INFLUENCE OF PHOTOPERIOD ON THE BEHAVIOR AND SERUM PROTEINS IN GREY QUAIL (*Coturnix coturnix*)

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ABSTRACT

The present investigation focuses on the effects of photoperiod on general behavior and serum proteins in Quails taking them as a reference for other vertebrates. The present conditions of altered natural circadian rhythm and activity due to anthropogenic activities are creating a general condition of emphasized restlessness in organisms including the Aves. The behavioral changes observed due to induced conditions of acute photoperiod in Quails included refusal for food and water, vigorous activity and deliberate efforts to keep eyes closed. The banding pattern in the gel surfaces obtained after SDS-PAGE revealed significant changes showing definite presence of proteins being formed or inhibited due to stress conditions.

KEY WORDS

Photoperiod, Circadian, SDS-PAGE, Gel Electrophoresis, Banding pattern

INTRODUCTION

Concerns regarding the welfare of animals now permeate all levels of society and are directed towards a diverse range of activities.

Extreme environmental conditions and stresses can have significant negative effects on physiological as well as life history traits of organisms. Analysis of such stressful conditions is a major focus in the development of the understanding of ecological adaptations and bio-geographical distribution of a species.

Animals are frequently exposed to a plethora of stress conditions. Various anthropogenic activities have accentuated the existing stress factors. All the stress factors are a menace for animals and prevent them from reaching to their full genetic potential. Various types of stressors, psychological, physical, and biological, abound in our environment and they occur in the working and social environments, in air, soil, water, food, and medicines etc.

A population may react and adapt to stressful conditions in two ways. Firstly, it may develop a capacity to make phenotypic compensations through acclimatization. Secondly, it may evolve macromolecules those are either more resistant to functional perturbation or better able to retain functional efficiency in the altered environment[1].

Much of stress research is based on the description of the General Adaptation Syndrome[2]. This syndrome identifies the three components of the

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stress response; the alarm reaction, followed by two phases of resistance and exhaustion. The latter two stages (the acute stress response) with the first component, the alarm reaction, are dominated by the HPA (hypothalamic pituitary adrenal) system. It influences the diversion of resources away from non-critical functions, such as reproduction; to those enabling a return to homeostasis for the animal and in extreme situations enables its survival[3].

A stress axis has been identified in animals and is assumed to play an important part in the animal's activities. The stress axis is supposed to be involved with controlling diurnal cycles, increased locomotion, exploratory behavior, increased appetite, and food-seeking behavior[4]. The stress axis also permits short-term adaptations and promotes survival in the face of acute, environmental stressors. Finally, the axis is central to certain long-term evolutionary adaptations to particular ecological and habitat pressures such as those encountered by the species inhabiting the cold regions in the north[5].

An animal's response to stressors occurs through behavioral, autonomic, neuroendocrine or immunological channels of the body[6].

The tolerance of stress by a species can influence its distribution and abundance[7] besides it can control the rate of population growth and also influence the outcome of interactions with other species[8].

Glucocorticoids, an indicative of the vertebrate stress response are released in response to a wide variety of unfavorable stimuli in laboratory animals[9, 10] and also under natural conditions. Daily fluctuations in basal glucocorticoid levels are believed to regulate the overall metabolism of the animal[11, 12]. Studies in several avian species like pigeons, chickens and white-crowned sparrows show daily corticosterone rhythms similar to the typical mammalian rhythm[13, 14, 15].

Short days have also been found to alter the function and cellular makeup of the immune system in hamsters and deer mice. Light intensity affects gonads of the deer mouse. Spleen weights of deer mice (*Peromyscus maniculatus*) [16] and Syrian hamsters (*Mesocricetus auratus*) [17] are elevated after exposure to short day lengths.

Stress is a big factor in determining the overall health of our birds[18, 19]. Poultry are prey and their genetic code predisposes them to the flight instinct, even when they're hand-reared and tame. When a bird is under stress, its whole body is affected. Respiratory infections, allergies, eating disorders, diarrhea, and skin and feather problems are a few of the outward symptoms of stress. Stress depletes nutrients from the body rapidly and the immune system becomes depressed. It also leads to hormonal imbalances of adrenal, pituitary, thyroid, thymus etc. that further interferes with immune function.

