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Photoperiod cycling and haematological evaluation of rainbow trout (*Oncorhynchus mykiss*)

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Abstract: The present research work aimed at the effect of five photoperiod regimes on the health status of rainbow trout (*Oncorhynchus mykiss*) assessed through hematological components. The results revealed no significant ($P<0.05$) changes in haemoglobin and RBC count as compared to normal in all the treatment groups. However significant ($P<0.05$) changes were recorded in immune component of fishes subjected to light duration ranging from 24h to 12 hours. Differential blood count, neutrophil and monocyte count was reduced in LD24 to LD12, as compared to control. However, thrombocytes showed higher titres in stress condition of fishes, subjected to longer light durations.
Keywords: *Oncorhynchus mykiss*, haemoglobin, RBC, LD24, LD12, thrombocytes.

INTRODUCTION

The changes in physiological behavior of fishes can be well understood by the alteration in haemo-biochemical profile, which is the best indicator of health [1-4]. Haematology and blood biochemistry are useful for assessing the physiological status of fish [5]. Blood cell indices (RBC, WBC and DLC counts) are good indicators of systemic response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood. Lymphocytes numbers are known to show variability according to the physiological condition of the fish [6]. Decreased lymphocyte numbers were observed under stressed conditions – hypoxia, cortisol induced or during handling and transport [7, 8].Valenzuela *et al.* [9] reported a decrease in lymphocytes count in trout exposed to constant illumination. Neutrophils on contrary show an increase under stressful condition. It is now agreed that in all the five vertebrate taxa including fish, natural stressors or exogenous administration of stressors in the environment elicit a stress response in their leukocyte profile [10].

Most of the fishes exhibit a daily light/darkrhythm tuned to the natural photoperiod with melatonin playing the key light perception hormone [11-13]. The fact that day-length has an important role is welldocumented. In a number of studies on juvenilefishes, exposure to increased day-length have produced such responses as stimulated growth, betterfeed conversion efficiency and early maturation [14-18] which hold lot of potential in fisherypractices. Intensive fish culture practices widelyutilize photoperiodic manipulation for obtainingdesired growth rates, spawning and early maturationof larval fish. In adult fishes, influence ofphotoperiod has been observed as changes in lightdependent circadian rhythimicity, melatoninproduction and its secretory patterns [19-21], anatomical andbiochemical changes in the pineal organ - the chieftransducer of environmental light in fish throughmelatonin [22-24].

Artificial photoperiod regimesare alterations in the natural light:dark cycles and anyalteration or manipulation of environmentalparameters such as temperature or light results insudden changes in the environment which may causestress thus altering the generalwell-being of the fish [1,1,2]. Recently some studies havefocused on stress related changes in fishes exposed toartificial photoperiod. Physiological changes in bloodcell indices, levels of lactate, glucose, plasmaproteins, cortisol, FFA have been observed in fishexposed to altered photoperiod conditions [25, 26, 9]. However, the results are varied and species– specific in most cases and observed after thephotoperiodic exposures that usually ranged from 30days to 3 months.

The rainbow trout (*Oncorhynchus mykiss*) is the best test animal to assess the alterations in blood profile on exposure to varied photoperiods. The present experiment was conducted with an aim to standardize a particular photoperiod to assess the impact on growth

parameters, thereby an investigations in hematological indices was mandatory to grab an idea about the kind of stress, a particular photoperiod regime poses on the test fishes.

MATERIAL & METHODS

Fingerlings of Rainbow trout (*Oncorhynchus mykiss*) used were obtained from homogenous source at the Department of Fisheries (Bandipora, J&K) farm and acclimatized for two weeks. They were fed twice daily during the period of acclimatization with pelleted feed @2% body weight. The fishes were maintained in fresh flowing water system (Temp: 12±1ºC), with an average flow rate of 25 L min^{-1}.

25 Rainbow troutfingerlings (average weight 10±0.45 g; average length 12±0.86 cm (n = 50)) were selected at random and placed in the six rearing tanks connected to the fresh stream water. The six tanks were assigned to five photoperiods namely twenty-four hours of darkness (DD24: LD00), eighteen hours of darkness: six hours of light (DD18: LD06), twelve hours of darkness twelve hours of light (DD12:LD12), six hours of darkness and 18 hours of light (DD06:LD18) and 24 hours of light (DD00:LD24). The light phase was achieved with the aid of an energy bulb (60W) emitting 150 lux intensity of light measured at the surface of water.

The fishes during the course of the experiment were fed 2% of their body weight with pelleted trout feed (10% Moisture, 12% Ash, 45% Crude protein, 9% Ether extract, 1.5% Crude fiber) for 90 days. The fishes were weighed fortnightly and sampling was done on monthly basis.

Blood collection and Analysis

At the end of the experiment, blood was collected from anaesthetized fish by cutting the caudal peduncle. Blood of two to three fish were pooled to obtain enough samples for hematological analysis. The collected blood was placed in coded 1.5mL heparinized plastic tubes, stored on ice according to the procedures established by Campbell and Murru [34], standard haematological procedures described by Blaxhall and Daisley [35] were employed in the assessment of the various blood parameters.

