

^{a,b,c}Means in the same column with different superscripts significantly differ(P< 0.05).
^{A,B} Means in the same column with different superscripts significantly differ (P< 0.05)

Table 7. Mean (LSM) of eosinophil percentage in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	9.24^{a}	8.53^{bc}	8.89^{A}
Fasting	8.53^{bc}	8.92^{ab}	8.72^{AB}
Protein deficiency	8.48^{bc}	8.78^{b}	8.62^{AB}
Overcrowding	8.85^{ab}	8.85^{ab}	8.86^{AB}
SEM	0.064	0.064	0.064

^{a,b,c} Means in the same column with different superscripts differ significantly (P< 0.05).
^{A,B} Means in the same column with different superscripts differ significantly (P< 0.05)

Table 8. Mean (LSM) of basophil percentage in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	8.87^{a}	8.24^{b}	8.55^{A}
Fasting	8.48^{ab}	8.15^{bc}	8.31^{A}
Protein deficiency	8.63^{a}	7.97^{c}	8.29^{A}
Overcrowding	8.39^{ab}	8.35^{ab}	8.37^{A}
SEM	0.060	0.060	0.060

^{a,b,c} Means in the same column with different superscripts significantly differ(P< 0.05).
^{A,B} Means in the same column with different superscripts significantly differ (P< 0.05)

As shown in Table 9, percentage of monocytes were not affected by treatment in males, however it increased (P<0.05) in overcrowded females. Monocytes exhibit the least population of leukocytes in fish. The values in males were; 2.0, 1.92, 2.04 and 1.91 and in females were; 1.98, 2.07, 1.78 and 2.91 for C, F, P and O, respectively.

Table 10 presents data of lymphocytes. In male tilapia there were no differences due to treatment on recent of lymphocytes. On the other side, in female's percent of lymphocytes significantly decreased in case of fasting and protein deficiency. Overcrowding didn't affect percentage of lymphocytes in both sexes. Overall, lymphocyte percentage was higher in males (52.87%) than in females (50.38%). Contrary to what exists in most mammals, the most dominant leukocyte in fish is lymphocytes and the second in order is neutrophil.

Table 9. Mean (LSM) of monocyte percentage in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	2.00 [b]	1.98 [b]	1.99 [B]
Fasting	1.92 [b]	2.07 [b]	2.00 [B]
Protein deficiency	2.04 [b]	1.78 [b]	1.91 [B]
Overcrowding	1.91 [b]	2.91 [a]	2.42 [A]
SEM	0.050	0.050	0.050

[a, b] Means in the same column with different superscripts differ significantly (P< 0.05).
[A, B] Means in the same column with different superscripts differ significantly (P< 0.05)

There appears that the increase of neutrophil and decrease of lymphocytes might be attributed to the elevated blood cortisol in the treated fish. It is also well known that neutrophil play an important role to resist stressors. Neutrophils have also the ability to invade blood capillaries and migrate to other tissues in the body to phagocytize the microorganisms. In contrast, the decrease in lymphocytes might be attributed to the toxic effect of ammonia on body organs and consequently the damage caused on the organs producing leukocytes.

3.3 Changes in Blood Cortisol and Glucose

The only stress factor that considerably increased (P<0.05) blood cortisol (Table 11) was overcrowding. The fold of increase of cortisol in case of overcrowding was 2.4 in males and 2.55 in females of the control value. On the other hand, none of the other tested stressors had shown differences (P>0.10) in blood cortisol. In a study by El-Khaldi on the effects of starving and overcrowding on tilapia fish reared for 72 hours and found significant increase in cortisol in both cases compared with control [10]. Due to the aggression of fish in the high density condition searching for normal motion, breath and feed, this provoked the hypothalamo-pituitary suprarenal axis to release excess cortisol. Bianca suggested that plasma cortisol increases quickly after exposure to an acute stress [29]. Cortisol is considered a bio-indicator for both long term and short-term stressors, it also influenced by species, feeding, reproductive cycles, seasonal cycles, photoperiod, husbandry condition and sampling [30], and multiple stress condition seems to amplify the cortisol response [31]. Recently, Sokołowska et al. [32] reported a linear increase in plasma cortisol by the increase of stocking density of round goby (*Neogobius melanostomus*). Apparently, tilapia fish (males and females) respond to stress by production of corticosteroids to cope with the improper environment. Typically, this observation was confirmed in round gobies [33,34].

