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EFFECT OF CONFINEMENT STRESS ON BEHAVIOUR, PERFORMANCE, CLINICOPATHOLOGICAL AND HISTOPATHOLOGICAL ALTERATIONS OF NILE TILAPIA CHALLENGED WITH AEROMONAS HYDROPHILA WITH REGARD TO THE BLUE LIGHT AS STRESS INHIBITOR

MAHMOUD, A.M*., ABEER H. ABD EL RAZEK, NASHWA, A. ABU-AITA*****

*Dept. of Pathology, **Dept. of Vet. hygiene and Management,

***Dept. of Clinical Pathology - Faculty of Vet. Med., Cairo Uni., Giza, Egypt

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SUMMARY

The present study was carried out to assess the effects of blue light as antistressor and its effect on susceptibility of Nile tilapia (*Oreochromis niloticus*) to Aeromonas hydrophila (*A. hydrophila*) infection. A total number of (120) *O. niloticus* was divided into 3 experimental groups (40 fish / group). Group 1 (G_1), was exposed to normal day light and considered as control group. Group 2 (G_2), was exposed to blue light. Group 3 (G_3), was exposed to white light (fluorescent illumination). After 15 days of light exposure, fish in groups 2 and 3 were confined into small area and then challenged with A. hydrophila infection. The obtained data revealed that during exposure to blue light, the fish of (G_2) recorded significant lower proportion in behavioural parameters including factors of fright behaviour "creeping, oblique plan position and air-gulping" than those of (G_3). On the other hand, (G_2) showed lower behaviour alterations for fright behaviour during confinement and infection than those of (G_3). Contrarily, it also had significantly higher proportions of aggressive behaviour during exposure to light, confinement and infection than (G_3). SO blue light improved behaviour and fish become more comfortable in (G_2) than (G_3) . Moreover, fish of (G_2) showed higher significant growth performance during exposure to blue light and little reduction in growth performance during exposure to both confinement and infection than those of (G_3). The clinicopathological studies revealed significant increase in the stress response indicators after exposure to confinement stress in (G_3). Marked elevation in the serum cortisol, glucose levels and significant leukocytosis associated with heterophilia and lymphopenia was observed in (G_3). On the other hand, non significant changes were observed in the measured stress parameters

in group (G_2) which exposed to the blue light. Exposure of stressed fish to *A. hydrophila* infection induced significant leukocytosis with heterophilia, monocytosis and lymphopenia in both (G_2&G_3). Analysis of serum biochemical constituents showed significant elevation in ALT, AST, BUN, creatinine and glucose while significant reduction in the total proteins, albumen and globulin was achieved. These changes were more pronounced and early detected in *A. hydrophila* infected stressed fish exposed to white light compared to those exposed to blue light. From pathological point of view, fish group which exposed to blue light and infection revealed early positive immune response "activation of melanomacrophage center of spleen" but with time dependent adverse pathological lesions. The lesions were noticed in spleen and gills after 2 weeks. Regeneration of the most observed lesions were detected after 4 weeks. On the other hand, the fish group exposed to white light and infection showed more advanced lesions including diffuse necrosis in hepatic tissue with additional changes in the kidneys. So, the blue light has a protective effect aganist stress in Nile tilapia.

INTRODUCTION

Stress is one of the most important factors that affects the health of fish especially in intensive culture system (Wedemyer 1997, Maule 1994 and Raheyshyam et al., 1993). Fish intensification under culture practices resulted in decrease of comfortable space area required for fish. Thus, fish in aquacultures are confined in narrow spaces along the time of rearing. This confinement stress contributes to physiological changes and exacerbates susceptibility to infection and initiate different pathological alterations (Roberts, 1989). An immunosuppression and disease outbreak in teleosts fish is usually accompanied with stressors in aquaculture (Moberg and Mench 2000, Roberts 1989, and Wechsler et al., 1986). Motile aeromoonas infection in fish is a classic example of a stress-born disease (Nooga, 1996). It is a gram-negative enterobacterium widely distributed in aquatic environment (Hazen et al., 1978., Holmes et al., 1996 and Massa et al., 2001) and it has long been known as a pathogen of different aquatic organisms including fish (Austin and Adams 1996., Cunningham et al., 1996 and Ogara et al., 1998). Also, this bacterial species has been reported to cause a wide variety of human infections (Altwegg and Geiss, 1989, Janda and Duffey, 1988 and Janda, 1991). Tissue alterations due to Aeromonas hydrophila infection was recorded in different organs including hepatic tissue, spleen, gills and kidneys (Roberts, 1998).

Fish responses to stress and infection are mediated through neuronal and endocrine pathways, known as the primary response. These in turn can influence secondary physiological features and tertiary a whole fish performance (Pickering, 1981). If the stressor is sever or long standing, the fish no longer able to cope with and enter a maladaptive state leading to sever alteration in its,

performance, clinicopathological conditions as well as increased susceptibility to infection or possibly death (Barton et al., 2002). For instance, stress induces variations in fish behaviour (Petrell and Ang 2001 and Almazn-Reuda et al., 2004) and decreases growth rate (Volpato and Fernandes, 1994). The effect of environmental colour on animal physiology and behaviour is a developing field. In fish, some studies have shown that environmental color affects schooling, fright reaction (Loukashkin and Grant 1959) and aggressive behaviour (Volpato, 2000) on the other hand, it affects growth (Dowing and Litvak 2000, and Head and Milson, 2000), food conversion rate (Papotsoglou et al., 2000) and stress (Volpato and Barreto, 2001).

On the level of clinical pathology, there are many indicators for stress response. Fish blood, it's hematological and biochemical parameters appear to be suitable means to indicate environmental influences and stress (Wendeleger-Bonga, 1997). Changes in serum constituents have been proved to be a useful indicator in diagnosis of stress and disease process (Aldrin et al., 1982). Typical primary response used for evaluating stress in fish includes determining circulating levels of cortisol and to a lesser extent, catecholamines. Secondry responses include measurable changes in blood glucose. (Barton et al., 2002). Moreover, hematological variables are considered good stress indicators in fish. (Ellis, 1981).

The concomitant relationship between stress and infection have been demonstrated for decades so, one of the most important goals of a fish keepers is to remove source of stresses whenever possible. Many trials were implemented to minimize stress factors in aquacultures, despite this; very few studies have been devoted to understanding the effects of background or light color on fish biology. On the other hand, some studies showed improvement of stressed fish after exposure to specific light regimens (Volpato and Barreto, 2001). From biological point of view, the visual environment of fish is blue, green or near infrared (Levine and MacNichol, 1982) and histologically fish have cone cells which make them able to discriminate colors (Nicol, 1963) and subsequently it could be have a positive effect against stress and infection. And thus, the present study was aimed to investigate the effect of blue light as stress inhibitor during confinement and susceptibility of Nile tilapia to *A. hydrophila* infection through evaluating the performance, behaviour, clinicopathological changes as well as histopathological alterations.

MATERIAL AND METHODS

Material

Experimental fish:

A total number of 120 apparently healthy Oreochromis niloticus fish with an average body weight of about 55 g were purchased from El-Wafaa fish

farm (Giza, Egypt) and transported in large oxygenated plastic bags. Fish were maintained on standard fish diet (3%/ Kg B.W. daily) at the Pathology department, Faculty of Veterinary Medicine, Cairo University (Giza, Egypt). After adaptation period of one week, fish were divided into 3 experimental groups (40 fish/group). The fish biomass was 16.6gL-1and each group was individually placed into a fish aquarium containing dechlorinated tap water (1 x0.3 x 0.5 meter aquarium). The water was changed one time / week and the average water temperature was 22 ± 3°C and the PH was in the range of 7.2 - 8.2.