Haemoglobin (HB) concentration was estimated as cyanmethemoglobin [29], Packed Cell Volume (PCV) was determined using microhaematocrit method of Snieszko [31]. The Red Blood Cell (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe [30]. Also the total White Blood Cell Counts (WBC) was enumerated with an improved NeubauerHaemocytometer using Shaw's diluting fluid [28]. The Red Blood Cell indices that include Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated using the formula mentioned by Dacie and Lewis [32].Unna-Ziehl staining was used for differentiating small, large lymphocytes and thrombocytes [33], € granulation staining was used for differentiating neutrophils by the standard method of Romeis [33]. ∂-granulation staining was used for differentiating monocytes as per the standard methods of Romeis [33].

Statistical Analysis

The results gotten were subjected to Analysis of variance using a computer software Gen stat discovery edition.

RESULTS

The haematological analysis of the blood sample of the fishes subjected to various photoperiods is presented in Table 1. The enumerated Hemoglobin (g/dL) in control group of fishes was 8.7±0.102. Out of all photoperiod regimes, the lowest haemoglobin(6.7±0.010) was recorded in treatment DD24:00LD, while as the highest (9.0±0.024) haemoglobin was recorded in treatment DD06:18LD, with a mean±SE value of 0.008. The lowest PCV (%) (26±0.105) was recorded in treatment DD24:00LD, and highest (35±0.105) in treatment DD12:12LD, as compared to control (36±0.226), with a mean±SE value of 0.033. The RBC (x 10^6/µL) showed lowest (2.85±0.007) count in treatment DD24:00LD, while as the highest (4.25±0.020) in treatment DD06:18LD, with a mean±SE value of 0.008, as compared to control (4.15±0.101).

The calculated MCV (fL) in control group of fishes was 86.74±0.316. The lowest (80.0±1.52) MCV value was recorded in DD06:18LD, while as the highest (91.80±2.3) value was recorded in treatment DD18:06LD, with a mean±SE value of 0.612. Similarly the lowest MCH (pg) (21.17±0.12) was recorded in treatment DD06:18LD, and highest (24.91±0.24) in treatment DD18:06LD, with a mean±SE value of 0.07, as compared to control (20.96±0.26). The calculated MCHC (g/dL) in control group of fishes was 24.16±0.03. The lowest MCHC (24.4±0.08) was recorded in treatment DD00:24LD, while as the highest (27.14±0.17) was recorded in treatment DD18:06LD, with a mean±SE value of 0.036.

The WBC (x10^3/µL) showed the lowest count (22.5±0.098) in treatment DD06:18LD, and highest (71.5±0.127) in treatment DD24:00LD, with a mean±SE value of 0.026, as compared to control (32.6±0.029). The small lymphocyte count (x10^3/µL) was 25.3±0.021 in control group, while as the lowest (18.3±0.024) was recorded in treatment DD06:18LD, and highest (55.9±0.024) in treatment DD24:00LD, with a mean±SE value of 0.016. However, large

lymphocyte count ($x10^3/\mu L$) showed the lowest (1.5±0.010) value in treatment DD06:18LD, while as the highest (7.8±0.018) in treatment DD24:00LD, with a mean±SE value of 0.005, as compared to control (1.5±0.020).

The lowest neutrophil count ($x\ 10^3/\mu L$) was recorded in treatment DD06:18LD (0.7±0.010), while as the highest (2.0±0.010) was recorded in treatment DD24:00LD, with a mean±SE value of 0.003, as compared to control (1.9±0.014). The monocyte count ($x10^3/\mu L$) was lowest (1.0±0.20) in treatment DD06:18LD and highest (2.5±0.010) in treatment DD18:06LD, with a mean±SE value of 0.013, as compared to control (1.65±0.021). Similarly, eosinophil count ($x10^3/\mu L$) in control group of fishes was 0.5±0.020, as compared to DD06:18LD, which recorded the lowest (0.59±0.020) eosinophil count and highest (0.8±0.001) in treatment DD24:00LD, with a mean±SE value of 0.002.

Table-1: Mean haematological parameters of *Oncorhynchus mykiss* subjected to various photoperiods for 90 days

