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Research Article

EFFECTS OF CHANGING PHOTOPERIODS IN PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES IN SUBTROPICAL BIRD BLACK HEADED MUNIA (*LONCHURA MALACCA MALA*CCA)

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Abstract

Aim: To find the Effects of Changing Photoperiods in Physiological and Biochemical Responses in Subtropical Bird Black headed Munia.

Method: This experiment was performed on the blackheaded munia during June month. A groups of photosensitive birds (n = 6-8 per group) was exposed to short day length (9L: 15D). After every 2 weeks, this group transferred to increasing photoperiod sequentially; 10L: 14D, 11L: 13D, 12L: 12D, 13L: 11D, 14L: 10D, 15L: 9D and 16L: 8D. The group received 460 lux light intensity during the day time at perch level. Observations on Physiological (body mass, body moults and moults primaries) and biochemical (Stress factor) parameter differences in blood at the appropriate samplings during the experiment.

Results: Male and female birds responded almost in similar fashion with some variations. Overall, our results show a gradual change over Photoperiod in responsiveness of the endogenous response system to stimulatory effects of long day length. They suggest roles of both long and short day lengths in regulation of seasonal cycles in subtropical bird Blackheaded munia.

Key words: Photosensitive; Photoperiod; Stress Factor; Endogenous Response; Subtropical and Seasonal cycles

1. Introduction

Day length regulates the seasonal responses in many vertebrates, including bird species. The seasonal change in day length provides the most reliable source of temporal information about the environment and has been adopted by birds in the course of evolution as the main proximate environmental cues for synchronizing reproduction, moult and migration with favourable environmental conditions. Some other ultimate factors serve as supplementary cues for fine-tuning the rate of gonadal growth and the timing of breeding with local phonological conditions [1,2,3,4]. In birds, the change in day length is the most important environmental cue used for synchronizing breeding, moult and migration with recurrent seasonal fluctuation in environmental conditions [5].

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According to Rowan (1925) [6] studies on slate-colored Juncos (*Junco hyemalis*), the role of day length (or photoperiod) as a major source of temporal information regulating seasonal responses has been demonstrated in many bird species [7,8,9,10,11,12]. Relatively less is known of photoperiodism in tropical birds [13,14]. Light is a major source of temporal information for circadian and seasonal responses in many species of birds. The regulation of annual periodicity in physiological and behavioral processes in wild birds depends upon the appropriate relationship with phase, among several endogenous components, including secretion of several hormones. Many, if not all, endogenous components need entrainment with the environmental variable(s), which is (are) precise in occurrence, to keep them in proper pace with seasons of the year.

Earlier findings suggest that the role of long day length in stimulation of fat deposition and/or gonadal growth in several species of temperate [15,16,7,17,8,18,19,11] and tropical bird species [20,21,22,23,24,25,26,27,28,10,29,30,31,32,33,34,35]. Relatively less attention is paid on photoperiod-induced effects on fat deposition [22,23,36,37,10].

In the photoperiodic literature, it appears that there is a threshold photoperiod for the gonadal response; testicular growth, within a range, occurs at a rate proportional to day length [38,39]. Fat deposition preceding breeding period (called vernal fattening), in contrast, appears to be example of an 'on-off' process, and hence the rate of photoinduced fattening is independent of day length [40]. Also, several studies have suggested that the photoperiodic response system has a minimum light intensity threshold [41]. In the earlier photoperiodic literature, different types of LD cycle paradigm (light and dark periods in different combinations in 24 h and non 24 h periods) have been used frequently to investigate how light information is used by the organisms in regulating their seasonal responses.

The testes are very small and usually avascular structure. It's shape, oblong or cylindrical, smooth on the surface and creamy-white in color, although they may be partially or totally pigmented. In a mature bird, the testes can vary in size and greatly enlarge during the breeding season. Basically testes perform two important functions, first to produce sperm and the secondary secrete the male hormone, testosterone which is responsible for various secondary sexual characteristics such as male sexual behavior (including song), color and feather formation (if different from the female) and also the development of a comb and wattles in some species. Testosterone is produced by cells, known as interstitial cells of Leydig. These cells are located in the spaces between seminiferous tubules.

