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ORIGINAL ARTICLE

Effect of different stress factors on some physiological parameters of Nile tilapia (*Oreochromis niloticus*)

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Abstract This study was conducted to determine the effect of different stress factors on some physiological measurements of Nile tilapia (*Oreochromis niloticus*).

A total number of 160 Nile tilapia, the body weight ranging between 100 and 120 g, were exposed to three stress factors of hypoxia, overcrowding and starvation for different periods 24, 72 and 144 h. The results of cortisol level were 134.15, 144.27, 154.12 ng/ml and 140.18 ng/ml for control, hypoxia, overcrowding and starvation, respectively, while after 144 h did not show significant difference among treatments compared with control group. In contrast, the values of T_3 and T_4 observed reduction with significant difference that T_3 ranged between the highest value 122.12 ng/ml for control group to lowest value of starvation group 94.35, 93.81 and 88.46 ng/ml after 24, 72 and 144 h. Also, similar trend of results observed in T_4 and blood glucose among treatments. And the enzymatic activity of lactate dehydrogenise (LDH) increased in hypoxic group, while a significant reduction appeared in overcrowding and starved fish compared to control group. The pyruvate kinase (PK) activity decreased in hypoxic group but increased in other group.

1. Introduction

Stress was defined as a state of decreased fitness, or any external agent which challenges the homeostatic power of any organism or threatens its survival (Colombo et al., 1990). In addition, the impact of aquacultural related stressors can also predispose fish to disease (Eddie and Norman, 2008).

Survival conditions and activities used during aquaculture practices cause stress (acute or chronic) and can involve a reduction of welfare, the mainly relevant factors for the welfare reduction of farmed fish can be fisted as following: genetic, environmental factors, stocking density during growth, malnutrition, starvation, cataracts, deformities, transport, handing, selection, overcrowding (Conte, 2004).

The magnitude and the type of behavioural and physiological response to stress can vary among fish species, but also at strain and individual levels (Schyolden et al., 2005).

A variety of physiological changes in response to stressor had been reported in fish as the primary stage involved

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neuroendocrine response such as catecholamine's release and activation of corticotrophin interrenal axis, followed by secondary response such as haematological, metabolic, blood enzymatic and osmoregulatory changes (Mazeaud et al., 1977; Sumpter, 1997).

As well as function involving stimulation of oxygen uptake and transfer, mobilization of energy substrates, reallocation of energy away from growth and reproduction and mainly suppressive effects of immune functions (Wendelaar Bonga, 1997), while the tertiary stage resulting from chronic exposure of fish to stressor is susceptibility to pathogens and behavioural changes (Pickering, 1981). On the other hand, (Coimbra and Reis-Henriques, 2007) found ovary and testicular alterations with decline in T_4 levels and normal levels of T_3 in Tilapia fish exposed to stress factors. In stress responses and behaviour which seem to indicate that also fish are able to feel pain and sufferance mainly at the end of their life (Lambooij et al., 2002; Braithwaite and Huntingford, 2004; Chandroo et al., 2004).

The stress axis is activated in response to food intake, but in contrast to mammals and birds, it is not activated during periods of food deprivation (Crespi et al., 2004). Thus, short-term overcrowding of Atlantic cod leads to a transient enhancement of in vitro serum antibacterial activity and enhanced transcriptional activity of glucose (Christopher et al., 2008).

In addition, the response varies with the nature of stress and the species of fish under investigation, stressor mediate changes in plasma cortisol as (Bianca, 2009) said that plasmatic levels of cortisol were increased quickly after exposure to acute stress and the standard conditions are restored in few hours.

Many workers have studied blood corticosteroid levels as an indicators of stress because the extreme sensitivity of the hypothalamo–pituitary interrenal (HPI) axis (Barton, 2002; Ortuno et al., 2002).