Experimental design:

Fishes within treatment groups were treated as follow :

Group1 (G_1) exposed to normal day light and considered as control.Group 2 (G_2) exposed to blue light (color was imposed by covering the fluorescent illumination by blue cellophane paper). Group 3 (G_3) was exposed to white fluorescent illumination. After 15 days of light exposure, fish in groups 2 & 3 were confined into small area in plastic bags measured 25 x 40 cm contained the same water of the aquarium (less than 10% of total aquarium volume) for 6 hours. After confinement period, fish were exposed to infection by *A. hydrophila*. The broth culture of *A. hydrophila* was diluted in 0.85% sterile saline and added to the water in aquaria to give a final concentration $5.2x10^6$ viable cells/ml according to Ventura and Grizzle, (1987). *A . hydrophila* isolate was kindly supplied from bacteriology department, Animal Health Research institute - Giza - Egypt.

Sampling:

Five fish were sampled each time for the following:

Blood samples:

Two blood samples were collected from caudal vertebral vein of each fish. The first one was collected into clean dry tube by a needle and syringe moisted with EDTA solution (10%) (disodium salt of ethylenediamine tetra-acetic acid) and used for hematological studies (Feldman et al., 2000). The second sample was collected into plain centrifuge tube for serum preparation and used for biochemical studies. The samples were collected at the end of confinement stress period for measuring the stress markers and then weekly for 4 consecutive weeks after *A. hydrophila* infection for hematological and biochemical analysis.

Tissue samples

Tissue samples were collected from gills, liver, spleen and kidneys after confinement stress and weekly after *A. hydrophila* infection for 4 weeks. The tissue samples were preserved in 10% buffered neutral formalin and used for histopathological examination.

Methods:

1-Behavioural and performance studies:

1.1-Behavioural measurement:

The behaviour was measured by using scan sampling (instantaneous sampling) according to

(Volpato et al., 2003). Fish was observed for 3 days/ week for 1 hr. / day. Behavioural observation was 10 minutes / group at 1 minute intervals. Observations were at 9.00 A.M. for (30 min.) and at 13.00 P.M. (30 min.).

a- Fright behaviour:

The detailed definition and description of behavioural elements have been published by (Csanyi et al., 1985, a, b). Briefly, we recorded the following elements:

1- Creeping (CRE):

The fish is propelled forward only by pectoral fin fanning, all other fins are closed, pectoral fins beat very quickly.

2- Oblique plan position (OBQ)

The body axis of the immobile fish is inclined 20-40° from the horizontal plan, the dorsal, caudal& anal fins are closed and the pectoral fins are quickly fanned as in (CRE).

3- Air-gulping :(A-G)

Being an anabantoid fish. Fish from time to time swim to the surface and gulp air.

b- Aggressive behaviour: was defined according to (Giaquinto and volpato (1997)

The following parameters representative the aggressive behaviour and was measured totally:

1- Approach: Swam closed to each other (decrease distance between 2 fish)

2- Rebuff: Mouth fighting and ramming.

3- Chase: One fish bit the flank of other fish.

4- Flee: Rapid movement that increase distance between 2 fish.

1-2- Body weight gain and growth performance: Performance of fish was calculated according to EL-Kholy et al., 2003.

1- Total weight gain (g/fish) = Final weight (g) - Initial weight (g).

2- Average daily gain (ADG).

ADG (g/fish/day) = (total weight gain,g/ period,day)

3- Relative growth rate (RGR%)

RGR% = (total weight gain, g/ initial weight, x100

4- Feed conversion ratio (FCR).

FCR = Feed consumed, g / weight gain,g

2- Clinicopathological studies:

2.1- Hematological studies:

Total leukocytic count was done as described by Stoskopf (1993) using hemocytometer counting chamber and Natt-Herrik solution. Stained blood films were prepared and absolute count of different leukocytes cells was calculated (Thrall, 2004).

2.2- Biochemical studies:

Serum was separated for determination of the following parameters, serum cortisol by radioimmunoassay (Rosalki, 1998), total proteins according to the biuret method after Weicheselbaun (1964), serum albumin after Dumas and Biggs (1972) and serum globulin which was calculated by

subtracting the obtained values of albumin from values of total proteins. Alanine (ALT) and aspartate (AST) amino transferases activities were performed according to the Reitman and Frankel (1957) method. Blood urea nitrogen was determined by an enzymatic method after Patton and Grauch (1977), serum creatinine according to Fabiny and Eringhausen (1971) and glucose (Trinder, 1959). Serum biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio-Laboratory, USA.

3- Histopathological studies:

Tissue samples from gills, liver, spleen and kidneys were preserved in 10% neutral buffered formalin, washed in tap water, dehydrated in ascending concentration of alcohol and cleared in xylene. After blocking using soft paraffin, serial sections of 4 μ thickness were done. The sections were stained using routine hematoxylin and eosin (H&E) stain (Bancroft et al., 1996).

4- Statistical analysis:

The system used for behavioural and performance assessment in this experiment was SPSS (statistical package of social science) version 12 for windows 2003. LSD at $p < 0.05$ was used for comparison between different groups within different treatments. For clinicopathological assessment, data were compared across groups using analysis of variance (ANOVA). Data were expressed as mean ± S.D. Levels of significance of $P < 0.05$ were chosen to identify the significant differences (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Stressors in aquaculture are unavoidable and cause many harmful effects. Depression of the defense system is one of the important effects of the fish's response to the stressors that resulted in increase the incidence of disease and mortality (Barton and Iwama, 1991 and Wendelaar Bonga, 1997). The present work was designed to investigate the efficiency of blue light as stress inhibitor and its effect on susceptibility of Nile tilapia to A. hydrophila infection.

Regarding the effect of stress on behaviour and performance of fish, fish behaviour under culture conditions holds important information for aquaculturist (Mcfarlane et al., 2004). Most physiological, environmental changes and handling process can induce variations in fish behaviour (Almazn-Reuda et al., 2004). Measuring the behavioural response have become potential alternative for assessing stress, and diseases (Kane et al., 2004).

The conditions that inducing behaviour differing from the standard pattern of species may be supposed to be stressor, thus indicating that behaviours are reliable indicators of stress in fish (Schreck, 1990). Our experiment, was chosen to

record fright behaviour "creeping, oblique plan position and air-gulping" as they are easily distinguishable -motor patterns that consistently appears in novel situation (Gervai and C. sanyi, 1986, Gerlai and C. sanyi, 1990 and Gerlai and C. sanyi 1994).

Analysis of these fright behaviour as affected by exposure to different light, confinement and A.hydrophila infection are shown in table (1). It was noticed that over all mean of proportion of fish of (G_2) at exposure to light had significantly ($P < 0.05$) lower proportions of creeping, oblique plan position and air-gulping than those of (G_3), where the over all values for creeping, oblique plan position and air-gulping were (27.2 ± 12.36, 14.08 ± 15.29 and 0.13 ± 0.35, respectively) for (G_2) while they were (31.05 ± 12.40, 41.08 ± 31.03 and 4.35 ± 3.85) for (G_3). Contrarily to results of exposure to light, the overall mean of proportion of fish of (G_2) had non significantly ($P < 0.05$) higher than fish of (G_1) during exposure to confinement and significantly ($P < 0.05$) higher during *A. hydrophila* infection. Meanwhile the fish of (G_2) showed lower behaviour alterations for creeping, oblique plan position and air-gulping than those fish of (G_3) (Table 1) where over all values for creeping, oblique plan position and air-gulping were (36.65 ± 0.50,45.38 ± 16.91 and 10.15 ± 1.88) during confinement and (48.06 ± 2.41,47.05 ± 0.50 and 18.65 ± 0.90) during infection for (G_2) while they were (63.34 ± 22.05, 61.75 ± 9.32 and 50.25 ± 0.50) during confinement and (54.05 ± 0.50, 55.25 ± 16.91 and 35.34 ± 22.05) during infection for (G_3) for the same parameters respectively (Table 1).

