Heat Stress Effect on Some Bacterial Infection in *Oreochromis niloticus*

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ABSTRACT

This work was made to investigate the effect of sudden change of water temperature on the immune status and susceptibility for bacterial diseases in *Oreochromis niloticus*. A total number of 180 apparently healthy *O. niloticus* were divided into 3 groups and were subjected to high temperature 35 °C and low 15 °C and group were served as control 25. Each group was subdivided to 3 subgroups 1st considered control and 2nd was contaminated with *Aeromonas hydrophilla* and the 3rd was contaminated with *Pseudomonas. floursences*. The results showed that *O. niloticus* subjected to low water temperature 15 °C had significantly higher mortalities and higher susceptibility to *P. floursences* infection while *O. niloticus* subjected to high water temperature 35 °C were more susceptibility for *A. hydrophilla*. The peak of serum glucose and cortisol had been reached after 6 and 12 hours, respectively. Also *O. niloticus* subjected to high water temperature 35 °C were rapidly acclimatized to changes in water temperature than those subjected to low temperature. Also, significantly lower RBCs, WBCs, TC, PCV, Hb and TP were detected in low water temperature group. It was concluded that sudden changes in water temperature had an adverse effect on immune status of *O. niloticus* and become more susceptible to bacterial infection.

Keywords: heat stress, *Oreochromis niloticus*, immunity, *Aeromonas hydrophilla*, *Pseudomonus floursences*

INTRODUCTION

However, absolute temperature ranges for health or survival do not exist because temperature tolerance depends on several factors, including the temperature to which the individual has been acclimated, salinity (for estuarine species), life stage, and reproductive status (Noga, 2010). Fish are subjected to stress from either rapid temperature fluctuations that preclude acclimation or inappropriate water temperature (beyond the high or low range of tolerance) (Harper and Wolf, 2009).

Sudden change in water temperature leading to heat stress which can cause temporary homeostasis modifications leading to physiological adjustments. These responses aimed at mobilizing energy by adrenergic system stimulation, release catecholamine and increase adrenocorticotropic hormone (ACTH) and plasmatic cortisol (Gamperl *et al.* 1994). 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). 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 (EL -Khaldi 2010).

In recent years the concept of stress as applied to fish has awaked the interest among scientists dedicated to the research of environmental influences on health (Barreto and Volpato-Braz 2006).The response to stress in fish is characterized by the stimulation of the hypothalamus, which results in the activation of the neuroendocrine system and a subsequent cascade of metabolic and physiological changes (Lowe and Davison 2005). These changes enhance the tolerance of an organism to face an environmental variation or an adverse situation while maintaining a homeostasis status (Pickering, 1981). This fact is due to an interaction between environment and immunological system of fish. Disease resistance is directly related to hypothalamic/pituitary/interrenal axis.

So this work was conducted to evaluate the impact of heat stress on the immune status of *Oreochromis niloticus*.

MATERIALS AND METHODS

***Experimental feeding system design* -١**

A total number of 180 apparently healthy of *O. niloticus* were collected from private fish farms at Kafr El-Sheikh Governorate- weighing 50 ± 2.5 gram. *O. niloticus* acclimated in fiberglass tanks for 15 days to laboratory conditions. Fish randomly distributed in glass aquarium (50 x 40 x 40 cm) containing about 60 liters of dechlorinated water and aquarium water temperature was adjusted at 25±2.5 ºC as well as continuous oxygen supply by air pump. *O. niloticus* were fed pelleted ration with daily percentage 3% of body weight six day per week. *O. niloticus* were subjected to sudden change in water temperature low 15 ±2.5 ºC and high 35±2.5 ºC for one week then return again to 25±2.5 ºC.

Clinical, Post mortem examination and mortality rate of O. niloticus -٢

The collected fish were examined for any abnormalities according to (Schaperclaus, 1992).

Cortisol , glucose and haemogram analysis of O. niloticus -٣

Serum samples were taken 7 times, after (1, 6, 12, 24) hours, 3 days, 7 days, 14 days for measure cortisol (stress hormone) and glucose. Glucose was determined calorimetrically as mentioned by Trinder (1969). Cortisol was estimated using radio immunoassay technique according to the method of (Pickering and Potinger, 1983) and Wedemyer, 1970).