Table 10. Mean (LSM) of lymphocyte percentage in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	53.67 [a]	53.11 [ab]	53.39 [A]
Fasting	53.56 [a]	49.15 [c]	51.37 [B]
Protein deficiency	52.00 [ab]	49.44 [c]	50.72 [BC]
Overcrowding	53.62 [a]	51.69 [b]	52.66 [C]
SEM	0.198	0.198	0.198

[a, b, c] Means in the same column with different superscripts significantly differ(P< 0.05).
[A, B, C] Means in the same column with different superscripts significantly differ (P< 0.05)

Table 12 presents data of serum glucose as influenced by various stressors. Fasting decreased (P<0.05) blood sugar by about 19%, however diet protein deficiency and overcrowding massively increased (P<0.05) blood sugar by 28-63% above control. Lack of feedstuffs decreased blood glucose; however lack of protein in the diet apparently was compensated by a higher carbohydrate metabolism raising blood glucose. Similar to what exists in cortisol, overcrowding increased blood glucose in males (+54%) and females (70%) above control. Similar finding was found in tilapia reared for 144 h [10]. In a study on Atlantic cod exposed to overcrowding there expressed two genes related to glucose transport through different tissues [35]. In fish the functions of these genes are related to providing glucose reserves during acute or chronic low oxygen challenges [36]. It is well known that hyperglycemia, which is a stress response, takes place due to the release of catechol amines [37] and is needed to augment the energy demands of the fish for swimming and continuous social adaptation.

Table 11. Mean (LSM) of serum cortisol concentration (ng/ml) in Nile tilapia after exposure to various stress factors

Treatments	Male	Female	Overall
Control	2.95 [b]	2.72 [b]	2.84 [B]
Fasting	4.12 [b]	3.61 [b]	3.87 [B]
Protein deficiency	3.53 [b]	3.62 [b]	3.58 [B]
Overcrowding	7.07 [a]	6.96 [a]	7.01 [A]
SEM	0.194	0.194	0.194

[a, b] Means in the same column with different superscripts significantly differ (P< 0.05).
[A, B] Means in the same column with different superscripts significantly differ (P< 0.05)

Table 12. Mean (LSM) of serum glucose concentration (mg/dl) in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	52.58 [a]	54.80 [a]	53.69 [C]
Fasting	42.41 [b]	45.03 [b]	43.72 [D]
Protein deficiency	64.71 [c]	73.13 [d]	68.92 [B]
Overcrowding	81.20 [e]	93.37 [f]	87.28 [A]
SEM	1.454	1.454	1.454

[a, b, c] Means win the same column with different superscripts significantly differ (P< 0.05).
[A, B, C, D] Means in the same column with different superscripts significantly differ (P< 0.05).

3.4 Changes in the Phagocytic Activity and Phagocytic Index of Nile Tilapia

Table 13 presents data of the phagocytic activity (%) in tilapia after exposure to stress factors. There exist significant (P<0.05) increases in this parameter in treated (males and females) compared to control fish. The values in males were 23.15, 24.41, 23.44 and 24.81% for C, F, P and O, respectively. The respective values in females were 23.27, 24.44, 23.26 and 25.08%. The increase in this parameter ranges between 5-10% in males and 5-8% in females.