These results are agreeable with those recorded by Moborg and Mench (2000), Barton, (2002) and Volpato et al., (2004) who found that the application of blue color in Nile tilapia improved fish behaviour although mechanisms involved are not completely understood. On the other hand, Volpato and Barreto (2001) found that most environmental changes, handling process and exposure to infection induce variation in fish behaviour (Lain Barber, 2007). Israeli and Kimmel (1996) and Petrell and Ang, (2001) indicated that the blue environment in Nile tilapia had a role in suppression of stress.

Therefore, as behavioural and physiological measures are welfare indicators and as the blue light improved the behaviour by lowering fear response of fish so the blue light had a protective role against stress and consequently improve fish welfare.

Results of aggressive behaviour are demonstrated in table (2). Data analysis revealed that, aggressive behaviour of fish of (G_2) showed over all mean of proportions significantly higher during exposure to light (39.2 ± 32.4), during confinement (23 ± 16.6), and at exposure to *A. hydrophila* infection (15.3 ± 13.8) than fish of (G_3) that recorded (20 ± 18.3, 0.25 ± 0.05 and 9.5 ± 17) for

the same treatments respectively (Table 2).

These results are agreed with Fanta (1995) who argued that a blue background increases aggression in the Nile tilapia and he suggested that this color is not adequate for holding containers for this species. Volpato (2000) studied the effects of light color on matrinxa, Brycon Cephalus and he found that this fish was more aggressive under green light and this finding supporting that this increased intraspecific aggression imply that these fish were unstressed. Volpato and Barreto (2001) found that intraspecific aggression in territorial species is a natural behaviour expected to be expressed when the fish are adjusted to their environment and concluded that the blue background increased this intraspecific aggression in the Nile tilapia and stressors might decrease this intra specific aggression and so that the fish were more comfortable. Therefore, the blue light provides the Nile tilapia with adjusted and comfortable environment and so we reinforce that the blue light improve welfare of such species.

Growth performance of the Nile tilapia as affected by environmental color is shown in table (3). The results revealed that blue color had significantly ($P < 0.05$) a positive effect on growth performance of the Nile tilapia. Where (G_2) showed higher significantly overall mean of the body weight (73.00 ± 2.9), total weight gain (11.5), average daily gain (1.62 ± 0.23), relative growth rate (19.17 ± 1.04) and feed conversion ratio (1.19 ± 0.31) than fish of (G_3) that recorded (62, 95 ± 3.2, 5.5, 0.79 ± 0.89, 9.72 ± 0.88 and 2.12 ± 0.48 for the same parameters respectively. (Table 3).

These results are in agreement with those obtained by Hinshaw (1986), Head and Malison (2000) who found that environmental grey color had positive effects on growth and feeding conversion rate in yellow prech. Papotsoglou et al., (2000) studied the effects of different colors of tanks on growth performance of scaled carp (Cyprinus carpio) and found that white adapted carp showed the highest specific growth rate and lowest feed conversion, whereas the black adapted carp exhibited the opposite pattern. Volpato (2000) recorded that matrinxa, Brycon Cephalus grew almost three time under green light.

The superiority of (G_2) in improving growth parameters may lead us to believe that the application of blue color provides the tilapia with more adjusted environment and so fish are more comfortable and fish grew almost more under their favourite color.

The performance of the Nile tilapia under confinement is shown in table (4). The obtained data indicated that, although fish of (G_2) and (G_3) are exposed to reduction in body weight and feed conversion rate in comparison to fish of (G_1) but the reduction level in (G_2) was lower than that of (G_3). The overall mean of the body weight and feed conversion for (G_3) were (75 ± 4.2 and -1.45

± 0.13) and (61.4 ± 5.2 and -2.2 ± 0.59) for (G_2), while were (67 ± 4.00 and 1.80 ± 0.08) for (G_1), respectively.

These results may suggest that the response of the Nile tilapia to confinement stress held in the blue environment, showing little reduction in growth parameters in comparison to that tilapia exposed to white color.

These results support the view of Volpato and Barreto (2001), who found that the environmental blue light has a protective role in the Nile tilapia against confinement stress than other color. Auperin et al., (1997), recorded that confinement stress reduced the level of growth hormone which had non linear correlation with growth rate in N.tilapia. Dver et al., (2004) recorded that, capture and confinement of wild tuna in sea-cages resulted in significant decrease in insulin like growth factor which was related to growth rate and also found that handling and isolation of silver perch (Bidyanus bidyanus) led to gradual decline in insulin growth factor I and so growth was reduced. Gunther et al., (2004) found that stress factor caused a notable worsening of both growth rate and feed utilization.

The effect of *A. hydrophila* infection on performance parameters for fish is shown in table (5). Data analysis revealed that, there are significant reduction in growth performance for both (G_2) and (G_3) and also reduction levels are more in (G_3) than (G_2). The fish of (G_2) showed lower significantly overall mean of the body weight (66 ± 3.15), total weight gain (-5), average daily gain (-0.35 ± 0.18), relative growth rate (-5.43 ± 0.87) and feed conversion ratio (-2.5 ± 0.74), while (G_3) were (50.50 ± 2.65, -6, -0.32 ± 0.12, -6.95 ± 1.57 and -3.08 ± 0.6) for the same parameters respectively (Table 5).

These results are agreed with Harms et al., (2003) who found that infected stripped bass (Morone saxatilis) with Mycobacterium marinum showing lower significantly expression of transforming growth factor beta (T.G.F. beta) which was related to growth than uninfected control.

The clinicopathological studies revealed significant increase in the stress indicators after exposure of fish to (6) hours confinement stress (Table 6). There was a marked elevation in the serum cortisol and glucose levels at the end of confinement stress period in the group of fish that maintained under white light (G_3). Moreover, significant leukocytosis associated with heterophilia and lymphopenia were observed in this group compared with control group. On the other hand, confinement stress induced non significant changes in the measured stress parameters in the group of fish which exposed to the blue light (G_2) comparing with the control group. Exposure to stressors elevates the concentration of cortisol and glucose as well as induces haematological changes that prejudice the fish immune system favoring the

pathogen installation (Yada and Nakanishi, 2002). Stress triggers a cascade of mechanisms that resulted in endocrine changes in the level of catecholamines and corticosteroids (Barton, 2002). Increase of plasma cortisol is a primary response in fish submitted to different kinds of stressors (Barton and Iwama, 1991 and Wendelaar-Bonga, 1997).

Blood glucose is the secondary response most used to quantify stress in fish. The observed hyperglycemia was reported in several teleosts during stress and it is primarily mediated by catecholamines and lately by cortisol (Wendelaar-Bonga, 1997). Glucocorticoids and catecholamines create peripheral insulin resistance and augment glucogenolysis thereby elevating plasma glucose (Sumpter, 1997 and Iwama et al., 2006). Our results are parallel with Carneiro and Urbinati (2002) and Urbinati et al., (2004) who recorded an increase in the level of blood glucose and cortisol in streesed fish.

Hematological parameters are considered good stress indicators in fish, since catecholamines and cortisol induce changes in blood cells. The observed leukocytosis with heterophilia and lymphopenia could be interpreted as stress response following increased corticosteroid level. Corticosteroids decrease stickiness and margination of neutrophil resulting in their retention in the circulation. Moreover, corticosteroids induce lymphocytes apoptosis and altering their patterns of recirculation causing lymphopenia (Zinkl, 1986). Our result is supported by Benfey and Biron (2000) and Ida and Kurogi (2001) who observed a decrease in lymphocyte and an increase in heterophil number in stressed fish.