Blood samples were taken three times, the first after (1, 7 and 14) days for measure Red blood cell (RBCs), Haemoglobin (Hb), Packed cell volum (PCV), White blood cell (WBCs), thrombocyte (TC) and serum total protein (TP).

Blood film was prepared according to the method described by Lucky (1977). RBCs and WBCs counts were counted by haemocytometer according to Stoskopf (1993).

The concentration of total protein (TP) Weichsellbaum (1946) and were measured by colorimetric methods

Experimental design -٤

A Total number of 180 apparently healthy ***O. niloticus*** were divided into 3 groups, 60 fishes each (groups 1, 2, 3). Each group was subdivided into 3 subgroup (A, B, C) for experimental infection (Table 1).

Fish in groups 2.B and 3.B were exposed to *A. hydrophilla* by immersion in 24 hours-old broth culture of *A. hydrophilla.* The fish in groups 2.C and 3.C were exposed to *P. fluorscence* by immersion in 24 hours - old broth culture of *P. fluorscence*. Bacterial strains were kindly supplied from department of fish diseases, faculty of veterinary medicine, Alexandria University (*A. hydrophilla*) and fish diseases department Animal Health Research Institute AHRI Agriculture Research Central ARC (*P. fluorscence*). Each bacterium (*A. hydrophilla* and *P. fluorscence)* were cultured on TSB and adjusted with water to give a final concentration of 5×10^6 colony forming units (CFU) ml after

Table (1*): Experimentally infected O. niloticus groups*.

Group	No. of fish	Subgroup No. of fish	Temp.	Type of infection
١	٦٠	**A**.١ fish ٢٠	ºC ±2.5ºC٢٥	Bath with sterile TSB (control)
		B.١ fish ٢٠		*Bath with ***A. Hydrophila***
		C.١ fish ٢٠		Bath with ***P. Fluorscence***
٢	٦٠	**A**.٢ fish ٢٠	ºC ±2.5ºC٣٥	Bath with sterile TSB (control)
		B.٢ fish ٢٠		*Bath with ***A. Hydrophila***
		C.٢ fish ٢٠		*Bath with ***P. Fluorescence***

٣	٦٠	A.٣ fish ٢٠	ºC ±2.5ºC١٥	(Bath with sterile TSB (control
		B.٣ fish ٢٠		*Bath with ***A. Hydrophila***
		C.٣ fish ٢٠		*Bath with ***P. Fluorescence***

.***Type of infection: bath with pure culture of A. Hydrophila and P. Fluorescence 5× 10^6 CFU/ml****

one hour (Ilhan *et al.*, 2006). The fish in groups 2.A and 3.A were kept as a control and exposed to the same amount of TSB without bacteria.

Fish were observed twice a day for 14 days according to Amos (1985) and described in Table (1). Experimentally infected *O. niloticus* were subjected to full examination. Mortality rate % (MR%) was recorded. Re-isolation of infected bacteria was carried out from the dead and sacrificed fish.

Statistical analysis-٥

Duncan's Multiple Range test DMRT (Duncan, 1955) was used to determine differences among means at significance level of 0.05. All statistics were run on the computer using the statistical and package for social science program (SPSS, 2004).

RESULTS

Observation of *O. niloticus* under heat stress revealed that the fish subjected to low and high temperature 15ºC and 35ºC were off food, emaciation, exhibited erratic movement and abnormal swimming behavior. Erratic movements were disappeared in group subjected to high temperature and restore normal behavior after 48 hours while, groups subjected to low temperature suffered from sluggish movement and loss of reflexes all over the period of experiment 7 days.

External examination that represented in figures (1, 2, 3 and 4) fish under heat stress of low temperature skin discoloration while those subjected to stress of high temperature showed no external or internal macroscopic changes. *Post* mortem examination showed in figures (5 and 6) revealed that *O. niloticus* infection with *A. hydrophilla* showed ascities, skin ulcers, spleenomegaly and hepatomegaly while those infected with *P. flourscences* haemorhagic dots on skin, spleenomegaly, distended gall bladder.

