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# BIOCHEMICAL METHODOLOGY FOR EFFECT OF PHOTOPERIOD ON BLOOD GLUCOSE LEVEL OF AN AIR BREATHING TELEOST, HETROPNEUSTES FOSSILS

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## ABSTRACT

**This paper show biochemical methodology for effect of photoperiod on blood glucose level of an air breathing teleost, *Hetropneustes fossils.* The present study reflects very surprising results in blood glucose level. There has been a significant incrrrease in plasma glucose level 7 days exposure whereas, decline in the blood glucose in 14 days exposure of light. The significant increase in blood glucose level in 7 days exposure points towards hypermetabolic stress of the fish in response of light illumination.**

**KEYWORDS:** Photoperiod, Blood Glucose Level, *Hetropneustes fossils*

The environmental factors play an important role in the life sustenance and harmony. There are number of environmental factors which impart from little to wide range of alteration on the living existence. In fact, the light is one of the essential requirements of living bodies to carry out their various vital activities like metabolism and nervous coordination as well as genital endocrine regulation. The photo periodic effect on living organisms in nature has been matter of concern since human developed sense of climatic effect. The photo periodic effect has been one of the important aspects of chronobiology that deals the matter of biological cycle or rhythms within the organism. Human being have always been trying to know the happenings in the body physiology and biochemistry by simple slight alteration in light intensity and duration.

The realization of light importance was noticed as early as the invention of electric light as Shakespeare once exclaimed that sleep meaning by darkness as “balm of hurt mind” .He meant the phrase to explain the importance of light duration and intensity.

Light is an important environmental factor having for reaching effect on living world directly or indirectly. Daly response of animal world to light condition is known as circadian rhythms, light effects locomotion, maturity growth pigmentation etc.

Human by nature curious has always been trying to unfold the mysteries of photoperiodic effect on different living organisms as well as on own selves. The artificial light has been designed variously to see the effect on various biochemical an physiological aspects of living organisms, so much so that human has been trying to know the effect of photoperiod on obesity, diabetes, hypertension and depression. In day to day affairs we always notice photoperiodic seasonal change like hair length, shedding of skin and others in nature.

The photoperiod effect on different animals have been carried out by some of the scientists namely Briz P.J. and Pienaar A.G., (1992), Carillo M., Bromage N., Zanuy S., Serrano R., Prat F. (1989), Adams C.F. and Torpe J.E. (1989). They suggested a role of light intensity on the growth ad survival of different animals.

Some scientist have suggested the positive effect of darkness on fish growth ( Piaia et al.,1999 and Aimense et al,.2000).has suggested that nocturnal and diurnal feeding habit impart effect on fish growth. The specific adaptation to light intensity influence behavior pattern and so feeding instinct and finally growth.

Studies on the effects on photoperiods on brown trouts were done by Brow (1946). Philips et al (1958) found that trout exposed to continuous light over a 20 week period grew at reduced rate. Pyle (1969) found that brook trout grow under continuous light increased their length at a constant rate. Quasim (1955) believed that the fish grew faster in darkness.

## MATERIALS AND METHODS

### Blood Biochemistry

At the end of photoperiod the fish were anaesthetized with 1: 4000 MS 222 ( tricane methane sulphonate, Sandoz) and were then processed for a quantitative estimation of plasma glucose, plasma protein, plasma cholesterol and plasma electrolytes.

### Sampling of Blood

Blood samples were collected in hepernized vials with the help of one mo disposable syring equipped with 22 gauge hypodermic needle by puncturing the ventral aorta. Blood taken from individual fish was centrifuged at

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3500 RPM for 10 minutes and plasma was collected indifferent vials and stored at 20* C until analyzed.

**Determination of Plasma Glucose**

Plasma glucose was determined by using O-toluidine reagent The Glucose was taken as standard and calculation was done as described. Cassone VM, Warren WS, Brooks DS, Lu J (1993)

Reagent:

(I) 0.1% aqueous benzoic acid solution.
(II) O- toluidine reagent.

1.5 gm thiourea was dissolved in 960 ml of glacial acetic acid 60ml O-toluidine was added to it and thoroughly mixed and stored in brown bottle. O-toluidine reagent so prepared was used after 24 hrs. of preparation.

Heteropneustes fossilis is an important live the plan is to observe following parameters under different photoperiods under laboratory conditions.

A. Blood Morphology, RBCs total count, WBCs differential count
B. Hemoglobin percent
C. Blood Ph,Blood Volume & Blood specific gravity
D. Blood biochemistry,
a. Plasma glucose
b. Plasma protein
c. Plasma cholesterol
E. Blood electrolytes – Na+ & K+
F. Effect on Pineal gland will be observed.

## OBSERVATION

**Plasma Glucose**

Both 7 and 14 days exposed fish exhibit interesting results in plasma glucose level. These are being mentioned below under separate subheadings.

**7 Days Exposure Period**

It shows hyperglycemic condition. The plasma glucose level elevates to 67.4 of blood against the control value of 60.88 of blood. This level shows an increase by 11%.

**14 Days Exposure Period**

Contrary to the observation of 7 days exposure period the fish of this period showed hypoglycalmic condition indicating exhaustion of glucose to meet high energy demand under light stress.

**Plasma Protein**

Similar to the observation of serum cholesterol decrease in protein content has been recorded in both 7 days and 14 days exposed group. The only difference is in the extent of protein depletion as mentioned below in respective sub heading.