Table 13. Mean (LSM) of phagocytic activity (%) in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	23.15 [c]	23.27 [c]	23.21 [C]
Fasting	24.41 [b]	24.44 [b]	24.42 [B]
Protein deficiency	23.44 [c]	23.26 [c]	23.35 [C]
Overcrowding	24.81 [ab]	25.08 [ab]	24.95 [AB]
SEM	0.105	0.105	0.105

[a, b, c] Means in the same column with different superscripts differ significantly (p< 0.05).
[A, B, C] Means in the same column with different superscripts differ significantly (P< 0.05)

Likewise, the phagocytic index (Table 14) as a measure of the ability of neutrophils to phagocytize the *candida albicans*, was found to be higher in treated than control fish. The highest value of this parameter was found in the overcrowding males and females. The values of phagocytic index in males were; 2.59, 2.95, 2.71 and 3.12 for C, F, P and O, respectively. The respective values in females were; 2.51, 2.91, 2.56 and 3.04. The increase in such an index in stressed male's ranges between 5-20%, however this range in females was found to be 15-22%. There found no significant differences in these two defensive parameters between males and female (Table 15).

Table 14. Mean (LSM) of phagocytic index in Nile tilapia after exposure to various stress factors

Treatment	Male	Female	Overall
Control	2.59 [d]	2.51 [d]	2.55 [B]
Fasting	2.95 [b]	2.91 [bc]	2.97 [A]
Protein deficiency	2.71 [c]	2.56 [d]	2.63 [B]
Overcrowding	3.12 [ab]	3.04 [ab]	3.09 [A]
SEM	0.026	0.026	0.026

[a, b, c, d] Means in the same column with different superscripts significantly differ(p< 0.05).
[A, B] Means in the same column with different superscripts significantly differ (P< 0.05)

Table 15. Differences in overall mean of phagocytic activity and phagocytic index between males and females of Nile tilapia

Sex	Phagocytic activity (%)	Phagocytic index
Male	24.29 [a]	2.86 [a]
Female	24.23 [a]	2.83 [a]
SEM	0.105	0.0263

[a, b] Means in the same column with different superscripts significantly differ (P< 0.05)

These results disagree with Haggag who reported that phagocytic activity and phagocytic index were decreased significantly in Nile tilapia after 8 weeks of starvation [38]. There appears that the impact of such stressors on the phagocytic leukocytes is due to treatments which caused increases in neutrophils. Such increases resulted in enhancement of the activity of these phagocytes. Overcrowding in males caused the highest response which might be attributed to their aggressiveness; this behavior was not clear in females. Moreover, this might also be a response to the excess release of cortisol in blood circulation in these stressed fish.

Lack of protein in fish diet has been a focus on various fish species. Protein considered as an essential nutrient for fish growth and maintenance of physiological functions representing between 50 and 70% of the total feed costs [39]. Nwanna et al. [40] indicated that in inadequate dietary protein retarded fish growth and nutrient utilization. In the present study the shortage of diet protein of 15% retarded the body weight to be 66% of the control. Overcrowding reduced the final body weight in the current study to reach 75% of the contemporary control. Fasting revealed a reduction in final body weight to reach 66% of the control. The least stressful environmental factor on tilapia growth is overcrowding. The highest release of cortisol in the overcrowded group might enhance carbohydrate metabolism.