Figure (1): O. niloticus suffering from ascities infected with A. hydrophilla high temperature group 2.B.

Figure(2): O. niloticus suffering from detached scales emaciation(off food) infected with P. floursences low temperature group 3.C

Figure (3): O. niloticus suffering from emaciation and

Figure (4): O. niloticus infected with P. flourscences suffering

discoloration body surface subjected to low temperature.	*from haemorrhagic dots on body surface subjected to low temperature group 3.C*
 *Figure (5): post mortem O. niloticus infected with P. flourscences suffering from spleenomegaly and empty intestine subjected to low temperature group 3.C.*	 *Figure (6): post mortem O. niloticus infected with A. hydrophilla suffering from distended gall bladder and empty intestine and darken liver subjected to high temperature group 2.B.*

Data presented in Table (2) cleared that MR% among experimented groups infected with *A. hydrophilla* and *P. flourscences* incorporation with heat stress. Showed significant high MR% (100%) that recorded in group (3.B) subjected to low temperature and infected with *A. hydrophilla.* Also, group (2.C) recorded 100% mortality. The results cleared significant ($P \leq 0.05$) lower MR% observed with groups not subjected to heat stress.

Data presented in Table (3) showed cortisol (stress hormone) levels in different fish groups. The highest cortisol level had reached the peak was after 6 hours of exposure 5.97 µg/dl in groups 3.B and 2.C. Bacterial infection had increased significantly the level of cortisol in fish serum but not as high as heat stress. The high level had extended for 24 hours then fish started to Acclimate to water temperature and the cortisol showed decline manner and had return to levels near control group after one week of stop exposure to different water temperature.

Measurement of glucose in fish serum showed the same trend of cortisol as changes in water temperature especially low temperature had the highest glucose concentration (Table 4).

Data in Table (5) cleared that RBCs count had significantly differences ($P \leq 0.05$) between fish groups following exposure to heat stress and bacterial infection. After one week of exposure and bacterial infection had lowered except RBCS for group exposed to high temperature which had restored normal state before one week while groups exposed to low temperature and all groups had infected didn't restore normal state even after stop exposure by one week.

Also, concerning Hb and TP content showed that all fish group had returned to normal values after stop exposure to heat stress. PCV had the same trend of Hb and TP except for group exposed to low water temperature.

WBCs and TC count had significantly differences between different fish groups. Group exposed to low temperature had lowest values after one week of exposure. TC recoded higher values with group exposed to high temperature and infected with *A. hydrophilla*.

Table (2): ***Mortality rate (Mean± SE and %) in fish groups subjected to heat stress***.

Treat	A.١	A.٢	A.٣	B.١	B.٢	B.٣	C.١	C.٢	C.٣
No death	e٠,٣٣ ٠,٣±	d١,٣٣ ٠,٣٣±	c٤,٦٧ ٠,٣±	de٠,٦٧ ٠,٣±	b٥,٦٧ ٠,٣±	a١٠ ٠,٠±	de٠,٦٧ ٠,٥٧±	a١٠ ٠,٠±	bc٥,٣٣ ٠,٣٣±

Maxi	١	٢	٥	١	٦	١٠	١	١٠	٦
Mini	٠	١	٤	٠	٥	١٠	٠	١٠	٥
%MR	٣,٣	١٣,٣	٤٦,٧	٦,٧	٥٦,٧	١٠٠	٦,٧	١٠٠	٥٣,٣

Treat = Treatment, Maxi = Maximum level, Mini = Minimum level and MR%= Mortality Rate %.

(Rows with different litters are significantly different at (P ≤ 0.05.

Table (3): Serum cortisol (Mean± SE µg/dl) in fish groups subjected under heat stress.