Fish are in constant interaction with their environment through the gills and skin, therefore water quality (Dissolved oxygen, salinity, NH_3, nitrites, PH, temperature, pollutants levels), is crucial for their welfare, (Bianca, 2009). Oxygen in its molecular state O_2 is essential for many metabolic processes that are vital to aerobic life, and aerobic organisms cannot exist without oxygen, which nevertheless is inherently dangerous to their lives. Like all aerobic organism, fish are also susceptible to the effect of reactive oxygen and have inherent and effective antioxidant defense (Rosa et al., 2005). In addition, Scapigliati et al. (1999) proved that low oxygen levels negatively influenced sea bas immunoglobin levels.

On studying the effects of low oxygen on the respiratory system of fish (Hughes, 1981) concluded that interference might occur in the main stages of oxygen transfer from a medium in which the fish was contained, via the blood and then to the sites of oxidative metabolism in the cells.

Crowding stress may be an important factor by which rearing density could affect the physiology of fish (Reynaldo et al., 1987; Ellis et al., 2002).

An intermediate level of density tends to increase growth efficiency and to decrease disease incidence (Ewing and Ewing, 1995).

Fine damage or erosion can also occurs as a results of aggressive interactions which may increase susceptibility to secondary infection (Turnbull et al., 1996).

North et al. (2006) reported that stocking density of 80 kg m^{-3} did not produce consistent effects of rainbow trout mean growth rate or physiological indicators, but fin erosion increased with increasing density (10, 40, 80 kg m^{-3}).

Fish starving is a practice used before the transport to reduce metabolic rate, stress response, oxygen consumption and production of discard products. Starving can be also a natural behaviour in fish according to the water temperature, the age, the species and the season (Einen et al., 1998).

Food deprivation, toxic polycycling aromatic hydrocarbons and hypoxia are environmental problems world wide and have been shown to impair growth for several fish species including red drum, *Sciaenops* ocellatus (Smith and Fuman, 2004), the orangespotted grouper, *Epinephelus coioides* (Heintz et al., 2000; Yeun and Au, 2006) and the marine medaka *Oryzias* melastigma (Picharan et al., 2000; Yu et al., 2006).

The aim of the present study was to clarify the effect of different stress factors like hypoxia, overcrowding and starvation on some physiological parameters of Nile tilapia (*Oreochromis niloticus*) including hormonal level of cortisol, T_3 and T_4, plasma levels of glucose, as well as glycogen content in the liver and muscle, in addition to the enzymatic activity of serum LDH and erythrocyte PK.

2. Materials and methods

In the present study, a total number of 160 *O*. niloticus fish obtained from sharkia governor fish farms, and ranging between 100 and 120 g weights were used. The fish were kept in a glass aquaria measuring $100 \times 50 \times 50$ cm^3 in well aerated and dechlorinated tap water with oxygen concentration 5.4 ± 0.4 mg/L, pH level 7.4 ± 0.2, temperature 3 °C salinity was 0.3 ppt, water hardness of 102 mg/L, as $CaCo_3$ to get acclimatized for 7 days before the beginning of the experiment. The fish were kept under natural day light and supplied with commercial pelleted ration at a rate of 1% of the body weight twice daily.

Prophylactic measurement were carried out for all fish to avoid parasitic and microbial infection. The fishes were divided into 4 equal groups, each contained 40 fish, which were located equally in glass aquaria (each containing 10 fish) as follows:

Group I: Control group, kept under optimal and normal environmental conditions.
Group II: Hypoxic group, hypoxic conditions were carried by removing aeration from the aquaria (Graham et al., 1987). The dissolved oxygen was 1.5 mg/L.
Group III: Overcrowding group, the fish were placed in glass aquaria with an average of one liter/2.5 cm of length.
Group IV: Starved group, the fishes had the normal constant environmental condition except that it was starved throughout the experimental period.

