EFFECT OF SOME PHYSIOLOGICAL FACTORS ON BLOOD PARAMETERS IN CATFISH (CLARIAS LAZERA)

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ABSTRACT

The effects of environmental changes on blood parameters of adult male and female catfish (Clarias lazera) were tested. The effects of fasting, dim light, high temperatures, and airless on the haemogram, leucogram, coagulation parameters and stress indicating tests (glucose and cortisol levels). The results revealed that, in control fish the average red blood cells (RBCs) count (was 1.55 X 10^6/ mm³, hemoglobin (Hb) concentration was 8.2 g/dl and packed cell volume (PCV) was 35.77%. The average white blood cell (WBCs) count was 1.89 X 10^4/ mm³ and the percentages of heterophils, basophils, eosinophils, monocytes and lymphocytes were 3.9%, 0.33%, 4.47%, 5.2% and 82%, respectively. The clotting time was 100 seconds, prothrombin time was 38.69 second, thrombocyte count was 3.33 10^4/mm³ and serum calcium level was 2.28 mmol/l. The average plasma glucose level was 3.77 mmol/l and cortisol level was 26.6 nmol/l. Environmentally induced changes in leukocytes and coagulation parameters were negligible. However, fasting and dim light induced a significant decrease in RBCs, Hb and PVC, whereas these parameters were increased markedly after exposure to high temperature and airless. Hyperglycemia was recorded after high temperature, while under fasting condition hypoglycemia was resulted. Cortisol level significantly increased under all conditions of the present study except in dim light it was significantly decreased. In conclusion of the environmental factors tested, airless condition and fasting caused the greatest changes in the blood parameters relative to dim light and high temperature.
INTRODUCTION

In Egypt, the availability of animal sources of dietary protein has not kept up with the tremendous increase in the human population. The freshwater catfish, Clarias lazera (Karmot) is one of the most popular and economic indigenous fish species in river Nile. Its growth rate and meat quality are very high (22). It is of low cost and suitable for the Egyptian consumers. Moreover, C. lazera has a high reproductive efficiency (30). However, these fish can be seriously affected by environmental factors such as airless, fasting, and high ambient temperature. The stress caused by water contaminants is also clearly reflected on the haemogram and leucogram (3). Rises in blood glucose levels can be used as a good indicator for metabolic stress (27).

The aim of this study is to understand the effect of some environmental factors (fasting, dim light, airless and high temperature) on the haemogram, leucogram, some coagulation parameters, glucose and cortisol levels in both sexes of catfish and apply this knowledge in efforts to enhance husbandry techniques.

MATERIALS AND METHODS

Fish handling. Sixty-three mature male and female C. lazera fish of more than 32 cm length and 320 g. body weight were obtained (freshly caught) from Nile river at Benha- Kalyoubia province - Egypt. Fish were transported in tanks containing dechlorinated tap water to the laboratory. The fish were kept (4 fish/aquarium) in glass aquarium (100 x 40 x 50 cm) with dechlorinated water which was oxygenated by electric air compressor (except in airless group). The fish were fed fresh earthworms at 3% of fish body weight. The fish were allocated into 4 groups, each group containing about 16 fish (male & female). Each group was then divided into two subgroups, one was used as a control and the other was subjected to one of the following environmental manipulations for two weeks: fasting, dim light (75 % less than control), airless (by stopping the aeration), high temperature by raising the aquarium temperature to 34 °C, while the control was remained at 24°C. At the end of the treatment period, blood were collected from the caudal vein between 1 and 3 PM using a plastic syringe to avoid the rapid clotting (35). Blood samples were divided into 4 tubes. One tube contained EDTA for complete blood count. The second tube contained sodium fluoride for glucose analysis. The third tube contained sodium citrate (3.8%) in a ratio of 1:9 for
prothrombin time measurement (plasma was obtained by centrifugation at 2500 g/10min). In the 4th plane tube, the blood was left to clot to obtain serum for calcium and cortisol determination. The supernatant fluids were stored at – 20 °C until used for analysis except complete blood count and prothrombin time were estimated within 2 hours from blood collection.

Hematological investigations. Blood hemoglobin was assessed by the cyanmethemoglobin method (Drabkin reagent-Biomerieux). The haematocrite value was obtained using the microhaematocrite capillary tube method. The RBCs and WBCs counts were determined using the method of Lehmann & Sturenberg (23). Blood indices MCV, MCH, and MCHC; were calculated from previous values. The differential leukocytic counts were recorded using Lieshmann stained blood film, and the thrombocyte count was measured indirectly by counting the number of thrombocytes/100 leukocytes in the blood film, then converting this number into absolute value. The coagulation time was obtained by placing one drop of blood on a warmed glass slide (37 °C), then recording the time elapsed for fibrin threads formation by gently touching the blood drop with a fine needle (checked every 30 second).