MATERIAL AND METHOD

Animal:

The present study deals with an analysis of **photoperiod** as a stress factor on the serum proteins and behavior of a species of Quails- *Coturnix coturnix*. Quails were the first choice for the study as they are rapidly maturing as an avian model for experimental studies.

Quail is a seasonal, migratory bird and its availability was an important factor to be assured. The species under observation i.e. *Coturnix coturnix* (Grey Quail) is available during winters (November-February) in this part of the India.

Experimental Design:

The birds were purchased from the local dealers and were checked for their health and activity before experimentation. The birds bought to the lab were kept in an open aviary under natural conditions for about a fortnight to acclimatize them after which the experiment was set up.

For the experiment simultaneously three cages with three birds each were kept in neat disinfected cages. They were given feed and water *ad. libitum*. They were provided uninterrupted light through out the experimental period (which was seventy two hours). The birds under observation were provided with natural light during the day and by a halogen (Power Lamp, 500 W) during the night. Along with these a set of three birds was kept as a control group through out the experimental period. They were given feed and water *ad. libitum.* Other environmental factors like temperature, humidity etc. was dependent on the season for both the groups.

The bird behavior was monitored day to day. After the completion of the experimental period blood was collected and serum was separated, the samples of the serum protein were marked and refrigerated. Electrophoresis (SDS- PAGE) was performed on the samples. The gel surfaces were run with one sample from control bird and the three samples from each one of the experiment. Following electrophoresis, gel surfaces were photographed and analyzed for the bandwidth of the serum proteins.

OBSERVATIONS

Behavioral Changes Observed during the Experiment in *Coturnix coturnix*

During the initialization of the experiment the birds showed normal behavior. They were active and their food and water uptake was normal. The birds initially sat in the centre of the cage with their activities decreased in the evening.

After 24 hrs of commencement of experiment and first night of light exposure, the light source was switched off early in the morning (at about 7o'clock), the birds were observed to scatter in the cage showing no movement, were very quiet, refused food and water. After about two hours they resumed normal activity and started movements, resuming feeding and drinking. It was also observed that there was the presence of a large amount of feacal matter in the cage as compared to the control group.

With 48 hrs of the experiment and the next night of exposure to continuous light the water intake for birds was insignificant. The birds seemed hyperactive and were observed to be running and jumping along in the cage darting towards the top of the cage in an attempt to escape.

Nearing the exposure to 72 hrs it was observed that the feed and water consumption had decreased significantly. Signs of fatigue in birds were quite evident with limited movements and no activity. They were also trying to keep their eyes closed deliberately.

The birds in the control group were provided normal conditions of temperature, humidity, photo cycle, and were given feed and water *ad.libitum*. They showed maximum activity during the early hours of the day and during late hours of the afternoons and during this time they consumed the maximum feed and water. For the rest of the day they showed normal movements and moved about effortlessly. Play behavior was also observed, crouching over one another during the evening hours and spreading out during the day.

Gel Surfaces Changes Observed during the Experiment in *Coturnix coturnix*

The right side of the surface shows roman numbers which denote the known marker (BSA-SHMT) with a limited range used along with the serum samples. The left side of the surface has numbering according to the major visible bands formed due to the control sample and they were used to compare the experimental samples for the presence or absence of the bands or the changes observed in the band width.

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I EXPERIMENT: (Plate No. 1). The first band as seen in the control group has corresponding appearance in P1, P2 and P3 (the three experimental samples). Same is observed for the second band. The third band marks its presence in P3 but is not found in P1 and P2. The fourth band observed in the control appears correspondingly in P1, P2 and P3 although this band is thicker in the experimental samples when compared to the control. The fifth, sixth, seventh and the eighth band observed in the control sample is similar and corresponding in all the experimental samples. The ninth band observed in the control is not in line with the experimental samples except with the P3 sample, in P2 it is situated a little above than the other two (control and P3) and in P1 it is positioned a little higher than P2.