2.1. Sampling

Blood samples were taken by serving the caudal vessels after 24, 72, and 144 h from the beginning of the experiment using heparin as anticoagulant. Before sampling, the fish were anesthetized using hemihydrates (1,1,1-trichl or 2-methyl–2-protopanol) ($C_4H_7CL_3O{\cdot}O$, $5H_2O$, Merk) with a concentration of 1.5% to prevent struggling (Bohl, 1968).

Samples of whole blood were used for determination of blood parameters.

The plasma was separated by centrifugation at 3000 rpm using and stored at −20 °C to be used for hormonal and biochemical analysis, after separation of the plasma, the packed cells were washed 3 times with 2 ml of 0.65% saline solutions, and then centrifuged at 3000 rpm for 10 min.

The erythrocytes suspension (free from leukocytes) was centrifuged in 2 ml redistilled water for 15 min, at 27 ± 3 °C to obtain a haemolysate which is used for analysis of PK activity. Samples of serum as achieved by allowing the blood to clot in refrigerator at 5 °C for 1 h. The clot was centrifuged at 4000 rpm for 15 min to separate the serum for determination of LDH enzyme on the same day of the blood collection. Samples of liver and muscle from each fish were excised and immediately taken on ice to be used for determination of glycogen.

2.2. Analysis

Hormonal assay of cortisol (ng/ml), T_3 (ng/ml), T_4 (ng/ml) were carried out by Radioimmuno Assay (RIA) technique using I^{125} Kits, obtained from Sorin Biomedica Diagnostic Division, according to, Vecsei (1979) for cortisol and kits obtained form Pentex Santa Monica, C.A 90404 for T_3 and T_4, respectively (Chopra, 1979).

Glucose level (mg/100 ml) was determined according to Trinder (1969) using glucose enzymatic PAP kits obtained from Bio-Merieux (France). Glycogen (mg/g) in the liver and muscles were estimated by methods of Johann and Lentini (1979). Serum LDH was estimated according to Klin (1972) using kits from bio-Merieux (France) and erythrocyte PK activity (mu/ery ml) was determined after (Beisenherz, 1953) using Bocheringer meannhein Gmb H. kit.

2.3. Statistical analysis

Analysis of data obtained was performed using Statistical package for the social sciences (SPSS12) computer program.

3. Result and discussion

As showed in Tables 1–3 results revealed that T_3 level decreased with no significant differences in hypoxic group, while a significant reduction in overcrowding and starved fish was observed. T_4 Level showed non significant differences among fish stressed by hypoxia and overcrowding after 24 and 72 h but significant compared to control group, while it decreased significantly in fish under starvation.

After 144 h, both the T_3 and T_4 level were decreased significantly.

The obtained level of thyroid hormones may result from the effect of stress by altering its metabolism (Colombo et al., 1990) who suggested that the suppressive effect of stress on thyroid physiology, which mediated through the direct action on the hypothalamo pituitary–thyroid axis through an effect on peripheral deiodination.

Bianca (2009) reported that fish stress would cause short time physiological changes (acute stress), mainly due to the action of some hormones and long time related physiological changes (chronic stress conditions) as scales loss, damaged fin, growth and reproduction, immunity defense and health.

In addition, oxidative stress as a welfare index could also be used as a new approach to fish quality evaluation (Bagni et al., 2007).

In agreement with the present results, Higgs and Eales (1977) reported that T_3 Level was low in the yearling brook trout starved for 12 days and added that, starvation also reduce T_4 degradation and deiodination. T_3 has a stimulating effect on the somatotropic axis (Wang et al., 2006) and has been shown more sensitive in response to food deprivation (Blake et al., 2006).

In addition, Dickhoff et al. (1989) suggested that in the Atlantic salmon the peak level of T_3 in ambient-fed group was significantly greater than in the starved group.

On the other hand, an inverse correlation of plasma T_4 level with increase in plasma T_3 concentration were strongly associated with time of feeding (Redaly and Leatherland, 2003)

Increase stocking density has been observed in both rainbow trout (Latherland and Cho, 1985) and coho salmon (Schreck and Patino, 1985).