4. CONCLUSION

In conclusion, tilapia fish might tolerate for some environmental stressors with various physiological mechanisms. Tested environmental parameters (i.e. high stocking density, shortage of protein in diet and lack of feedstuffs) are great challenges facing the growth and productivity of Tilapia farming. For better growth, health and consequently for best tilapia farming economic feasibility, there must avoid stress factors that hinder fish production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Murat JC, Plisetskaya E, Woo NYS. Endocrine control of nutrition in cyclostomes and fish. Comparative Biochemistry and Physiology. 1981; 68(Part A):149-158.
2. Mahajan CL, Dheer TR. Haematological and haematopoietic responses to starvation in an air-breathing fish, *Channa punctatus* (Bloch). Journal of Fish Biology. 1983;22:111-123.
3. Lane HC, Rolfe AE, Nelson JR. Changes in the nucleotide triphosphate/hemoglobin and nucleotide triphosphate/red cell ratios of rainbow trout, *Salmo gairdneri Richardson*, subjected to prolonged starvation and bleeding. Journal of Fish Biology. 1981;18:661-668.
4. Shalabi SI. Some physiological studies on the Nile tilapia in response to stress conditions. M.Sc. Thesis, Faculty of Agriculture, Zagazig University; 1992.
5. Shoemaker CA, Klessius PH, Lim C, Yildirim M. Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. Journal of Fish Diseases. 2003;26:553-561.
6. Pirhonen J, Schreck CB, Reno PW, Ogut H. Effect of fasting on feed intake, growth and mortality of Chinook salmon, *Oncorhynchus tshawytscha*, during an induced *Aeromonas salmonicida* epizootic. Aquaculture. 2003;216:3-38.
7. Benhaïm D, Marie LB, Samuel P, Chatain B. Effect of fasting on self-feeding activity in Juvenile Sea bass (*Dicentra chuslabrax*). Applied Animal Behavioral Science. 2012;136(1):63-73.
8. Costas BC, Aragao I, Ruiz JL, Conceicao EC. Feed deprivation in Senegalese sole (*Solea sengalensis*) Kaup, (1858) juvenile: Effects on blood plasma metabolites and free amino acid levels. Fish Physiology and Biochemistry. 2011;37:495-504.
9. Caruso GM, Denaro GR, Genovese F, Marichielo G. Short fasting and refeeding in red porgy (*Pagruspagrus* L 1758): Response of some hematological, biochemical and non- specific immune parameters. Marine Environmental Research. 2012;81:18-25.
10. EL-Khaldi ATF. Effect of different stress factors on some physiological parameters of Nile tilapia (*Oreochromis niloticus*). Saudi Journal of Biological Sciences. 2010;17:241-246.
11. Trenzado CE, Morales AE, Manuel DLH. Physiological effects of crowding in rainbow trout, *Oncorhynchus mykiss*, selected for low and high stress responsiveness. Aquaculture. 2006;258: 583-593.
12. Montero D, Izquierdo MS, Tort L, Robaina L, Vergara JM. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead sea bream, *Sparusaurata*, juveniles. Fish Physiology and Biochemistry. 1999;20:53-60.
13. Huang WB, Chtu TS. Effect of stocking density on survival, growth, size variation, and production of tilapia fry. Aquaculture Research. 1997;28:165-173.