Thus in the first experiment conducted on *Coturnix coturnix* the control sample showed a band whose absence was marked in the experimental samples, a protein band was also found to be thicker in the experimental samples as when compared to the control sample.

II EXPERIMENT: (Plate No. 2). The first band as seen in the control group has corresponding appearance in P1, P2 and P3 (the three experimental samples). Before the second band in the control sample a band is observed in P1 and P2 although it cannot be traced in the P3. The second band observed in the control sample has uniform presence in all the experimental samples. The third and the fourth band observed in the control are not found in P1, P2 and P3. The fifth band observed in the control is a thick band indicating a high concentration of the related protein but the corresponding bands in the experimental samples are thicker. The sixth band observed in the control is found to have similar incidence in all the samples.

Thus in the second experiment conducted on *Coturnix coturnix* the experimental samples showed a band whose absence was marked in the control sample. The control samples showed two bands whose absence was marked in the experimental sample. A protein band was also found to be thicker in the experimental samples as when compared to the control sample.

III EXPERIMENT: (Plate No. 3). The first band as seen in the control group has corresponding appearance in P1, P2 and P3 (the three experimental samples). The second and the third bands observed in the control have equal correspondence in all the experimental samples. The fourth band found in the control sample is thin and although it is not distinct in P1 but in P3 and P2 it is a bit thicker. The fifth band present in the control has similar presence in P3 but is absent in P1 and P2. The sixth band observed in the control sample is not marked in the experimental samples. The seventh band observed in the control sample shows similar appearance in P1 and P3 although in P2 it is a bit thinner than the rest. The eighth band observed in the control sample is corresponding in position in all the experimental samples except in P1 where it is placed a little lower than the others. The ninth band observed in the control sample also corresponds in all the samples although the band width is a bit thicker in the experimental samples.

Thus in the third experiment conducted on *Coturnix coturnix* the control sample showed two bands whose absence was marked in the experimental samples. Three protein bands were also found to be thicker in the experimental samples as when compared to the control sample.

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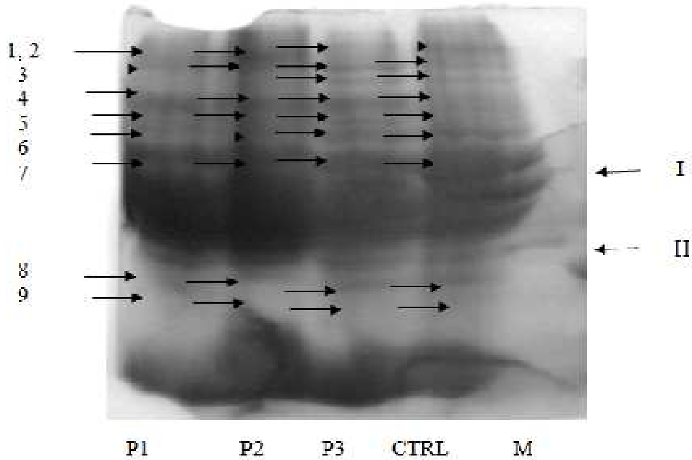
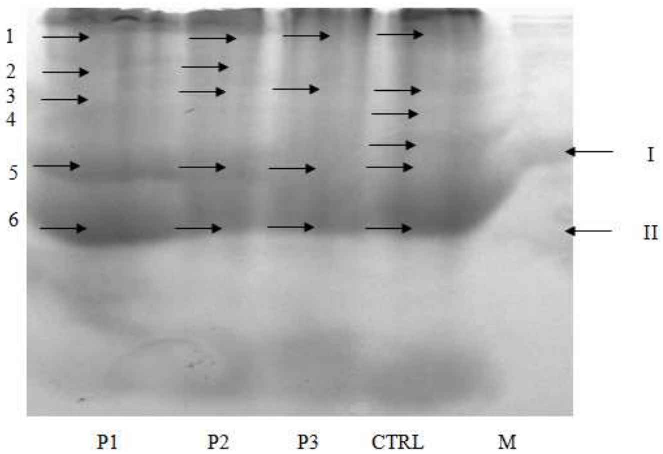