Treat	A.١	A.٢	A.٣	B.١	B.٢	B.٣	C.١	C.٢	C.٣
h١	B٠,٨١ ٠,٠١±	dAB٠,٨٥ ٠,٠١±	eAB٠,٨٧ ٠,٠٢±	cB٠,٨١ ٠,٠٦±	dAB٠,٨٤ ٠,٠٥±	dAB٠,٨٦ ٠,٠٣±	cAB٠,٨٦ ٠,٠٢±	fA٠,٩١ ٠,٠٣±	eAB٠,٨٧ ٠,٠٤±
h٦	٠	aC٢,٩٩ ٠,١١±	aB٥,٥ ٠,١٥±	cD٠,٨٢ ٠,٠٢±	aB٥,١٧ ٠,١±	aA٥,٩٧ ٠,١±	cD٠,٨٦ ٠,١±	aA٥,٩٧ ٠,١±	aB٥,٤ ٠,٣±
h١٢	٠	bD١,٣٩ ٠,٠٣±	bC٢,١٧ ٠,٠٢±	cE٠,٨٥ ٠,٠١±	bB٣,٦ ٠,٢±	bB٣,٩ ٠,٢٤±	bE٠,٩ ٠,٠٥±	bA٤,٥ ٠,١±	bB٣,٧ ٠,١±
h٢٤	٠	cD١,١ ٠,١±	cC١,٩٨ ٠,٠٤±	cD٠,٩ ٠,٠٥±	cB٢,٦ ٠,٣±	cA٣,٣ ٠,٢±	bD٠,٩٢ ٠,٠٧±	cB٢,٨ ٠,٢±	cB٢,٨ ٠,٠٦±
d٣	٠	dE٠,٨٦ ٠,٠٢±	dD١,٤٧ ٠,٠٤±	aA٢,٢ ٠,١٢±	dBC١,٩٧ ٠,٠٧±	٠	aC١,٨٣ ٠,٠٣±	dBC١,٩٢ ٠,١±	dAB٢,١ ٠,٠٦±
d٧	٠	dC٠,٩١ ٠,٠٢±	eC٠,٩٩ ٠,٠٦±	bAB١,٤٧ ٠,١١±	e١,٣٣ ٠,٠٧±	٠	bC٠,٨٨ ٠,٠١±	eA١,٦ ٠,١٢±	٠
d١٤	٠	dB٠,٨٩ ٠,٠٣±	eB٠,٩٢ ٠,٠٦±	cB٠,٨٧ ٠,١±	edA١,١ ٠,٠٧±	٠	bB٠,٨٩ ٠,٠٢±	٠	٠

Treat=Treatment, 1h= 1hour, 6h=6 hours, 12h= 12 hours, 24h=24 hours, 3d= 3 days, 7d=7 days and 14d=14 days.

0= not measured.

Different small letter in the same column and different letter capital in the same rows are different significantly (P ≤ 0.05).

Table (4): *Serum glucose (Mean± SE mg/dl) of fish groups under heat stress*.

Item	A.١	A.٢	A.٣	B.١	B.٢	B.٣	C.١	C.٢	C.٣
h١	D٢٤,٧ ٠,٨٨±	deD٢٨,٧ ١,٨±	cD٣٣,٣ ١,٨±	bD٢٦,٣ ١,٥±	fBC٣٥,٣ ٢±	dA٤٣ ١,٥±	bcD٢٦ ١,٥±	fA٣٩ ٠,٦±	dD٢٨ ٠,٦±
h٦	٠	bB٨١,٣ ٢,٣±	bB٨٧,٣ ٥,٨±	bC٤٣,٧ ٢,٢±	cB٨٩,٧ ١,٢±	cB٨٠,٧ ٤,١±	bcB٢٥,٧ ٠,٩±	aA١٣١,٧ ١٠,٢±	cB٨٠ ٧,٩±
h١٢	٠	aE٩٣,٣ ٢±	A١٢٣,٣ bc ٦±	bF٣٣,٣ ٠,٩±	aD١٠٩ ٢,١±	aA١٤٥,٣ ٤,٤±	cF٢٤,٨ ٠,٥±	b١١٤,٣ CD ٤±	aB١٢٥,٣ ٥,٤±
h٢٤	٠	bD٨١,٧ ±١,٢	bB١٠٠,٧ C ٤,٨±	bE٢٩ ٠,٦±	bBC٩٨,٧ ٤±	b١٢١,٧ A ٤,٤±	bcE٢٦,٦ ٠,٦±	cC٩٢ ٣±	bB١٠٦,٣ ٥,٤±
d٣	٠	cC٦٠,٣ ٢,٧±	cBC٦٩,٧ ١١,٣±	bD٢٦ ٠,٦±	dAB٧٧ ٤,٣±	٠	aD٣٢,٣ ١,٢±	dAB٧٦ ٢,٦±	cA٨٦,٣ ١,٤±
d٧	٠	dDE٣٣,٧ ٢,٧±	dBC٤٠,٣ ٠,٩±	aCD٢٥,٧ ٠,٩±	eB٤٤,٣ ٦,٧±	٠	bE٢٨,٣ ١,٢±	eA٥٤,٣ ٥,١±	٠
d١٤	٠	eBC٢٦,٧ ١,٢±	dB٢٨,٣ ٠,٩±	bC٢٤,٧ ٠,٩±	fA٣٣,٧ ٤,٧±	٠	bcB٢٥,٢ C ٠,٦±	٠	٠