Contrarily to our results, Salonius and Iwama (1993) who observed an increase in the lymphocyte percentage in wild and cultivated salmon 4 hours after stress.

The response of fish held in blue environment is interesting, showing inhibition in the serum cortisol and glucose levels and non significant changes in leukocytic count when compared with control group. Few studies have been devoted to the effect of color on fish. Groneveld et al., (1995) found that the response of the melanin- concentrating hormone to background color is not strictly related to the pituitary internal axis in tilapia fish. However, the present study supports the idea that color influences this axis, thus may affect different biological system.

The effect of *A. hydrophila* infection on the leukogram of stressed fish is shown in (Table 7). Data analysis revealed significant increase in the total leukocytic count in both group (2&3) compared with control group. This leukocytosis was accompanied with heterophilia, monocytosis and lymphopenia from the 1st week post infection till the end of experimental period in group (3). The leukogram of *A. hydrophila* infected stressed fish in group (2) showed the same pattern of alteration achieved in (G_3) except for lymphopenia which is

recorded from the 2nd week post infection. The recorded heterophilia and monocytosis may be attributed to the bacterial infection with *A. hydrophila* to attack and engulf the micro-organism. Furthermore, heterophilia with lymphopenia could be interpreted as stress response following *A. hydrophila* infection. These results are in agreement with Stoskopf (1993) who recorded an increase in the total leukocytic count and differential count of neutrophils, monocytes and a decrease in the lymphocytic count in the blood of common carp after infection with *A. hydrophila* infection.

In the present investigation, it is noticeable that the lymphocytopenia was detected earlier in group (3) which exposed to the white light comparable with those maintained in blue light. This finding may be an important link between stress response and the onset and susceptibility of Nile tilapia to *A.hydrophila* infection. Our finding is agreeable with Wiik et al., (1989) who assumed a correlation between the reduction of the number of circulating lymphocyte and susceptibility of Atlantic salmon to Vibrio salmonicida infection.

Results of biochemical analysis of serum constituents of stressed fish challenged with *A. hydrophila* infection are illustrated in Tables (8 and 9).

Analysis of serum proteins (Table 8), revealed a significant drop in the total proteins concentration on the 3rd and 4th week post infection in both group (2&3). The observed hypoproteinemia was associated with hypoalbuminemia and hypoglobulinemia. The hypoglobulinemia may be ascribed to the decreased circulating immunoglobulins due to *A. hydrophila* infection (Evenberg et al., 1986). This finding is supported by the recorded lymphopenia in peripheral blood and necrosis of lymphoid tissues of spleen in our study. Furthermore, the hypoalbuminemia and hypoglobulinemia may be referred to decreased synthesis resulted from hepatic damage induced by *A. hydrophila* infection. Similar results were obtained by Rehulka (1996) and Yildiz (1998).

Concerning activities of hepatic enzymes, significant elevation of alanine amino transferase (ALT) activity was achieved on the 4th week in both groups (2&3). Whereas, elevation of aspartate amino transferase (AST) activity was recorded on the 2nd and 3rd week post infection in group 3&2 respectively and persisted till the end of the experiment. Such changes reflect hepatic damage induced by *A. hydrophila* infection. These findings are in accordance with Brenden and Huizinga (1986).

In the present investigation, marked elevation was observed in BUN and serum creatinine concentration on the 2nd and 3rd week in group (3) and (2) respectively till the end of experiments. Elevation of BUN is more likely associated with gill disease in most fish as it is predominated over kidney as a major organ of urea excretion (Stoskopf, 1993).

This result is supported by the histopathological alterations of the gills in our study. Meanwhile, creatinine elevation reflects kidney damage as it is mainly excreted through kidneys in most fish. So elevated creatinine level in our study is assumed to be related to the renal damaged induced by *A. hydrophila* infection. Our results coincide with Cipriano (2001) who mentioned that kidneys are one of the target organs of *A. hydrophila* infection.

Analysis of serum glucose revealed conspicuous hyperglycemia on 1st and 2nd week in group (3) and (2) respectively which continued till the 3rd week post A. hydrophila infection. While non significant changes were obtained on the 4th week. This hyperglycemia could be referred to the stress induced by *A. hydrophila* infection as the increased level of blood glucose considered a good indicator of stress in fish (Hattingh, 1976).

From the previous results it is clearly obvious that the clinicopathological alterations were earlier in the group of fish maintained under white light in comparable with those exposed to blue light. So, we could concluded that stressed fish kept in white light is more susceptible to the *A. hydrophila* infection comparable with those exposed to blue light.

The histopathological examination of different groups revealed the following, in group two (G2) (exposed to blue light, confinement and infection), the early pathological alterations were noticed in spleen just after confinement where marked activation of melano macrophage centers was noticed (Fig.1a). In such samples, the melanophores were impacted with intense melanin pigments and showed large number of lymphocytes aggregation. At this time, many melanophores were also noticed around the sinusoidal capillaries (Fig.1b). This changes in spleen indicated early immune response. In this concern, Wolk, 1992 considered the increase in number of melanophores in melanomacrophage centers is indication for stress while Roberts, 1989 considered it as favorable reaction to compete any bacterial infection by liberation of free oxygen radicals. Our pathological findings in this group revealed time dependent lesions where after one week post infection, areas of necrosis in spleen were noticed with fragmentation of some melanophores (Fig.1c). At the time of two weeks post infection, mild pathological lesions were observed in gills and liver.

In gills, focal lamellar telangectasis (focal dilation of branchial blood vessels) was obseved. The telangectasis could be a vascular inflammatory response of the gill tissue against the infection (Noga, 1996) or secondary as a result of increased oxygen demands due to lamellar tissue alterations (Roberts, 1989). The telangectasis is demonistrated in (Fig.1d). Many eosinophilic granular cells (Fig.1e) were detected in the area of gill rackers. The role of the eosinophilic granular cells in teleosts tissue reaction is unclear but such cells were recorded in some chronic lesions in fish and thus

it could be considered as inflammatory cell (Ferguson, 1989). The hepatic tissue showed few melanophores infiltrating the area of hepatopancreas (Fig.1f).

After 4 weekspost infection, the parenchymatous organs were apparently normal but, marked melanophores aggregation around the main blood vessels nourishing spleen (Fig. 1g), the branchial tissue showed focal areas of hemprrhage at the base of gill lamellae (Fig..1h).

The induced lesions could be attributed to *A.hydrophila* infection. The lesions of this disease were discussed in detail by many authors (Bach et al., 1978, Huizinga et al., 1979 and Roberts, 1989). The time dependent lesions could be attributed to the organ priority to attacking the bacterial infection. In this concern, Mahmoud, 1996 isolated *A. hydrophila* from spleen and kidneys earlier than any other organ after experimental infection. Also, Roberts, 1989 explained the early isolation of *A. hydrophila* from spleen on the base indicated that the affinity of haemopoietic tissue to early attack the microorganisms due to presence of two main defensive structures in such organs (melano-macrophage centers and splenic ellipsoids). Species variation could be playing a role in target organ susceptibility after experimental *A. hydrophila* infection. Thus, Soliman et al., (1989) isolated *A. hydrophila* from all organs in experimentally infected stripped mullet after 24 hours post infection.

In group three (G_3) (exposed to white light, confinement and infection), the lesions were more advanced where after 1 week marked vacuolar degeneration of hepatocytes was noticed with mononuclear cells infiltration in hepatic tissue (Fig..2a). Diffuse necrotic parts were observed. After two weeks. The area of hepatopancreas showed marked infiltration with melanophores. peripancreatic hemorrhage and dilated hepatoportal blood vessels was common (Fig.2b).