Photoperiod as a Stress Factor in Serum Protein

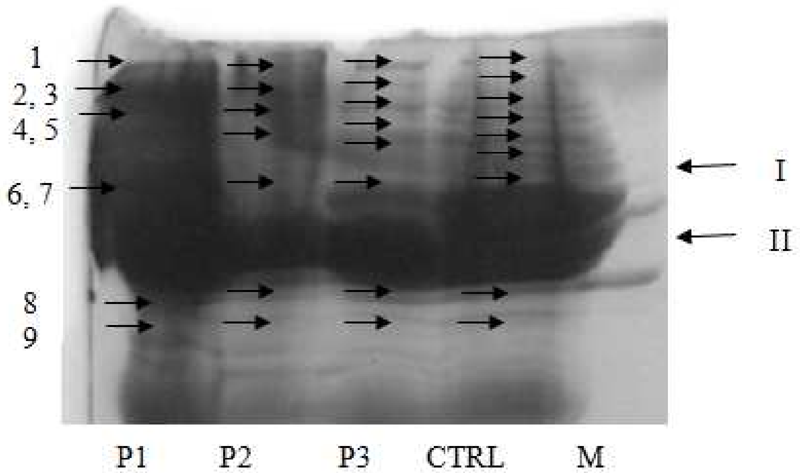
PLATE NUMBER 1



Photoperiod as a Stress Factor in Serum Protein

PLATE NUMBER 2

INFLUENCE OF PHOTOPERIOD ON THE BEHAVIOR AND SERUM PROTEINS IN GREY QUAIL (*Coturnix coturnix*)



Photoperiod as a Stress Factor in Serum Protein

PLATE NUMBER 3

DISCUSSION:

The choice of photoperiod for this study as a factor was based on the fact that natural light and dark cycle of day and night for the organisms has been altered due to increasing use of electricity in urban and rural areas in India. This has altered the daily circadian rhythm and activity in the animals and so can be assumed to be inducing some sort of deleterious changes. The annual cycle of changing photoperiod is a very precise temporal cue for determining the time of year.

The mammalian pineal gland can be influenced by a variety of exogenous stimuli among which light and darkness play important roles. Moreover, it has become clear that nonphotic stressful stimuli modulate the secretion of melatonin, which has extremely widespread effects on possibly every organ in the body[20].

Glucocorticoid release is considered to be the hallmark of the vertebrate stress response. Glucocorticoids are released in response to a wide variety of noxious stimuli in both laboratory animals and under natural conditions[21]. Recent studies done by workers have used many nontraditional avian species as models for glucocorticoid release under more naturalistic conditions[22].

Individuals use photoperiodic information to enhance immune function in anticipation of challenging energetic conditions that may otherwise compromise immune function. Recent evidences also indicate that photoperiod alters integrative measures of organism's immune function.

Similarly early healing was observed in the birds in the control group where the wound inflicted due to the cage on their head, while trying to fly out was healed within a period of 24 hours as compared to experimental birds under continuous exposure to light

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where it took a longer time than 24 hours to heal the wound.

There is also some evidence that housing under different light sources alters bird behavior. The type of lighting significantly affected the degree to which turkeys peck and pull feathers[23]. Laying hens were more active under fluorescent, as compared to incandescent lighting[24].

Adaptation to short days attenuates the magnitude and duration of the febrile response to bacterial infection in Siberian hamsters (*Phodopus sungorus*), likely via diminution of proinflammatory cytokine (IL- 1b, IL-6) secretion. Short days also enhance B cell dependent delayed-type hypersensitivity responses[25].

The experiment on birds conducted for analysis of continuous exposure to light, produced effects which were observed in their behavior pattern. They showed little movement and were very quiet. They refused food and water. The birds under observation produced an increased amount of feacal matter in the cage and with termination of experiment feed consumption decreased with simultaneous decrease in water intake Five bands on the whole in *Coturnix coturnix* are such which are seen to be present in the control samples but are not to be traced in the experimental samples indicating towards proteins whose synthesis are checked when conditions are not favorable. Similarly one band can be traced in the experimental samples which cannot be seen to be present in the control sample thus indicating towards a protein whose synthesis is initiated when conditions are not favorable.

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