١ *h= 1hour, 6h=6 hours, 12h= 12 hours, 24h=24 hours, 3d= 3 days, 7d=7 days and 14d=14 days.*
Different small letter in the same column and different letter capital in the same rows are differnent significantly ($P \leq 0.05$).

Table (5): *Different blood parameters Mean± SE of fish groups subjected to heat stress*.

Trea	A.١	B.١	C.١	A.٢	B.٢	C.٢	A.٣	B.٣	C.٣
RBCs ١ ٢ ٣	a±0.3٤,٣ - -	a±0.1٤,١ 7 bc±0.٢,٥ 1 b±0.1٣,٣	a±0.1٤ bc±0.٢,٥ 4 b±0.4٣,٣	a±0.1٤,٢ a±0.15٤ a±2٤,٢	a±0.0٣,٩ 5 a±0.2٣,٨ a±0.1٤,٢ 2	a±0.1٤ b±0.2٢,٧ 5 ٠	a±0.1٤,٣ 5 c±0.15٢ b±0.3٢,٧	a±0.07٤ ٠ ٠	a±0.0٤,١ 6 ٠ ٠
Hb ١ ٢ ٣	a±0١١,٦ .8 - -	ab±١٠,٨ 0.5 c±0٧,١٤ .1 ab±١٠,٣ 0.1	ab±١٠,٥ 0.3 c±1.4٧,٨ b±1.2٩,٣	ab±0.6١١ a±0١٠,٤ .2 a±0.6١١	b±0.2١٠ ab±0.8١٠ a±0١٠,٨ .2	ab±١٠,٤ 0.4 bc±1.٨,٣ 2 ٠	ab±0.4١١ c±0..٦,٦ 3 c±0.5٧,٦	b±0١٠,٢ .3 ٠ ٠	ab±0.١١,٣ 04 ٠ ٠
PCV ١ ٢ ٣	a±4٣٥,٦ .3 - -	ab±٣٢,٤ 1.4 b±0.4٢٠ bc±0.4٣٠	ab±٣١,٦ 0.9 b±3.8٢٢ cd±3.4٢٧	ab±1٣٤ a±0٣١,٥ .4 a±1٣٥,٢	b±0٣٠,٣ .3 a±1٣١,٣ .4 ab±٣٢,٤ 1.3	ab±1٣٢ ±٢٤,٤ ٤,٣ ٠	ab±٣٣,٣ 1.1 b±0١٩,٦ .8 d±0٢٣,٨ .9	ab±٣٢,٣ 0.6 ٠ ٠	ab±٣٣,٨ 0.2 ٠ ٠
WBCs ١ ٢ ٣	abc±٤٠,٦ 0.6 - -	a±0٤٢,٥ .8 a±0٣٩,٣ .2 ab±0.1٤٠	a±1٤٢,٧ .2 a±0٣٩,٥ .4 ab±٤٠,٧ 1.2	e±1٣٤,٣ .3 b±0٣٥,٨ .8 b±1٣٩,١	bcd±٣٨,٧ 0.5 a±0٤١,٢ .7 a±0.8٤١	de±٣٦,٨ 1 b±1٣٤,٥ .5 ٠	cd±٣٧,٨ 0.7 c±0٢٠,٨ .3 c±٣٤,٩ 0.6	ab±٤١,٧ 0.4 ٠ ٠	bcd±٣٨,٩ 1.6 ٠ ٠
TC ١ ٢ ٣	a±0٦١,٨ .7 - -	cd±0.6٥٠ b±1٤٤ bc±5.7٥٢	cd±٤٨,٨ 0.7 b±1٤٣,١ c±3٥١,٣ .2	bc±٥٣,٧ 0.9 a±0٥٢,٥ .8 a±0.6٦١	d±4٤٤,٣ a±0٥٣,٧ .9 ab±٥٧,٧ 2.8	e±2٣٧,٩ .9 c±2٣٧,٧ .2 ٠	ab±٥٦,٢ 2.3 d±1.5٣٣ c±2٤٨,٧ .7	h±0٣١,٣ .7 ٠ ٠	eh±٣٤,٥ 1 ٠ ٠
TP ١ ٢ ٣	a±0.1٦,١ - -	a±0.1٦,٣ 2 ab±0.٤,٥ 1 b ±0.25 ٥	a±0.2٥,٩ bc±0.٤,٢ 2 b ٥,١ ±0.2	a±0.6٥,٤ a±0.2٤,٩ a±0.1٦	a±0.0٥,٩ 6 ab±0.٤,٥ 1 b ٥,٢ ±0.1	a±0.0٥,٩ 6 bc±0.1٤ ٠	a±1.2٥ c±0.4٣,٩ b±0.6٥	a±0.2٥,٩ ٠ ٠	a±0.1٦ ٠ ٠