After 3 weeks, the changes were noticed in spleen and liver in the same manner of one week in addition to marked aggregation of melanophores around spleen ellipsoids (Fig.2c).

In our concept, the necrotic lesions, hemorrhages and leukocytic infiltration could be attributed to many virulence factors excreted by *A. hydrophila* microorganism. Among which, proteolytic enzymes and haemlysins (Kimya and Yasuki, 1986., Easa et al., 1988, and Mahmoud, 1996). The kidneys of fish in this group showed peritudular hemorrhage (Fig. 2d) and vacuolar degeneration of the tubular epithelium (Fig. 2e). After 4 weeks, same lesions were noticed as after 3 weeks.

The histopathological examination indicated that the lesions of this group (G_3) showed more severe and advanced lesions than in group two. additional renal tissue response was also noticed in this group. Thus, the exposure of fish to blue light prior to *A. hydrophila* infection could reduce the

Table (1): Proportion of frighting behaviour ($\bar{X}\pm$LSD) for all treated groups:

Treatment	Light			After Confinement			Infection		
Groups / Behavioural patterns	G1	G2	G3	G1	G2	G3	G1	G2	G3
CRE	31.7^{a} ±12.25	27.2^{b} ±12.36	31.05^{a} ±12.40	34.58^{a} ±24.60	36.65^{a} ±0.50	63.34^{b} ±22.05	31.05^{a} ±0.05	48.06^{b} ±2.41	54.05^{c} ±0.50
OBQ	42.50^{a} ±20.58	14.08^{b} ±15.29	41.08^{a} ±13.03	43.88^{a} ±0.75	45.38^{a} ±16.91	61.75^{b} ±9.32	40.4^{a} ±7.33	47.05^{b} ±0.05	55.25^{c} ±16.91
A-G	5.55^{a} ±4.12	0.13^{b} ±0.35	4.35^{a} ±3.58	7.95^{a} ±0.50	10.15^{a} ±1.88	50.25^{b} ±0.50	8.45^{a} ±9.86	18.65^{b} ±0.90	35.34^{c} ±22.05

Mean within the same column having different superscripts are significantly different at ($P < 0.05$)

CRE: Creeping

OBQ: Oblique plan position

A-G: Air- gulping

Table (2): Proportion of aggressive behaviour ($\bar{X}\pm$LSD) of treated groups during exposure to light, confinement and *A.hydrophila* infection.

Treatment	Light			Confinement	Infection		
Period in week / Groups	1st week	2nd week	Over all	End of 6 hour	2nd week	4th week	Over all
G1	19.20^{a} ±14.60	25.00^{a} ±15.10	22.10^{a} ±15.60	21.00^{a} ±14.70	20.40^{a} ±11.50	22.50^{a} ±20.10	21.40^{a} ±16.40
G2	28.30^{a} ±12.60	50.00^{b} ±44.40	39.20^{b} ±32.40	23.00^{a} ±16.60	18.70^{a} ±19.00	11.90^{b} ±17.30	15.30^{a} ±13.80
G3	9.90^{b} ±3.80	30.40^{a} ±22.00	20.26^{a} ±18.30	0.25^{b} ±0.05	8.30^{b} ±16.60	10.70^{b} ±21.00	9.50^{b} ±17.00

Mean within the same column having different superscripts are significantly different at ($P < 0.05$)

Table (3): Performance parameters of treated groups ($\bar{X}\pm$LSD) during exposure to different light.

Parameters	F.B.W. (gm.)			T.W.G. (gm.)			A.D.G. (gm.)			R.G.R. %			F.C.R.		
Period in week / Groups	1st week	2nd week	Over all	1st week	2nd week	Over all	1st week	2nd week	Over all	1st week	2nd week	Over all	1st week	2nd week	Over all
G1	60[a] ±2.90	64[a] ±3.10	62[a] ±3.00	5	4	4.5	0.71[a] ±0.09	0.59[a] ±0.08	0.68[a] ±0.12	9.11[a] ±0.93	6.64[a] ±0.69	8.20[a] ±1.80	2.10[a] ±0.50	2.62[a] ±0.31	2.36[a] ±0.41
G2	68[b] ±3.10	78[b] ±2.70	73[b] ±2.90	13	10	11.5	1.90[b] ±0.09	1.43[b] ±0.83	1.62[b] ±0.23	23.64[b] ±1.20	14.70[b] ±0.88	19.17[b] ±1.04	1.00[b] ±0.05	1.37[b] ±0.56	1.19[b] ±0.31
G3	60[a] ±3.50	65.9[a] ±2.90	62.95[a] ±3.20	6	5	5.5	0.86[a] ±0.04	0.72[a] ±0.05	0.79[a] ±0.89	11.11[c] ±0.93	8.33[a] ±0.83	9.72[a] ±0.88	1.75[c] ±0.04	2.50[c] ±0.42	2.12[a] ±0.48

Mean within the same column having different superscripts are significantly different at ($P < 0.05$)

F.B.W.= Final body weight

T.W.G.= Total weight gain

A.D.G.= Average daily gain

R.G.R.= Relative growth rate

F.C.R. = Feed conversion ratio

Table (4): Performance parameters of treated groups ($\bar{X}$±LSD) during confinement.

Parameters / Group	Body weight (gm.)			R.G.R %	F.C.R
	Before confinement	After confinement	Over all		
G1	64.00[a] ±3.10	70.00[a] ±2.40	67.00[a] ±4.00	9.10[a] ±0.84	1.80[a] ±0.08
G2	78.00[b] ±2.70	72.00[b] ±3.10	75.00[b] ±4.20	-7.20[b] ±0.90	-1.45[b] ±0.13
G3	65.90[a] ±2.90	57.00[c] ±2.10	61.40[c] ±5.20	-12.07[c] ±0.83	-2.20[c] ±0.59

Mean within the same column having different superscripts are significantly different at ($P < 0.05$)

Table (5): Performance parameters of treated groups ($\bar{X}$±LSD) during exposure to *A.hydrophila* infection.

Parameters	F.B.W (gm.)			T.W.G (gm.)			A.D.G			R.G.R %			F.C.R		
Period in week / Groups	2nd week	4th week	Over all	2nd week	4th week	Over all	2nd week	4th week	Over all	2nd week	4th week	Over all	2nd week	4th week	Over all
G1	75.00^{a} ±3.60	80.00^{a} ±4.10	77.50^{a} ±3.85	6	5	5.5	0.24^{a} ±0.41	0.77^{a} ±0.09	0.51^{a} ±0.29	7.11^{a} ±0.70	6.70^{a} ±0.89	6.91 ±0.77	2.30^{a} ±0.60	2.40^{a} ±0.70	2.36^{a} ±0.61
G2	68.0^{b} ±3.10	64.00^{b} ±3.20	66.00^{b} ±3.15	-6	-4	-5	-0.19^{b} ±0.08	-51^{b} ±0.09	-0.35^{b} ±0.18	-5.10^{b} ±0.80	-5.70^{b} ±0.9	-5.43 ±0.87	-2.10^{b} ±0.59	-2.90^{b} ±0.77	-2.50^{b} ±0.74
G3	52.0^{c} ±3.10	49.00^{c} ±2.20	50.50^{c} ±2.65	-8	-5	-6	-0.22^{b} ±0.07	-0.41^{b} ±0.08	-0.32^{b} ±0.12	-8.30^{a} ±0.90	-5.60^{b} ±0.78	-6.95 ±1.57	-3.11^{c} ±0.69	-3.06^{c} ±0.74	-3.08^{b} ±0.66

Mean within the same column having different superscripts are significantly different at ($P < 0.05$)

Table (5): Performance parameters of treated groups ($\bar{X}\pm LSD$) during exposure to *A.hydrophila* infection.