The electrophoresis patterns of avian serum proteins

More than 50 years, it has been known that particular proteins characterize every species of plants and animals and their phylogenetic relationships are reflected in protein structure. The first application of this fact to taxonomic studies was by [42] who used the precipitin reaction of immune sera to test degrees of relationship in over 500 species of animals. With refinements in technique have come many more serological studies and the results have justified the statement by [43] that 'chemical differences parallel the variation in structure' and hence are useful in classification.

The development of other methods for protein characterization has suggested that these too might be applied for systematics. Soon after [44] described his apparatus for the electrophoretic separation of colloidal mixtures [45] used it to compare the egg albumins and hemoglobins of five species of birds. Within the next few years there followed the studies by [46,47,48]. These authors investigated the plasma proteins of several species of reptiles, amphibians, fish, birds, mammals, and some invertebrates. They showed that electrophoresis could detect the species specific qualities of proteins and that similarity in proteins paralleled evolutionary relationships. With the development of filter paper electrophoresis the procedure has been simplified and the study of [49] on the plasma proteins of more than 100 species and subspecies of reptiles and amphibians has been the most extensive to date. Others who have used paper electrophoresis include [50] who found specific characters in the serum proteins of turtles of the genus Pseudemys, [51] who published the serum protein patterns of several species of sharks. [52] used starch gel electrophoresis in a study of the sera of 19 species of invertebrates. The egg white proteins of birds have also been shown to be species specific and to produce excellent electrophoretic profiles. The papers by [53] and [54] are the principal ones to date. The latter reported on 37 species of birds and concluded that the method was applicable to taxonomic problems. Sibley (1960) [55] has used paper electrophoresis in a study of the albumin proteins of egg of more than 300 species and has found the conclusions of [54] fully justified.

Methods

Adult male and female munia were captured from wintering and kept in an outdoor aviary (3.6 m × 3.6 m × 2.5 m) for 2 weeks, aviary receiving natural light-dark cycle. Study of annual seasonal cycles was conducted on these birds. A experiment was performed on the blackheaded munia (both male and female) during 20 June. Groups of photosensitive birds (n = 6-8 per group) were exposed to short day length 9L: 15D and then transferred to increasing photoperiod sequentially for each month to 10L: 14D, 11L: 13D, 12L: 12D, 13L: 11D, 14L: 10D, 15L: 9D and 16L: 8D photoperiod (figure 1). All experimental birds were received 460 lux light intensity during the daytime at perch level. Observations on body temperature, Cloacal protuberance, body mass, body moults and moult primerase was taken at the beginning and at appropriate intervals during the experiment. The blood sampling was performed in time to time during the experimental period; in responsive changing photoperiodic group and control group beginning, mid and at the end.

Collection of blood samples and Bimolecular (protein) SDS-PAGE analysis- The blood was sampled from groups of all birds (male and female) on the start of experiment, pre–reproductive phase(P1&P2), reproductive phase(M1&M2) and post reproductive phase(F1&F2). From left wing over the puncture site by using cotton dipped with distilled water. Swabbing the site with a small amount of 70% alcohol may help visualize the vein. Insert the needle slightly anterior to the vein and in the direction of the tip of the wing (i.e. against the flow of blood). Re-direct the needle to puncture the vein. Thus brachial vein was pricked with a needle and about 5-10 µl of blood was collected in micro centrifuged tubes, with the help of heparinized capillary tube.The blood samples placed for centrifugation for 10 minutes at 2,000-3,000 rpm using a refrigerated (40c) centrifuge. And for deplete platelets in the plasma sample centrifugation for 15

minutes at 2,000 rpm. Than SDS-PAGE was performed according to Laemmli [56].