Rows with different litters are significantly different P≤0.05.

DISCUSSION

In Egypt fish culture performed in semi intensive farms depending on fertilizing water and subjected to changes of water temperature. There are standard environmental temperature (SET) ranges for individual fish species that define the temperatures for optimal growth.

Results obtained concerning survival rate agreed with those obtained by EL-Sherif and EL-Feky (2009) and Saber *et al*. (2004) when they stated that water temperature 25-30 ºC were more suitable for culture of *O. niloticus* fingerlings to obtain optimum growth performance and survival rate were 100% at temperatures 20, 25 and 30°C this is mainly due to the acclimation of tilapia prior to the start of stocking in aquaria this experiment started with Nile tilapia with an initial weight of 19±1.0 g; such size show high tolerance to the unfavorable conditions (Saber *et al.*, 2004) and the ecological conditions throughout the

experimental period were suitable for tilapia rearing especially the average water temperature of 20, 25 and 30°C. Low survival rate in *O. niloticus* group exposed to sudden change water temperature to 15 °C were similar to those obtained with EL-Sherif and EL-Feky (2009) who mentioned that survival rate of fish decreased (75%) at temperature 15°C at the end of the experimental period. Our findings explained by Khouraiba (1989) who stated that poor survival of fish observed at 15°C was attributed to poor environmental conditions of the fish especially the prevailing low water temperature 15°C. Also, our results agreed with findings of Mei *et al.* (2010) who conducted a study on the tolerance of *O. niloticus* to low temperature stress and its duration. *O. niloticus* was continuously stressed by artificial decreasing temperatures and the lethal time of *O. niloticus* under different low temperature stress was recorded. The results showed that the semi-lethal duration of *O. niloticus* was 12.25, 17.00, 25.00 and 31.50 h under low temperatures of 7, 8, 9 and 10°C, respectively. Our results agreed with those obtained by (Schaperclaus, 1979) who stated that the mortality of *O. niloticus* was higher at lower temperature stress. *P. fluorescens* is associated with fin or tail rot in which the infected are is eroded away. Also, Ahne *et al.* (1982) agreed with obtained findings as he mentioned that visual signs of disease included haemorrhagic lesions on the skin at the base of the fins, ascitic fluid accumulated in the peritoneal cavity, and petechial haemorrhages were evident in the gills, kidney, liver, in the lumen and submucosa of the gut, i.e. a typical generalized bacterial septicaemia. Also, Yardimci and Aydin (2011) found that *A. hydrophila* in the macroscopic examination the liver was seen to be yellowish brown and crispy with haemorrhagic and greyish white foci on the surface. The gall bladder was tightly full with emerald green bile. Austin and Austin (2007) had agreed with our findings as he stated that haemorrhagic septicaemia (also referred to as motile aeromonas septicaemia) is characterised by the presence of small surface lesions (which lead to the scales sloughing off), local haemorrhages particularly in the gills and vent, ulcers, abscesses, exophthalmia and abdominal distension.