In the present study plasma levels was increased significantly after exposure to acute stress in all groups. The changes in plasma cortisol, thyroid hormone levels were reflect their concerted actions on energy metabolism (Edwin et al., 2006).

In agreement with the present study Bianca (2009) suggested that plasmatic levels of cortisol increase quickly after exposure to an acute stress and the standard conditions are restored in few hours.

Cortisol (evaluated by RIA) is widely used both as long term and as short-term stress condition index, even if it may be influenced by species, feeding, reproductive cycles, seasonal

Table 1 Changes in some parameters of Nile tilapia (*Oreochromis niloticus*) after 24 h from the exposure to hypoxia, overcrowding and starvation.

Parameters groups	Cortisol (ng/ml)	T_3 (ng/ml)	T_4 (ng/ml)	Glucose (mg/100 ml)	Glycogen		LDH (u/l)	PK (mu/ery ml)
					Liver (mg/g)	Muscle (mg/g)		
I Control	134.15 ± 1.20	122.12 ± 0.40	6.75 ± 1.01	105.72 ± 1.18	7.02 ± 1.02	0.45 ± 0.09	1971.91 ± 1.38	490.50 ± 1.25
II Hypoxia	149.27 ± 1.08**	115.31 ± 0.92	5.45 ± 0.83*	111.0 ± 0.26	3.45 ± 1.19**	0.099 ± 0.18**	2325.20 ± 1.44**	260.18 ± 1.72**
III Overcrowding	154.12 ± 0.90**	111.07 ± 0.15*	5.82 ± 1.40*	124.70 ± 0.33*	4.16 ± 0.95**	0.089 ± 0.42**	892.16 ± 1.09**	630.00 ± 1.26*
IV Starvation	136.18 ± 1.04*	94.35 ± 0.22**	5.00 ± 1.17**	98.14 ± 0.42**	3.15 ± 0.63**	0.072 ± 0.61**	788.62 ± 1.62**	680.12 ± 1.45**

±SE = standard error of mean.
* $P < 0.05$.
** $P < 0.01$.

Table 2 Changes in some parameters of Nile tilapia (*Oreochromis niloticus*) after 72 h from the exposure to hypoxia, overcrowding and starvation.

Parameters groups	Cortisol (ng/ml)	T_3 (ng/ml)	T_4 (ng/ml)	Glucose (mg/100 ml)	Glycogen		LDH (u/l)	PK (mu/ery ml)
					Liver (mg/g)	Muscle (mg/g)		
I Control	I Control	121.17 ± 0.42	6.80 ± 1.11	105.18 ± 0.12	6.98 ± 0.09	0.45 ± 0.02	1905.88 ± 1.14	485.40 ± 1.84
II Hypoxia	148.27 ± 1.08**	113.28 ± 0.92	5.62 ± 1.12	113.0 ± 0.63*	2.97 ± 1.15**	0.097 ± 0.35**	2410.10 ± 1.41**	255.18 ± 1.91**
III Over-crowding	153.14 ± 0.92**	111.09 ± 0.18*	5.74 ± 1.62	125.40 ± 0.07*	4.26 ± 1.14**	0.84 ± 0.52**	884.12 ± 1.14**	597.00 ± 1.34*
IV Starvation	140.18 ± 1.04*	93.81 ± 0.75**	4.34 ± 0.09*	97.14 ± 0.15**	2.66 ± 0.72**	0.069 ± 0.88**	754.60 ± 1.15**	635.11 ± 1.55**

± SE = standard error of mean.
* $P < 0.05$.
** $P < 0.01$.

Table 3 Changes in some parameters of Nile tilapia (*Oreochromis niloticus*) after 144 h from the exposure to hypoxia, overcrowding and starvation.