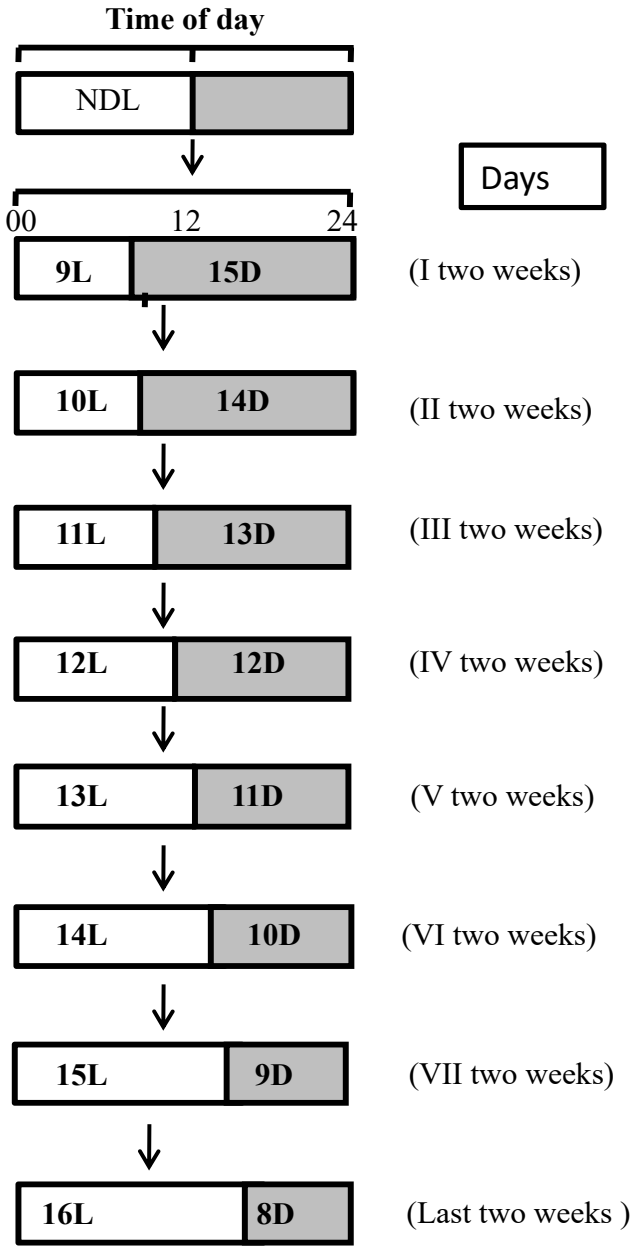

Fig. 1: Schematic representation of experimental protocol. Each bar covers 24 h period. The SD-LD (9L:15D-16L:8D) illumination has changed on eight photoperiodic phases sequentially (Day 1-128). During light phase all experimental birds were exposed 460 lux light intensity at and dark time received dim light illumination (0.3 lux). The blood sampling was performed in time to time during the experimental period; in beginning pre-reproductive period (male P1 & female P2), mid reproductive phase (male M1 & female M2) and at the end of experiment post reproductive phase (male F1 & female F2) of experiment in responsive changing photoperiodic group and control group