14. Gray AW. Physiological response of juvenile Coho salmon (*Concorhyn chuskisutch*) and Rainbow trout (*Salmogairdneri*) to handling and crowding stress in intensive fish culture. Journal of the Fish Research Board of Canada. 2011;33(12):2699-2702.
15. Ajani EK, Setufe SB, Oyebola OO. Effects of stocking density on hematological functions of juvenile African catfish (*Clarias gariepinus*) fed varying crude protein levels. African Food Science. 2015;9(2): 65-69.
16. Saraiva A, Costa J, Eiras JC, Cruz C. Histological study as indicator of juvenile farmed turbot, *Scophthalmus maximus* L., health status. Aquaculture. 2016;459:210-215.
17. Shyong W, Huang C, Hon-Cheng C. Effects of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata*. Aquaculture. 1998;167:35-42.
18. Yang S, Liou C, Fu-Guang L. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). Aquaculture. 2002;213:363-372.
19. Webb KAJ, Gatlin DM. Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum *Sciaenops ocellatus*. Aquaculture. 2003;225:17-26.
20. Seiverd CE. Hematology for medical technologists. Leo and Fibiger, Philadelphia. USA. 1964;334.
21. Kawahara E, Ueda T, Nomura S. *In vitro* phagocytic activity of white spotted shark cells after injection with *Aeromonas salmonicida* extracellular products. Gyobyo Kenkyu, Japan. 1991;26:213-214.
22. Soliman MK. Depression of phagocytosis in carp (*Ctenopharyn godonidella*) following chemical stress. Alexandria Journal of Veterinary Science. 1997;13:81-86.
23. Foster LB, Dunn RT. Single-antibody technique for radioimmunoassay of cortisol in un-extracted serum or plasma. Chemistry. 1974;20:365-368.
24. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry. 1969;6:24.
25. SAS. SAS user's guide: Statistics version eight. SAS Institutes. Inc. Cary. NC, USA; 2000.
26. Steel JD, Torrie JH. Principals and procedures of statistics. A Biometrical Approach 4th Edition. McGraw Hill. USA; 1984.
27. He J, Jun Qiang J, Naftal Gabriel N, Xu P, Yang R. Effect of feeding-intensity stress on biochemical and hematological indices of Gift Tilapia (*Oreochromis niloticus*). Turkish Journal of Fisheries and Aquatic Sciences. 2015;15:303-310.
28. Johansson ML, Dave G, Larssone A, Lewander K, Lidman U. Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla L*. II. Comparative Biochemistry and Physiology Part A. 1975;52:431-434.
29. Bianca MP. Farmed fish welfare-suffering assessment and impact on product quality. Journal of Animal Science. 2009;8:139-160.
30. Barton BA. Stress in fishes a diversity of responses with particular reference to changes in circulating corticosteroids. Integrated Comparative Biology. 2002;42: 517-525.
31. Ortuno J, Angeles Esteban M, Meseguer J. Lack of effect of combining different stressors on innate immune responses of sea bream (*Sparus aurata L*.). Veterinary Immunology and Immunopathology. 2002;84:17-27.
32. Sokołowska E, Kleszczyńska A, Kalamarz-Kubiak H, Arciszewski B, Kulczykowska E. Changes in brain arginine vasotocin, isotocin, plasma 11-ketotestosterone and cortisol in round goby, *Neogobius melanostomus*, males subjected to overcrowding stress during the breeding season. Comparative Biochemistry and Physiology, Part A. 2013;165:237-242.
33. Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MAW, Blokhuis HJ. Coping styles in animals: Current status in behavior and stress-physiology. Neuroscience Biobehavior Review. 1999;23:925-935.
34. Øverli Ø, Sørensen C, Pulman KGT, Pottinger TG, Korzan W, Summers CH, Nilsson GE. Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian

vertebrates. Neuroscience Biobehavior Review. 2007;31:396-412.

35. Caipang CMA. Potential molecular biomarkers of crowding stress in Atlantic cod, *Gadus morhua* and their importance in health management. ABAH Bioflux. 2012;4(2):79-83.
36. Hall JR, Richards RC, MacCormack TJ, Vanya Ewart K, Driedzic WR. Cloning of GLUT3 cDNA from Atlantic cod (*Gadus morhua*) and expression of GLUT1 and GLUT3 in response to hypoxia. Biochimica et Biophysica Acta. 2005;1730:245-252.
37. Andersen DE, Reid SD, Moon TW, Perry SF. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences. 1991;48:1811-1817.
38. Haggag SA. The effect of some environmental factors on the immune response of the cultured fish and its relation with the diseases. PhD. Thesis. Faculty of Veterinary Medicine. Alexandria University; 2004.
39. Nwanna L. Performance of hybrid clariid catfish fingerlings (male *Heterobranchus Bidorsalis x* female *Clarias gariepinus*) fed poultry layer waste diets in glass tanks. Journal of Applied Aquaculture. 2002;12(3):99-106.
40. Nwanna LC, Omojola I, Nwanna E, Abiodune E. Effect of protein deficient diets on the growth and carcass protein ash ratio of African catfish *Clarias gariepinus* (Burchell 1822). Journal of Applied Sciences and Environmental Management. 2014;18(3):537-541.

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