Parameters	F.B.W (gm.)			T.W.G (gm.)			A.D.G			R.G.R %			F.C.R		
Period in week / Groups	2nd week	4th week	Over all	2nd week	4th week	Over all	2nd week	4th week	Over all	2nd week	4th week	Over all	2nd week	4th week	Over all
G1	75.00^{a} ±3.60	80.00^{a} ±4.10	77.50^{a} ±3.85	6	5	5.5	0.24^{a} ±0.41	0.77^{a} ±0.09	0.51^{a} ±0.29	7.11^{a} ±0.70	6.70^{a} ±0.89	6.91 ±0.77	2.30^{a} ±0.60	2.40^{a} ±0.70	2.36^{a} ±0.61
G2	68.0^{b} ±3.10	64.00^{b} ±3.20	66.00^{b} ±3.15	-6	-4	-5	-0.19^{b} ±0.08	-51^{b} ±0.09	-0.35^{b} ±0.18	-5.10^{b} ±0.80	-5.70^{b} ±0.9	-5.43 ±0.87	-2.10^{b} ±0.59	-2.90^{b} ±0.77	-2.50^{b} ±0.74
G3	52.0^{c} ±3.10	49.00^{c} ±2.20	50.50^{c} ±2.65	-8	-5	-6	-0.22^{b} ±0.07	-0.41^{b} ±0.08	-0.32^{b} ±0.12	-8.30^{a} ±0.90	-5.60^{b} ±0.78	-6.95 ±1.57	-3.11^{c} ±0.69	-3.06^{c} ±0.74	-3.08^{b} ±0.66

Mean within the same column having different superscripts are significantly different at ($P < 0.05$)

Table (8): Effect of blue light exposure on Protein profile of stressed Nile tilapia (Oreochromis niloticus) challenged with *A. hydrophila*.

weeks	Groups	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G (Ratio)
1	G.(1)	3.71 ± 0.39 [a]	1.77 ± 0.14 [a]	1.94 ± 0.18 [a]	0.92 ± 0.17 [a]
	G.(2)	3.36 ± 0.30 [a]	1.63 ± 0.12 [a]	1.73 ± 0.45 [a]	0.94 ± 0.16 [ab]
	G.(3)	2.86 ± 0.34 [a]	1.65 ± 0.13 [a]	1.22 ± 0.36 [a]	1.41 ± 0.24 [a]
2	G.(1)	3.74 ± 0.46 [a]	1.82 ± 0.09 [a]	1.92 ± 0.60 [a]	1.00 ± 0.09 [a]
	G.(2)	3.46 ± 0.43 [a]	1.62 ± 0.08 [a]	1.83 ± 0.37 [a]	0.90 ± 0.07 [a]
	G.(3)	3.20 ± 0.26 [a]	1.55 ± 0.23 [a]	1.65 ± 0.13 [a]	0.94 ± 0.08 [a]
3	G.(1)	3.89 ± 0.25 [a]	1.87 ± 0.10 [a]	2.02 ± 0.15 [a]	0.92 ± 0.03 [a]
	G.(2)	2.56 ± 0.08 [b]	1.23 ± 0.26 [b]	1.33 ± 0.33 [b]	0.93 ± 0.42 [a]
	G.(3)	2.30 ± 0.15 [b]	1.10 ± 0.25 [b]	1.20 ± 0.15 [b]	0.94 ± 0.34 [a]
4	G.(1)	3.84 ± 0.39 [a]	1.78 ± 0.05 [a]	2.06 ± 0.04 [a]	0.88 ± 0.18 [a]
	G.(2)	2.76 ± 0.34 [b]	1.17 ± 0.20 [b]	1.59 ± 0.05 [b]	0.75 ± 0.41 [a]
	G.(3)	2.37 ± 0.28 [b]	1.03 ± 0.19 [b]	1.34 ± 0.05 [b]	0.76 ± 0.09 [a]

Values represent means ± SD

[a-b] Values with different letters at the same column are significantly different at $p < 0.05$

Table (9): Effect of blue light exposure on on some serum biochemical parameters of stressed Nile tilapia (Oreochromis niloticus) challenged with *A. hydrophila*.

weeks	Groups	ALT (IU/L)	AST (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)
1	G.(1)	14.97 ± 2.75 ^{a}	40.45 ± 1.50 ^{a}	6.17 ± 0.40 ^{a}	0.98 ± 0.13 ^{a}	36.65 ± 1.78 ^{b}
	G.(2)	14.81 ± 2.45 ^{a}	38.37 ± 1.57 ^{a}	5.79 ± 0.60 ^{a}	0.88 ± 0.08 ^{a}	40.68 ± 3.62 ^{ab}
	G.(3)	15.32 ± 2.99 ^{a}	41.79 ± 2.27 ^{a}	6.83 ± 0.46 ^{a}	0.93 ± 0.08 ^{a}	45.16 ± 2.90 ^{a}
2	G.(1)	15.22 ± 1.95 ^{a}	36.45 ± 2.42 ^{b}	6.37 ± 0.27 ^{b}	1.05 ± 0.13 ^{b}	38.19 ± 0.80 ^{c}
	G.(2)	14.73 ± 2.55 ^{a}	38.75 ± 3.15 ^{ab}	6.73 ± 0.25 ^{b}	1.17 ± 0.25 ^{b}	43.29 ± 1.55 ^{b}
	G.(3)	15.60 ± 3.00 ^{a}	44.11± 3.18 ^{a}	7.55 ± 0.53 ^{a}	1.67 ± 0.13 ^{a}	57.15 ± 2.40 ^{a}
3	G.(1)	16.68 ± 2.35 ^{a}	43.37 ± 3.23 ^{c}	6.36 ± 0.39 ^{b}	0.97 ± 0.13 ^{b}	42.02 ± 3.07 ^{c}
	G.(2)	16.66 ± 0.97 ^{a}	54.33 ± 2.78 ^{b}	7.83 ± 0.20 ^{a}	1.77 ± 0.25 ^{a}	57.27 ± 2.72 ^{b}
	G.(3)	17.73 ± 1.38 ^{a}	68.18 ± 2.65 ^{a}	8.03 ± 0.09 ^{a}	2.08 ± 0.34 ^{a}	76.00 ± 4.05 ^{a}
4	G.(1)	18.68 ± 0.39 ^{c}	44.2 ± 0.92 ^{c}	6.40 ± 0.45 ^{c}	0.87 ± 0.03 ^{b}	51.25 ± 3.59 ^{a}
	G.(2)	22.16 ± 0.93 ^{b}	51.57 ± 1.85 ^{b}	8.05 ± 0.18 ^{b}	1.80 ± 0.20 ^{a}	50.40 ± 2.23 ^{a}
	G.(3)	27.83 ± 1.10 ^{a}	70.78 ± 2.87 ^{a}	8.79 ± 0.26 ^{a}	2.02 ± 0.43 ^{a}	52.34 ± 3.00 ^{a}

Values represent means ± SD

$^{a-c}$ Values with different letters at the same column are significantly different at $p< 0.05$