**7 Days Exposure Period**

The plasma protein level in control fish has been estimated as 8.18 of blood whereas exposed fish shows 6.24 of blood as the level of plasma protein which is equivalent to a loss 23.78% and has been estimated to be significant at $P<0.01$.(table)

**14 Days Exposure Period**

The plasma protein level of light exposed fish depletes to 4.49+_0.21 (gm/100 ml) a compared to control value of 7.03+_0.37 (gm/100ml) of blood. This has been found to be significant at $P<0.001$ and amount to loss of 36.02%.

**Pineal Protein**

**7 days exposure period**

The pineal protein of control fish was measured 61.85+/_ 2.01 ug/100gm where as exposed fish show value 54.26+/_ 1.27ug /100gm which is significant at $P<0.01$ . (table).

**Plasma Cholesterol**

The results are shown in table. The plasma cholesterol In control fish has been estimated to be 162.50 +/_ 0.90 mg/100ml of blood as against this value significant loss 144.34 +/_ 1.57 equivalent to 11.16% has been recorded in exposed fish. (table).

**Plasma Electrolytes**

In present study plasma sodium (control 132.50 +/_ 1.71 MEq/L; exposed 127.50+/_ 0.80 MEq/L) showed 3.78% decrease in 7 days exposure. And in 14 days slight increase was observed (control 127.90 +/_ 0.72 MEq/L ; Exposed - 131.50 +/_ 0.55) Significant at $P<0.001$. The level of potassium showed 2.02% increase.

## RESULTS AND DISCUSSION

The hematological and biological profile due to artificial photoperiodic illumination of light for 7 days and 14 days exposure periods have presented very interesting results. The present study has attracted to think the alterations in the light of pineal gland modification

melatonin alteration along with alterationin biochemical and endocrinological capability of fish.

The haematological parameters in present study has presented very surprising results as there is a decline in RBC count for both the exposure periods, which can be related to the altered respiratory activities. The present investigation can also be related to the melatonin impacts on erythropoiesis in haemopoietic organ.

The haemoglobin contents study reflects decline results for both the periods. The severe anaemoic condition has been marked to explains impairment in haemopoiesis. The anaerobic condition in present investigation seems to have created haemolytic crisis. The decline haemoglobin show fall in erythropoietin and decline of RBC number.

The WBC total count in present investigation reflects altered physiological crisis accounting for increase in total count of WBC. It seems that the immune system response and adrenal emergency hormone release are real factors emergency hormone release are real factors for present increase.

The differential count in present study have shown surprising result as lymphocytes, neutrophils, basophile and eosinophils have elevating trend whereas there has been decline in monocytes.

The present study reflects very surprising results in blood glucose level. There has been a significant incrrrease in plasma glucose level 7 days exposure whereas, decline in the blood glucose in 14 days exposure of light. The significant increase in blood glucose level in 7 days exposure points towards hypermetabolic stress of the fish in response of light illumination. In fact there is a glucose hunger in fish to meet excessive demand of energy in initial illumination stage. Alison L. N. (1951): Delay of spawning of eastern brook trout by means of artificial prolonged light intervals.

In present investigation Plasma Protein of Heteropeustes fossilis under 7 days and 14 days light illumination exposure reflects a progressive and significant decline in the level of plasma protein ($P<0.01$ in 7 days exposed fish and $P<0.001$ for 14 days exposed fish). The level of protein in controlled fish further indicates that the protein are larger contributor to the weight of the tissues after water.

In present investigation the impared carbohydrate metabolisim may have stimulated gluconeogenesis process by utilizing protein for meeting energy demands. Bjornsson B. Th., Halldorsson, O (1998): Photoperiod control of sexual maturation on the Atlantic halibut (Hippoglossus): Plasma thyroid hormone and calcium levels Aquaculture.

The continuous illumination of light for 7 days and 14 days in present study showed decreased level of protein in pineal tissue. In fact the pineal gland is the only gland in organism which activity is directly dependent upon the photic information reaching it via eyes (Reiter 1974) the continuous light causes distributed pinealcytes activity (Srivastava 2003). Further investigations are required in the line to illucidate the role of pineal in metabolic status of fish.

In the present study exhibited the reduction in plasma cholesterol under the artificical illuminating exposure. In present investigation the plasma cholesterol level has been observed in decline state as there is a loss of 11.16% and 13.26% In 7 days and 14 days exposure respectively.

In present study decline in the plasma sodium in 7 day exposure 3.78% whereas there is a increase in the plasma sodium on 14 days exposure by 2.35%. both are significant at <0.05 and 0.001 respectively. Davies B, Bromage N and Swanson P (1999): The brain pituitary gonadal axis of female rainbow trout oncorthynchus mykiss: effects of photoperiod manipulation.

**Table: Profile of Plasma Glucose, Plasma Protein, Plasma Cholesrol and Pineal Protein in Normal and Light Illuminated Exposed Fish Hetrophneustes Fossilis. (Value are +/- SE of Five Fish in Each Group)**

| Parameter | 7 Days | | Student 't' Test P value | % (+) or (-) | 14 Days | | Student "t" Test P value | % (+) or (-) |
|---|---|---|---|---|---|---|---|---|
| | Control | Exposure | | | Control | Exposure | | |
| Plasma Glucose mg/100 ml | 60.88 | 67.4 | <0.01 | +11% | 62.69 | 37.78 | <0.001 | -39.72% |
| Plasma Protein g/100 ml | 8.18 | 6.24 | <0.01 | -23.78% | 7.03 | 4.49 | <0.001 | -36.02% |
| Plasma Cholestrol mg/100 ml | 162.50 | 144.34 | <0.01 | -11.16% | 160.50 | 139.20 | <0.001 | -13.26% |
| Pineal Protein *u* g/100 gm | 61.85 | 54.26 | <0.01 | -12.27% | 62.70 | 55.26 | <0.001 | -11.87% |

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