Although motile aeromonads appropriately receive much notoriety as pathogens of fish, it is important to note that these bacteria also compose part of the normal intestinal microflora of healthy fish. Therefore, the presence of these bacteria, by itself, is not indicative of disease and, consequently, stress is often considered to be a contributing factor in outbreaks of disease caused by these bacteria (Trust *et al.*, 1974, Kaper *et al.*, 1981, Len, 1987 and Khalil and Mansour, 1997). Abrupt temperature change, handling, crowding, inadequate feed and oxygen are known to be the predisposing factors which contribute to the infection of *A. hydrophila* (Leung *et al.*, 1994 and Roberts 2001).

Data concerning high suscepitability of *O. niloticus* to bacterial infection could be explained by heat stress lowering humoral immunity these results agreed with findings of Ndong *et al.* (2007) who conducted study on *O. mossambicus* acclimated to 27 ºC then held at 19, 23, 27 (control), 31 and 35 ºC, and were examined for non-specific cellular and humoral responses after 12-96 h, total WBCs decreased significantly when fish were transferred to 19 and 23 degrees. Also, Ndong *et al.* (2007) concluded that transfer of tilapia *O. mossambicus* from 27 ºC to low temperatures (19 and 23 ºC) after 12h, and transfer of fish from 27 ºC to high temperatures (31 and 35 ºC) reduced their immune capability and tilapia under temperature stress at 19 and 35 ºC from 27 ºC decreased its resistance against *S. iniae.* whereas phagocytic activity and phagocytic index and lysozyme activity decreased significantly when fish were transferred to low temperatures (19 and 23 ºC) and high temperatures (31 and 35 ºC) over 12-96 h.

In agreement with our results Harper and Wolf (2009) stated that a rapid temperature decrease limits a fish's ability to produce antibodies integral to an immediate immune response, and a delay in the immune response may enable pathogens to colonize, reproduce, and establish an infection. Very cold temperatures may inactivate defensive functions of nonspecific leukocytes known as natural killer (NK) cells, although there is some evidence from studies in common carp (*Cyprinus carpio*) that NK cells may be able to accommodate temperature changes over time (Kurata *et al.*, 1995).

The rate of cortisol clearance is another step in the cortisol cycle that may be influenced by environmental factors. However the efficiency of that process is reported to be altered by stress, salinity, maturity, nutritional state (Mommsen *et al.*, 1999). In most fishes, cortisol reaches highest concentration 1 hour after being stressed, and returns to basal levels after 6 hours (Iwama *et al.*, 2006). Cortisol levels of red drum during some handling procedures grew rapidly, but decreased to the basal state within 48 hours (Robertson *et al.*, 1987). Porchas *et al.* (2009) mentioned that cortisol test is a good option in acute stress experiments, but it is indispensable to measure cortisol immediately after stress and over time, because a single and/or a late test will have a high probability to be far from the real response. When an organism undergoes suboptimal conditions for a considerable period of time, the release of cortisol decreases because the interrenal tissue of stressed fishes becomes less sensitive to the action of ACTH or other pituitary hormones (Mommsen *et al.*, 1999). Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways