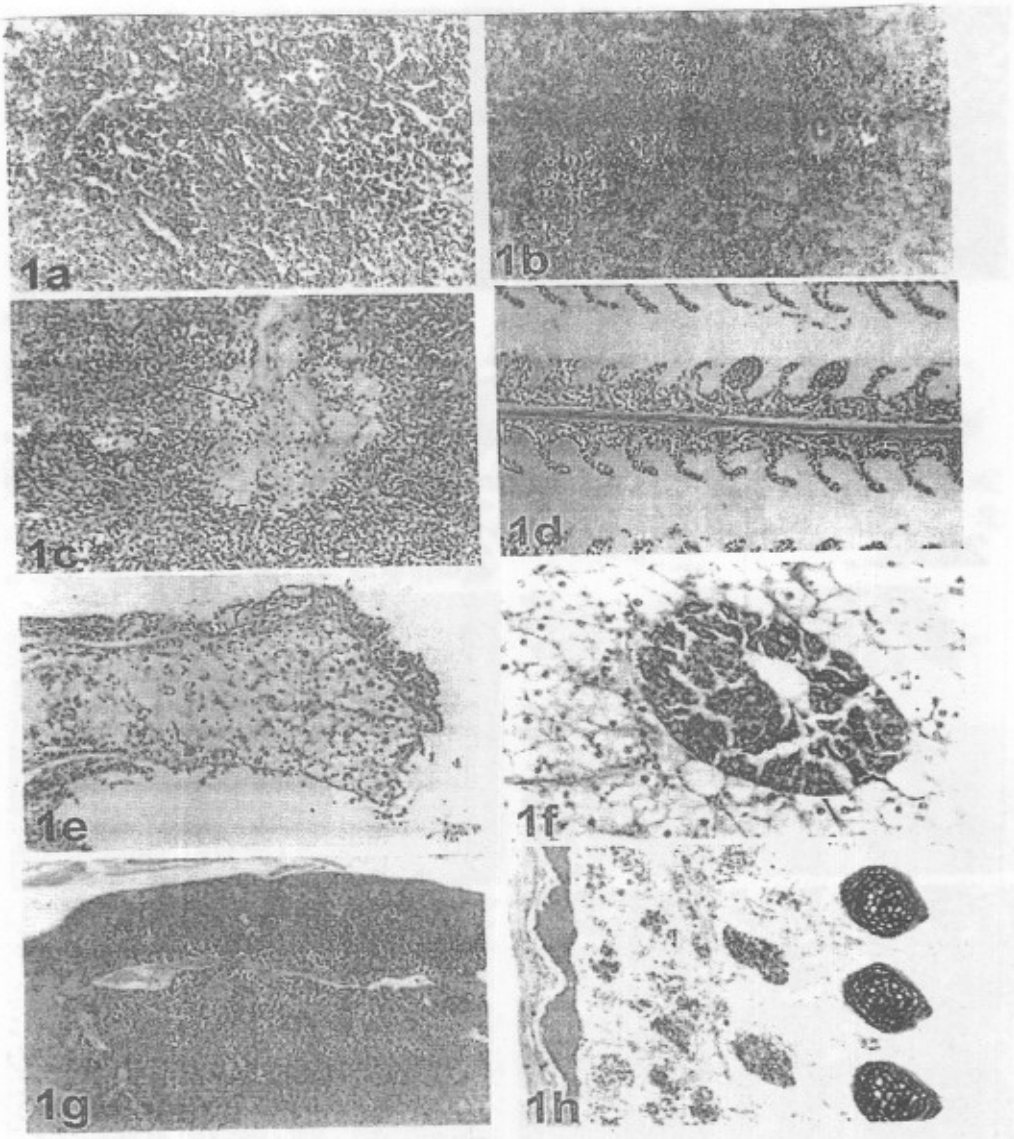

Fig.1: Tissue samples of group two (*O.niloticus* exposed to blue light, confinement and infection showing:

a- Spleen showing marked activation of melanomacrophage centers (H&E stain x 200)

b- Spleen showing melanophores aggregation around ellipsoidal capillaries (H&E stain x 100)

c- Spleen showing area of necrosis (arrow) and fragmented melanophores. (H&E stain x 200)

d- Gills showing focal lamellar telangectasis. (H&E stain x 200)

e- Gills showing eosinophilic granular cells aggregation in gill rackers. (H&E stain x 200)

f- Liver showing large number of melanophores in the area of hepatopancreas. (H&E stain x 400)

g- Spleen showing marked aggregation of melanophores around the main splenic blood vessels. (H&E stain x 40)

h- Gills showing focal area of hemorrhage at the base of gill lamellae.(H&E stain x200)

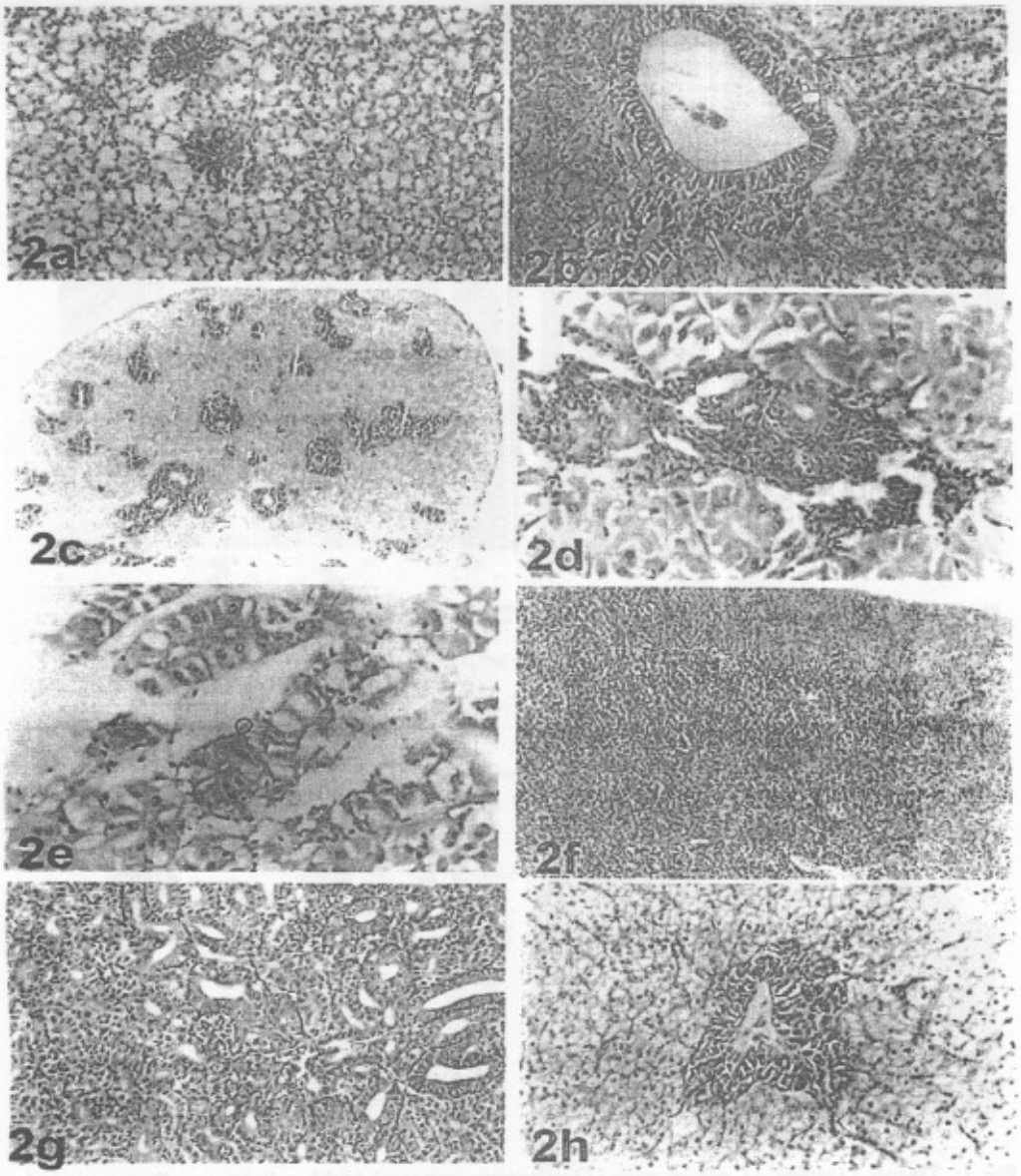

Fig.2:

a- Liver of *O.niloticus* from group 2 (exposed to white light) showing mononuclear cells infiltration. (H&E stain x 200)

b- Liver of *O.niloticus* from group 2 showing melanophores aggregation in hepatopancreas and peripancreatic hemorrhage (arrow).
(H&E stain x 200)

c- Spleen of *O.niloticus* from group 2 showing melanophores aggregation around the blood capillaries of splenic ellipsoids. (H&E stain x 40)

d- Kidney of *O.niloticus* from group 2 showing peritubular hemorrhage.
(H&E stain x 400)

e- Kidney of *O.niloticus* from group 2 showing vacuolar degeneration of tubular epithelium. (H&E stain x 400)

f- Spleen of control group (without treatment) showing normal structure.
(H&E stain x 400)

g- Kidney of control group (without treatment) showing normal structure.
(H&E stain x 100)

h- Liver of control group (without treatment) showing normal structure.
(H&E stain x 200)

induced lesion. This result was go parallel with the findings of Volpato and Barreto, 2001 who indicated preventive effect of blue light on stress conditions in Nile tilapia. The control group (G_1) showed no obvious pathologoical lesions in different organs (Fig. 2f, 2g&2h).

Our studies clarified that blue light has a preventive effect of stress in Nile tilapia. It improves the fish welfare by lowering fear response and increasing growth performance. Moreover, blue light exposure reduces the clinicopathological and pathological alterations induced by *A. hydrophila* infection in stressed Nile tilapia. So, it recommended to be used in interior aquarium, wall of indoor fish production and during transportation.

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تأثير إجهاد الإحتجاز على السلوك والأداء و التغيرات الباثولوجية الإكلينيكية والتغيرات النسيجية فى البلطى النيلى المعرض لميكروب الأيروموناس هيدروفيلا وتأثير الضوء الأزرق كمثبط للإجهاد

محمود على محمود[1]، عبير حماده عبد الرازق[2]، نشوى عادل أبو عيطه[3]
١ ـ قسم الباثولوجيا ٢ ـ قسم الصحه و الرعايه البيطريه ٣ ـ قسم الباثولوجيا الأكلينيكيه
كلية الطب البيطرى ـ جامعة القاهره

لقد أجريت هذه الدراسه لتقييم تأثير الضوء الأزرق كمثبط للأجهاد ومدى حساسية أسماك البلطى النيلى للعدوى بميكروب الأيروموناس هيدروفيلا عند التعرض لذلك الأجهاد. و لذلك فلقد تم أستخدام عدد ١٢٠سمكه بلطى تم تقسيمهم الى ٣ مجموعات حيث أحتوت كل مجموعه على ٤٠ سمكه و لقد أعتبرت المجموعه الأولى مجموعه ضابطه و تعرضت للضوء العادى بدون معاملات أما المجموعه الثانيه فقد تعرضت للضوء الأزرق و المجموعه الثالثه تعرضت للضوء الأبيض الفلوريسينتى. و بعد ١٥ يوم من التعرض للأضاءه تم أحتجاز أسماك المجموعتين الثانيه و الثالثه فى حيز ضيق ثم تعريضهم لميكروب الأيروموناس هيدروفيلا. و قد أوضحت النتائج أنه أثناء التعرض للضوء فأن أسماك المجموعه الثانيه (ج٢) قد سجلت أقل نسبه فى الأنماط السلوكيه. هذا وقد أشتملت هذه الأنماط على كل من سلوك الخوف (الفزع) و السلوك العدوانى. وعلى الجانب الأخر قد سجلت نفس هذه المجموعه أقل خلل فى سلوك الخوف أثناء الأحتجاز و العدوى بالمقارنه بأسماك المجموعه الثالثه (ج٣) و على العكس فقد سجلت هذه المجموعه (ج٢) أعلى نسب معنويه للسلوك العدوانى أثناء التعرض لكل من الضوء و الأحتجاز و أخيرا أثناء تعرضها للعدوى بالمقارنه بالمجموعه الثالثه (ج٣). أما بالنسبه لكفاءة النمو فى الأسماك فأن أسماك المجموعه الثانيه (ج٢) قد حققت أعلى مستوى معنوى من هذه الكفاءه أثناء التعرض للضوء و نقص بسيط فيها أثناء كلا من الأحتجاز و العدوى بالمقارنه بمجموعة الأسماك الثالثه (ج٣). ولقد تم تجميع عينات الدم و السيرم لقياس دلالات الأجهاد وفحص خلايا الدم و أجراء بعض القياسات البيوكيميائيه للسيرم. و لقد أظهرت النتائج أن الأسماك المعرضه للأجهاد فى الضوء الأبيض قد لوحظ بها زياده معنويه فى دلالات الأجهاد حيث سجلت زياده معنويه فى نسبة الكورتيزول و الجلوكوز و زياده فى عدد كرات الدم البيضاء وخاصة الهيتيروفيل مع نقص خلايا الليمفوسيت بالمقارنه بالأسماك التى تعرضت للأجهاد فى الضوء الأزرق. و لقد وجد أن قابلية البلطى للأصابه بميكروب الأيروموناس هيدروفيلا قد زادت فى الأسماك المجهده و التى تعرضت للضوء الأبيض مقارنة بالأسماك المجهده و المعرضه للضوء الأزرق حيث أوضح فحص خلايا الدم زياده معنويه فى عدد كرات الدم البيضاء و خاصة الهيتيروفيل و المونوسيت مع نقص خلايا الليمفوسيت و التى سجلت فى فتره مبكره فى الأسماك المعرضه للضوء الأبيض. و بفحص مكونات الدم الكيميائيه أظهرت النتائج زياده معنويه فى كل من أنزيمات الكبد و البولينا و الكرياتينين و الجلوكوز مع أنخفاض معنوى فى البروتين الكلى و الألبومين و الجلوبيولين و هذه التغيرات قد لوحظت فى فتره مبكره و أشد ضراوه فى الأسماك المجهده و المعرضه للضوء الأبيض. أما الفحص النسيجى فقد أوضح أن المجموعه المعرضه للضوء الأزرق و العدوى لوحظ بأنسجتها أستجابه أيجابيه مبكره خاصة بالأنسجه المناعيه، ولقد تمثلت تلك الأستجابه فى حدوث نشاط واضح بمراكز تجمع الميلانين بالطحال. أما فحص الأنسجه الأخرى فقد لوحظ بها حدوث تأثيرات نسيجيه غير مرغوبه و لكن ظلت هذه التغيرات مرتبطة بوقت التعرض و لقد لوحظت تلك التغيرات فى أنسجة الطحال و الخياشيم بعد أسبوعين من بداية التعرض للأضاءه كما لوحظ التئام معظم التغيرات النسيجيه بعد أربعة أسابيع. و من ناحية أخرى فأن الأسماك التى تعرضت للضوء الأبيض مع العدوى قد حدث بأنسجتها تغيرات أشد حده من المجموعه السابقه تمثلت فى حدوث تنكرز واسع الأنتشار بالنسيج الكبدى مع تغيرات بأنسجة الكلى.
و لذلك فقد أوضحت الدراسه أن تعرض الأسماك للأضاءه الزرقاء أسفر عن تحسن ملحوظ سواء فى السلوك و كفاءة النمو و كذلك التغيرات الباثولوجيه الأكلينيكيه و التغيرات النسيجيه.