Biochemical studies. Test kits from Bio Merieux (France) were used for determination of prothrombin time (2), glucose (6), calcium (38). Cortisol levels were determined by radioimmunoassay (11). Statistical analysis was carried out using Student t-test (12) between the appropriate control and treatment groups.

RESULTS

There were no significant differences between control males and females in their haemogram profiles (RBCs, Hb, PCV, MCV, MCH & MCHC). The haemogram (except blood indices) was decreased under fasting and dim light conditions (\( P < 0.05 \)) and increased under airless (\( P < 0.05 \)) and high temperature (\( P < 0.01 \)) conditions (Table 1 and 2). No changes were recorded in blood indices except under dim light, where MCV & MCH showed significant decrease (\( P < 0.05 \)).

Fasting induced lymphopenia (\( P < 0.05 \)) and monocytosis (\( P < 0.05 \)) in male catfish. High temperature induced lymphopenia (\( P < 0.05 \)) and monocytosis (male, \( P < 0.01 \) female, \( P < 0.05 \)) in male and female catfish.
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Clotting time significantly decreased \((P<0.01)\) under fasting and high temperature conditions in both sexes of fish. Thrombocyte count significantly increase \((P<0.05)\) under the same environmental conditions only in female fish. Under all experimental conditions of the present study, calcium level showed significant decreases \((P<0.05)\) except in male fish under airless condition where no change was recorded (Table 1). High temperature induced hyperglycemia \((P<0.01),\) whereas fasting induced hypoglycemia \((P<0.05)\) relative to control groups. Under all conditions tested, cortisol levels were significantly increased \((P<0.01)\) except under dim light where cortisol was significantly decreased \((P<0.01)\).

**DISCUSSION**

Starvation of catfish in the present study resulted in a significant decrease in haemogram profiles except the blood indices, which did not change. This effect may be due to reduction of erythropoiesis. These findings are in accordance with the studies on Atlantic cod (17), rainbow trout (18), air-breathing fish *Clarias maculate* (24) and Nile tilapia (33). In contrast, Larsson & Lewander (20) reported no changes in European eel after prolonged starvation. Moreover, Johansson et al. (15, 16) found a significant increase after starvation due to the release of RBCs from erythropoietic storage. The haemogram was significantly decreased after exposure of catfish to dim light. This result may be due to a reduction in erythropoiesis by insufficient stimulation to brain - hypothalamus - anterior pituitary – pathway controlling the release of ACTH, thus affecting the inter-renal gland that secretes corticosteroid which might be the cause of blood picture changes.

The increase in RBCs count under airless condition is one of the physiological adjustments necessary to compensate for the reduction in oxygen availability. Contraction of smooth muscle of spleen and other organs (liver & kidney) may cause RBCs count elevation. The increased PCV may also due to swelling as a result of water shift into the cells from plasma. These results are similar to that obtained in pinfish (4), Striped mullet fish (5, 14, 41), channel catfish (32), and yellow tail fish (42). In contrast, in dogfish (8) and air-breathing *Clarias maculate* fish (43) airless induced a significant decrease in PCV and Hb. These observations were presumed to be caused by an increase in plasma volume or haemodilution from elevated water permeability of the gills or body surface.
Blood indices in the present study did not change in both sexes under airless condition. The same findings were obtained in studies with other species such as dogfish (8), rainbow trout (40) and air-breathing fish *C. maculate* (43). In contrast, airless produce a significant reduction in MCHC in Lamprey fish (28) probably due to cellular swelling. In the present study, there was a significant increase in haemogram in both fish sexes after raising the water temperature from 24°C to 34 °C (Tables 1 & 2) but without a change in blood indices. These results may be due to dehydration of blood as a consequence of osmotic water shifting from blood to muscles at higher temperature. Also, this effect may be attributed to the metabolic activation that resulted after the release of RBCs from its stores. These data are supported by other studies on different fish species such as, pinfish (37), river shiner *Notropis blenius* (3) and *Tilapia zilli* (10). In contrast, Anthony (1) found an inverse relationship between RBCs count and temperature in gold fish (in *Salvelinus fontinalis* and brook trout, respectively).

Fasting did not cause significant changes in leucogram in both sexes. Similar results were obtained in eel by Johansson *et al.* (15) when were starved fish during the spawning season. In contrast, prolonged fasting induced a decrease in leucocytic count as a result of impaired immunological defense in burbot fish (34), European eel (16) and *Tilapia nilotica* (33). No detectable changes in leucogram occurred in dim light conditions in the present study. The leucogram was also unaffected under airless condition. This result differs from those obtained with dogfish (8, 9) and Nile tilapia (33) where leucopenia and heterophilia were reported under airless condition. The reason for the difference between these latter species and catfish may be that catfish is well adapted to hypoxic environments. High temperature s.1. exposure did not affect leucogram in catfish. The same observation was reported with *Tilapia zilli* (10).