(Iwama *et al.*, 1999). 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. Also, Porchas *et al.* (2009) mentioned that blood samples can be extracted during the chronic experiments, but if the experimental units are limited and all the samples have to be taken from the same tanks, it is not recommendable to measure cortisol over time, because the consequent handling and manipulation of organisms may lead to erroneous results in the future samples. Nevertheless, if it is necessary to measure glucose over time, it is recommended that sampling is not very frequent, while a limited number of samplings should be established. In agreement Porchas *et al.* (2009) mentioned that cortisol may be useful only in acute stress experiments and monitored throughout time and to be used as .stress indicator, the physiological status of organisms should be standardized

Regardless of the wide use of glucose as an indicator of stress, some authors (Flodmark *et al.*, 2001) emphasized that care has to be taken when using plasma glucose as the only indicator. It has been reported that glucose content is a less precise indicator of stress than cortisol (Pottinger, 1998). On the other hand as previously stated, stress hormones such as catecholamines, cortisol and others may be influenced by internal or external conditions in the history of the fish (anoxia, pollution, nutritive stress, physical stress) (Reid *et al.*, 1998). To this respect, it has been demonstrated that catecholamines itself can increase glucose levels (Wagner *et al.* 2003). Porchas *et al.* (2009) claimed that sometimes no significant changes in plasma glucose may be observed, because under stress the fish is rapidly consuming the energetic substrates generated (glucose) since the main function of the central nervous system (CNS) is to maintain homeostasis. West *et al.* (1993) argued that during peak activity glucose use can increase by almost 30- fold. In agreement Porchas *et al.* (2009) stated that normally the increase of glucose in plasma is not as rapid as for cortisol. An increase was documented of glucose minutes or days after the stress (Falahatkar and Barton 2007) because cortisol triggers glucose production. Measuring glucose just after an acute experiment is considered a source of error, because there is the probability of not measuring any change. These results appeared probably because the change in blood glucose levels .(might occurs minutes, hours or even days later (Langiano and Martinez, 2008

Data concerning blood analyses showed that PCV pointed that stress of low temperature15°C associated with low PCV values this could be explained by it decreased feed consumption and appearance of anemia (Rodrigues *et al.*, 2003). An opposite findings obtained Sherif and EL-Feky (2009) who found hematocrit value at the end of the experimental period was 22.0, 24.2, 25.0 and 24.5% for the groups .under 15, 20, 25 and 30°C, respectively

Low Hb concentration similar to those obtained with Sherif and EL-Feky (2009) who stated that Hb concentration in the experimental groups 20 and 30°C was not significantly different while decreased at 15°C. It could be attributed to decreased feed consumption and fishes were anemic (Rodrigues *et al.*, .(2003

So it was concluded that stress of sudden change of water temperature considered an important factor that causes increase the susceptibility of *O. niloticus* to bacterial infection *A. hydrophilla*, *P. flourscences*. Also, we suggested that this impact could be due to lowering of the immune status of stressed .*O. niloticus*

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تأثير الأجهاد الحراري علي العدوي البكتيرية في اسماك البلطي النيلي

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تمت هذه الدراسة لدراسة تأثير الاجهاد الحراري علي الحالة المناعية وكذلك الاصابة ببكتريا الايروموناس هيدروفيلا والسيدوموناس فلوروسنس. تم اقلمة ١٨٠ سمكة بلطي نيلي علي درجه حرارة ٢٥ درجة مئويه بوزن ابتدائي ٥٠ جرام في احواض زجاجية ثم تم تقسيمها الي ٣ مجموعات اعتبرت الاولي مجموعه ضابطه وتم تعريض الثانيه الي ارتفاع مفاجئ في درجة حرارة الماء الي ٣٥ درجه مئويه والثالثة لأنخفاض مفاجئ الي ١٥ درجه مئويه. كل مجموعه تقسم الي ثلاث تحت مجموعه تعدي الاولي ببكتريا الايروموناس هيدروفيلا والثانية ببكتريا السيدوموناس فلوروسنس والثالثة لا يتم العدوي ببكتريا. اوضحت النتائج المتحصل عليها من الدراسة ان التغير المفاجئ لدرجة حرارة الماء تسببت في انخفاض مناعة الاسماك ومن ثم اصبحت اكثر قابليه للأصابة بالعدوي البكتيرية.