Treatment	Control	DD24:00LD	DD18:06LD	DD12:12LD	DD06:18LD	DD00:24LD	±SEM
Parameters							
Haemoglobin (g/dL)	8.7±0.102	6.7±0.010	7.6±0.010	8.7±0.010	9.0±0.024	7.8±0.010	0.008
PCV (%)	36±0.226	26±0.105	28±0.105	35±0.105	34±0.028	32±0.105	0.033
RBC (x 10^6/µL)	4.15±0.101	2.85±0.007	3.05±0.007	4.05±0.007	4.25±0.020	3.58±0.017	0.008
MCV (fL)	86.74±0.316	91.22±2.10	91.80±2.3	86.41±3.12	80±1.52	89.38±2.9	0.612
MCH (pg)	20.96±0.26	23.5±0.24	24.91±0.24	21.48±0.32	21.17±0.12	21.78±0.22	0.07
MCHC (g/dL)	24.16±0.03	25.7±0.18	27.14±0.17	24.8±0.18	26.47±0.08	24.4±0.08	0.036
WBC (X 10^3/µL)	32.6±0.029	22.5±0.098	24.5±0.027	31.5±0.127	45.5±0.127	26.2±0.521	0.026
Small lymphocytes (X 10^3/µL)	25.3±0.021	39.9±0.024	46.3±0.084	60.3±0.084	83.3±0.024	51.3±0.084	0.016
Large lymphocytes (X 10^3/µL)	1.5±0.020	1.0±0.018	1.2±0.018	1.8±0.018	1.5±0.010	1.4±0.018	0.005
Neutrophils (X 10^3/µL)	1.9±0.014	1.1±0.010	1.3±0.010	1.7±0.010	2.0±0.010	1.8±0.010	0.003
Monocytes (X 10^3/µL)	1.65±0.021	0.94±0.010	1.1±0.010	1.1±0.020	1.7±0.20	1.2±0.010	0.013
Eosinophils (X 10^3/µL)	0.5±0.020	0.1±0.001	0.3±0.001	0.35±0.001	0.59±0.020	0.4±0.001	0.002
Thrombocyte like cells (X 10^3/µL)	1.8±0.021	4.5±0.014	4.4±0.014	2.4±0.014	1.7±0.020	3.4±0.014	0.004
Thrombocytes (X 10^3/µL)	34.9±0.025	48.3±0.014	45.9±0.014	42.9±0.014	40.8±0.152	45.9±0.014	0.011

Note: Values are means±SD of three replications (d.f. 5, 17). Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with same superscript are not significantly different ($P > 0.05$).* No statistical analysis was possible as determinations were performed on pooled samples

DISCUSSION

The present study revealed that rainbow trout showed improved haemoglobin content in DD06:LD12 group, which was 9.0±0.024, as compared to control (8.7±0.102). The least Hb was observed in LD24:DD00 group (6.7±0.010), which reveals the level of stress due to excessive stress. Similar pattern was recorded in RBC's, which showed the highest value (4.25±0.020) in DD06:LD18, as compared to control (4.15±0.101). Haematological parameters are often used as health status and stress indicators in fish. However, information on the effects of artificial photoperiods on these parameters is scarce and ambiguous. An important contribution to the effect of photoperiod on rainbow trout is that of Silva&Klempau [27]. The authors reported that at the end of phase 1, photoperiods LD 14:10 and 24:0 induced higher production of immature (late basophilic) erythrocytes ($0.06–0.08 \times 10^{12}$ cells/l, $P < 0.05$) and elevated EPI (young erythrocytes = 1.6 in LD 14:10, 2.25 in LD 24:0; late basophilic erythrocytes = 4.1–4.9 in LD 14:10, 3.3 in LD 24:0) than in controls (late basophilic erythrocytes = $0.02–0.04 \times 10^{12}$ cells/l; EPI young erythrocytes = 0.2–1.0; EPI late basophilic erythrocytes = 1.4–2.7). During phase 2, only reduced numbers of lymphocytes and thrombocytes were observed at day 150 in the LD 14:00 groups. An increase in the EPI (1.9) of control young erythrocytes was found at day 150. This stands true for our experiment also, which showed significant ($P<0.05$) changes in erythrocytes on exposure of trout to more light hours.

In yet another effort by Valenzuela et *al*. [9] on the effects of different artificial photoperiods and temperatures on haematological parameters of rainbow trout (*Oncorhynchus mykiss*), it was reported that the LD 24:0 photoperiod (independently of temperature) increased the haematocrit and the number of erythrocytes at days 7, 14, and 30 ($P < 0.01$). The LD 24:0 photoperiod (also independently of temperature) lowered the number of lymphocytes only after 14 days of experimentation ($P < 0.01$). These results resemble the effects of stress caused by the application of continuous light photoperiods, indicating that survival risks may develop in trout farming.Our observations are documented by the work of Srivastava and Sanjeev [36], who reported that the total RBC and WBC counts were unaffected by the artificial photoperiod regimes. However, lymphopenia ($p<0.05$) and neutrophilia ($p<0.05$) were observed under 24L:0D photoperiod. However during the present research work, lymphocyte count (small & large lymphocytes) showed reduced titres in other treatment groups except DD06:LD18, where lymphocyte titres were equal to or more than control. Neutrophil count was also reduced in other treatment groups, except LD06:DD18 (2.0±0.010), which showed higher value than control group (1.9±0.014).Haematological analyses has been routinely used in determining the physiological state of animals and known to be affected by different environmental factors, Solomon and Okomoda [37] therefore designed to assess the effect of 24 hours of light (00D: 24L), 24 hours of darkness (24D: 00L) and 12 hour light / 12 hours darkness (12D: 12L) photoperiod on the haematological parameters of the African Catfish. At the end of the six weeks experiment, it was observed that some haematological parameters such as Mean Corpuscular Haemoglobin Concentration (MCHC), the Mean White Blood Cells (WBC), Mean Red Blood Cells (RBC), Haemoglobin content (HGB), Platelet count (PLT) showed significant difference ($P<0.05$), which stands true for our experiment as well.

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