Parameters groups	Cortisol (ng/ml)	T_3 (ng/ml)	T_4 (ng/ml)	Glucose (mg/100 ml)	Glycogen		LDH (u/l)	PK (mu/ery ml)
					Liver (mg/g)	Muscle (mg/g)		
I Control	I Control	121.72 ± 0.62	6.75 ± 1.09	105.00 ± 0.64	6.70 ± 1.14	0.45 ± 0.12	1904.29 ± 1.35	485.0 ± 0.17
II Hypoxia	141.00 ± 1.09	105.12 ± 0.08**	4.69 ± 1.13*	120.12 ± 0.51**	1.79 ± 1.08**	0.088 ± 1.03**	2460.15 ± 1.21**	212.00 ± 0.62**
III Ove-rcrowding	144.12 ± 0.71	108.12 ± 0.44**	4.95 ± 1.18*	124.12 ± 0.60**	2.79 ± 0.22**	0.082 ± 0.80**	792.14 ± 1.17**	641.02 ± 0.25**
IV Starvation	135.01 ± 0.16	88.46 ± 0.09**	3.88 ± 1.64**	86.22 ± 0.27**	1.98 ± 0.75**	0.061 ± 0.45**	669.08 ± 1.26**	691.18 ± 0.24**

± SE = standard error of mean.
* $P < 0.05$.
** $P < 0.01$.

cycles, photoperiod, husbandry condition and sampling (Barton, 2002), and multiple stress condition seems to amplify the cortisol response (Ortuno et al., 2002). In addition, teleostean fishes lack aldosterone and mineral regulatory processes seems under dominant control by cortisol (Ellen et al., 2008).

The results of the present work revealed that plasma glucose levels was increased significantly in both hypoxic and overcrowding group which might be result from the increased level of catecholamines and cortisol as they are considered the principle hormones in controlling carbohydrate metabolism.

These results are in agreement with the finding of Pickering et al. (1982) who proved that stress might increase secretion of catecholamines which initially suppressed insulin secretion and subsequently increasing plasma levels of glucose (Duong, 1974). Because Tilapia do not tent to consume large quantities of glucose in the wild, it is not surprising that they have involved without a mechanism to move glucose rapidly from the blood stream into muscle and fat (James et al., 2000).

The glycogen content of both liver and muscles significantly deplated after exposure to different stress factors which might be due to glycogenolysis during excessive metabolic activities and increased required for energy providing elements (glucose).

Milligan and Wood (1986) found that rainbow trout exposed to exhaustive exercise, showed non significant change in hepatic glycogen content after 24 h of exercise, indicating that due to anaerobic metabolism or accumulation. Of lactate, pyruvate, ATP and creatine phosphate stores, as well as, fluid shift from extracellular to intracellular. On the other hand, Morata et al. (1982) suggested that the reduction of hepatic glycogen content might be directly related to increase glycogen phosphorylase activity.

Serum LDH activity showed a significant increase in hypoxic group which is probably due to increased formation of lactic acid production in the muscle that have been oxidized in the liver and this situation calls for and increase in LDH activity (Skyervold et al., 2001; Poli et al., 2002, 2004).

The PK activity in erythrocytes significantly decreased in hypoxic group which may be due to glycolytic rate in erythrocytes, as well as, oxygen deficiency leading to anaerobiosis.

A similar conclusion was proposed by Sauer and Haider (1977) in rainbow trout exposed to low oxygen concentration.

In addition, Bremmer and Edwards (1985) emphasized that hypoxia induced inhibition of mitochondrial activity with subsequent increase in LDH activity.

The conclusion of the present study is that fish are able to feel pain in the same way as humans or mammalian animals. Fish are able of strong and unconscious behavioural, physiology and hormonal response to the stressor which, if intense and lasting enough, can be deterimental for their health. Therefore, the aim to reach should be to minimise and keep under control infra vitam, pre-slaughter and slaughter stress.

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