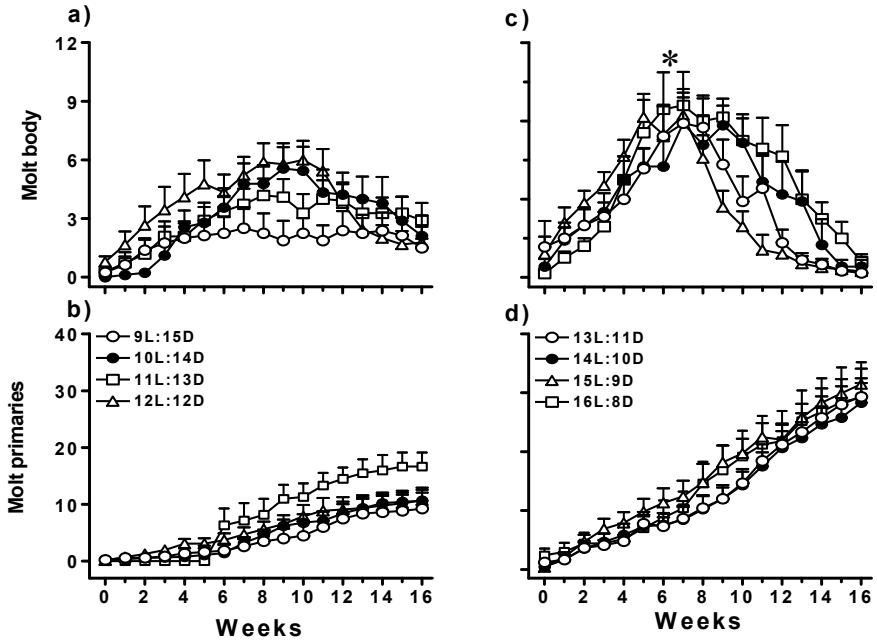

Fig.2: Mean (±SE) molt body (a and c) and molt primaries (b and d) of the male blackheaded munia exposed to 9L:15D, 10L:14D, 11L:13D, 12L:12D, 13L:11D, 14L:10D, 15L:9D and 16L:8D at 460 lux intensity. Asterisks on the symbol indicate significance of difference compared to initial value (*= 16L :8D; 1-way RM ANOVA).

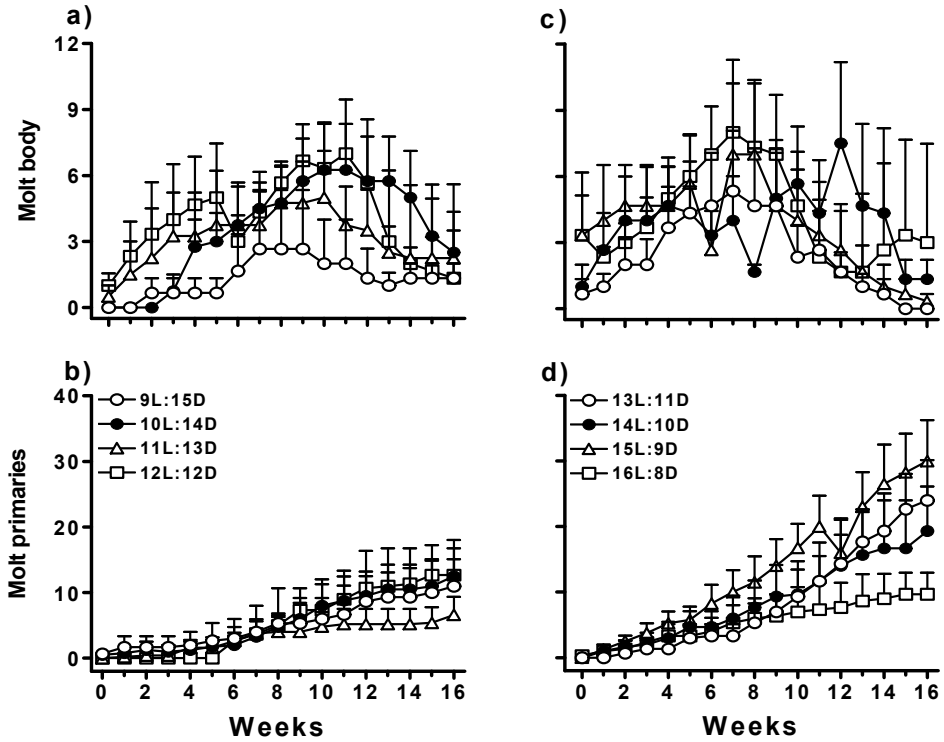

Figure 3. Mean (±SE) molt body (a and c) and molt primaries (b and d) of the female blackheaded munia exposed to 9L:15D, 10L:14D, 11L:13D and 12L:12D, 13L:11D, 14L:10D, 15L:9D and 16L:18D at 460 lux light intensity. Asterisks on the symbol indicate significance of difference compared to initial value (*= 16L:8D; 1-way RM ANOVA).