Analysis of coagulation parameters showed negligible changes except in serum calcium level which decreased significantly under most of experimental conditions of the present study. Although the significant decrease in calcium level was recorded in the present study but not reached to the level that affect clotting and prothrombin times. This could be explained according the ability of catfish to overcome stress factors in relation to the coagulation mechanisms. Plasma glucose level in both fish sexes was decreased significantly under fasting condition (Table1 & 2). This observation
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is in contrast to the suggestion of Murat et al. (26) that blood glucose in fish is largely maintained during starvation by glyconeogenesis and that glucocorticoids might play an important role in promoting gluconeogenesis, for example, during periods of migration. The glucose level under dim light and airless condition did not changed significantly in catfish, possibly because catfish are highly adapted to these environments and thus are not perceived as stressful. The hyperglycemia observed in the present investigation in both sexes of catfish under high temperature is similar to that obtained in rainbow trout, *Salmo gairdneri* (21), and *Labeo rahita* (29).

Cortisol levels in both sexes of catfish were significantly increased under most experimental conditions of the present study, except in dim light. Similar results were recognized as an indicator of stress after starvation (24) and airless (36) in other species stressful stimuli lead to rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue (25). However, cortisol level significantly decreased in dim light. In other vertebrate taxa, the secretory rate of corticotrophic releasing hormone, ACTH and cortisol are all high in the early morning but low in the late evening (13). If this concept can be applied to fishes, then it might help in explainig the effects of dim light on cortisol levels in catfish. Also, it has been shown that the cortisol response to stress in salmonids is attenuated in dark.

In summary, the results of this study suggest that husbandry practices for catfish should avoid airless conditions and include well-designed nutritional practices. Conditions of light and temperature are of lesser importance within the ranges tested in the present study.
REFERENCES


تأثير بعض العوامل الفسيولوجية على قياسات الدم في سمك القراميط

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تم في هذا البحث دراسة تأثير تغير بعض العوامل الفسيولوجية (القص، قلة الضوء، نقص التهوية وارتفاع حركة الوسط الخارجي) لمدة أسبوعين متتاليين على القياسات الدموية (عد الدم الكلي، بعض دلالات التحلل، بعض دلالات الحالة الإجهاد (الجلوكوز وهرمون الكورتيزول) لذكور وإناث سمك القراميط (كلاريس لازير) الناضجة. وقد أظهرت متوسطات النتائج للمجموعة المضادة للكيرات الدم الحمراء، والهيموجلوبين، وحجم الخلايا المرصوصة، خلايا الدم البيضاء، والنسب المنوية "لينيروفيل- الفاقدية- الحمضة- الحمضة- الليمفاوية- القيم الأمثل على الترتيب:

1.55 X 10^6/ mm^3, 8.2 g/dl, 35.77%, 1.89 X 10^4/ mm^3 و "3.9% - 0.33% - 4.47% - 5.2% - 82%".

وقد سجلت النتائج لزنم التحلل 100 ثانية، وزمن الورثة 38.69 ثانية وعدد الصفائح الدموية 3.33 X 10^4/mm^3، والكالسيوم في المصل 2.28 mmol/l، والجلوكوز وهرمون الكورتيزول فسجلت 26.6 mmol/l و 3.77 mmol/l.

وقد أوضحت النتائج التي:

- لم تغير عدد خلايا الدم البيضاء ودلالات التحلل معنوي تحت تأثير تغير العوامل الفسيولوجية (تحت الدراسة).
- أدى الصوم وقلة الضوء فعالي معنوي على قيم "كرات الدم الحمراء، الهيموجلوبين و حجم الخلايا المرصوصة" بينما ارتفعت هذه القيم معنوي مع مدة التهوية وارتفاع حركة الوسط الخارجي. أما مستوى الجلوكوز وهرمون الكورتيزول فقد أظهرت ارتفاعًا معنوي مع تأثير الصيانة.
- أُجريت تجارب تأثير تغيرات الكورتيزول تحت تأثير تغير العوامل الفسيولوجية (تحت الدراسة) عدا عامل قلة الضرر فقد أنخفضت معنوي.
- أستصحب الباحث (مع التحذير لمربى مزارع الأسماك) ان قلة التهوية والصوم لهما الأثر الأكبر لتغييرات قياسات الدم في سمك القراميط بالمقارنة بتأثيرات قلة الضوء وارتفاع حرارة الوسط الخارجي.