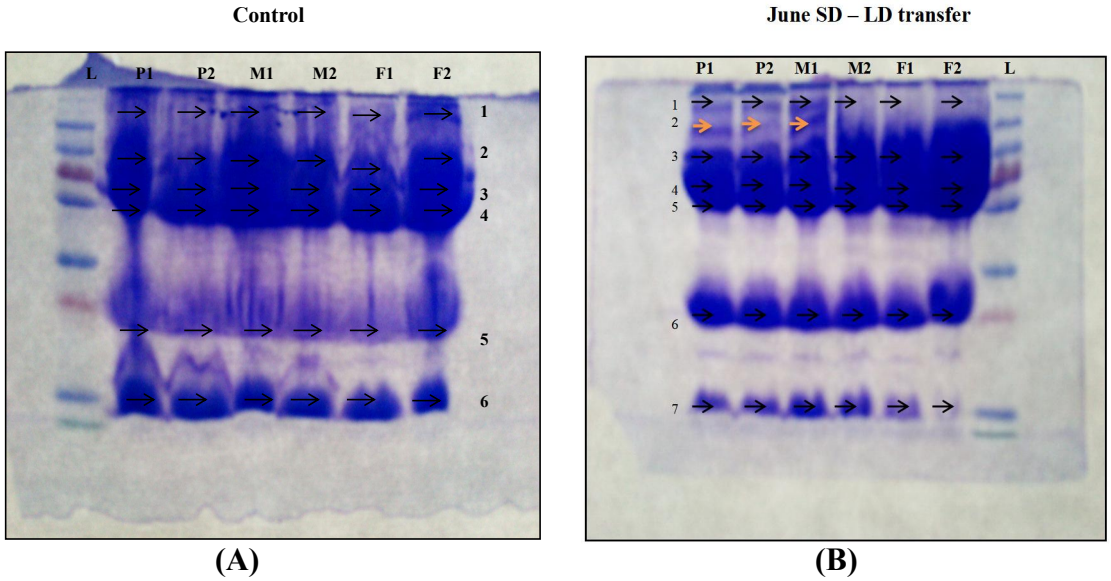

Fig.4: Polyacrylamide gel electrophoresis patterns of plasma proteins of black headed male and female munia kept under control and SD to LD transfer in June, light intensities 460 lux. The illuminations were changed sequentially, 1 hr after every two weeks in both groups. In both groups L-Marker (Ladder), P1 and P2 column are initial, M1 and M2 are mid , F1 and F2 column are final male and female plasma proteins bands patterns.

Results:

Results are shown in figure 2 the molt cycle of both (body feathers and wing primaries) in male blackheaded munia followed testicular cycle. Complete molt cycles of the feathers occurred in both SD to LD transferred male group (body feathers, $F_{40,160}$=27.21, P<0.0001; wing primaries, $F_{40,160}$=32.76, P<0.0001: 1-way RM ANOVA (figure 2 a, c, b and d). In SD to LD transferred female groups (body feathers, $F_{40,160}$=35.70, P<0.0001; wing primaries, $F_{40,160}$=69.88, P<0.0001, 1-way RM ANOVA) (figure 11a,c,b, and d).

Besides body molt and molt primaries, the blood plasma proteins are an obvious choice for investigation of different photoperiodic response because it was easy to collect and because a great deal is known about their properties and functions. Plasma is the fluid portion of blood in which the blood cells are suspended. It is a complex mixture of proteins, carbohydrates, lipids, steroids, and free ions whose composition varies with sex, age, starvation and seasons, etc. [57,58,59,49,60] to test different photoperiodic stress factor, blood was collected of March group in both (LD to SD and SD to LD transfer group) and a September (LD-SD transfer) group. Birds of March group showed pre-breeding phase. No any stress protein was observed, in LD-SD transfer group. The stress factors (130_{kDa} protein bands) were observed in male and female munia by LD-SD photoperiodic induction.

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CONCLUSION:

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