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Impact on Behaviour and Serum Proteins of *Coturnix coromandelica* Induced due to Acute Photoperiodic Stress

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ABSTRACT

Extreme environmental conditions and stresses can have significant negative effects on physiological as well as life history traits of organisms.In response to the stress factors various genes are up regulated in the body, which can mitigate the effect of stress and lead to adjustment of the cellular milieu and animal tolerance. Birds can be easily subjected to stress.An external stressor sets off a rapid cascade of responses in vertebrates to deal with the threat and then to reestablish homeostasis.Stress stimulates several hormonal responses.The present study deals with an analysis of photoperiod as a stress factor on the serum proteins of Coturnixcoromandelica. For the study the birds were divided into unstressed or the control group and the stressed or the experimental groups consisting of three sets of three birds each.

The experiment aimed at providing acute photoperiodic stress for seventy two hours followed by observations in changes in behavior and serum proteins. Serum proteins were subjected to electrophoresis and changes in band width with increase or decrease in bands of proteins were analyzed later. The experimental birds showed marked changes in behavior after the completion of the experiment.

Key words: *Photoperiod, Coturnixcoromandelica, Acute stress,Serum proteins, Electrophoresis*

INTRODUCTION

The survival pressure on our planet as well as its flora and fauna have been increasing during past few decades. Animals are finding it increasingly difficult to survive due to various reasons like deforestation, lack of adequate aforestation, pollution, rapid industrialization, destruction of original habitats, lack of shelter places and breeding areas, decreased food availability etc.

To counteract this survival pressure animals have two options either they find new inhabitable areas where they can reestablish themselves and utilize the available resources orif they are unable to migrate rapidly enough then resort to other options to survive. In that case they will have to adapt to the new environment.

To help them in this type of adaptation animals have a diversity of genes within their populations which are otherwise dormant, but undergo activation and help to develop new traits as per the needs of the new environment.

Animals are frequently exposed to a variety of stress conditions which prevent them from reaching to their full genetic potential. Extreme environmental conditions and stresses can have significant negative effects on physiological as well as life history traits of organisms. To understand ecological adaptations and biogeographical distribution of a species detailed analysis of stressful conditions is a must.

Evolution of stress has been a consistent feature among populations and can be categorized as chronic, acute or/ and adaptive stress.Chronic stress is associated with hypertension, infertility, etc. Acute stress affects endocrine, cardiovascular and immune systems whereas adaptive stress is an adaptive method to help the animal resist damages produced due to stress.

A population may react and adapt to stressful conditions in two ways. Firstly they may develop a capacity to make phenotypic compensations via acclimatization. Secondly, they may evolve macromolecules those are either more resistant to functional perturbation or better able to retain functional efficiency in the altered environment[3].

Not all individuals respond in the same way or through the same channel to the same stimulus and also, different stimuli, may provoke different responses by the same individual as observed by Honessa and Marin[9].

In response to the stress factors various genes are up regulated in the body, which can mitigate the effect of stress and lead to adjustment of the cellular milieu and animal tolerance. In higher vertebrates chronic or intermittent stress causes psychoneuroendocrine and emotional disruption, while in lower vertebrates' normal behavior patterns are disturbed as observed by Pottinger[18]. From an evolutionary perspective, animals have evolved behavioral responses to avoid prolonged stress exposure[12].

Studies shows that altered affective state of the animals reflects the "hopelessness" state andthese models are interpreted as "learned helplessness" model, which are the best animal model of depression[13].

Literature Review

Birds can be easily subjected to stress. They can be under stress due to severe cold, heat, poor diet causing, genetic factors, metabolic disorders, exposure to toxins, social stress, fear, insufficient light, insufficient rest or sleep deprivation etc.

Stress can be defined in many different ways[17]. It can be defined as adaptive physiological changes which result from a variety of environmental stressors as explained by Seyle[25].

The stress response is found to be highly conserved throughout the vertebrate taxa, thus emphasizing its adaptive value[15]. The stress system has evolved and functions to regulate homeostasis in the face of a dynamic and changing external milieu[6].

An external stressor sets off a rapid cascade of responses in vertebrates to deal with the threat and then to reestablish homeostasis[21]. Chronic stress results in an imbalance in an organism's energy budgetand it leads to a down-regulation of the immune system[1]. It also causes increased susceptibility towards diseases[16].

Stress stimulates several hormonal responses, and in birds one of these is the release of the adrenal glucocorticoid hormone corticosterone[2] via activation of the hypothalamic–pituitary–adrenal (HPA) axis.Birds respond to environmental changes by modulating their levels of plasma corticosterone, a stress hormone produced by the adrenal glands[27].

Ulett *et al.*,[25] were the early workers who studied the photic stimulation as an index of anxiety proneness. Lewis and Morris[11] recorded the responses of domestic poultry to various light sources.

Glucocorticoids, an indicative of the vertebrate stress response are released in response to a wide variety of unfavorable stimuli in laboratory animals[20] and also under natural conditions.

The secretory pattern of melatonin allows individuals to ascertain the time of year and anticipate seasonal environmental changes[19]. Melatonin is an indole-amine hormone that is found throughout the animal kingdom and its best-known biological function is to provide annual day length information.

Maintenance of a positive energy balance is critical for survival and reproductive success[4]. In many bird species, it has been demonstrated that high levels of corticosterone result in birds abandoning all their normal activities and initiating behaviors directed toward immediate survival[28].

MATERIAL AND METHOD

The present study deals with an analysis of photoperiod as a stress factor on the serum proteins of a species of Quail which is commonly known as rain quail- *Coturnix coromandelica*.

Quail is a seasonal, migratory bird and its availability was during summers in this part of the India i.e. during July- August. The experiment was conducted at the Laboratory of Reproductive Biology situated

at Dayanand Girls Degree College, Kanpur. For the study the birds were divided into two categories: unstressed or the control group consisting of three birdsand the stressed or the experimental groups consisting of three sets of three birds each.

Prior to the initialization of the experiment the lab was prepared,disinfected and cleaned.The birds were purchased from the local dealers, checked for their health and activity before experimentation. The birds when bought to the lab were kept in an open aviary under natural conditions for about a fortnight to acclimatize them after which the birds were placed in different cages.

The duration of the experiment was seventy two hours for photoperiod as an acutestress factor.For the experiment three birds each were kept in three neat disinfected cages. They were given feed and water *ad. libitum*. They were provided uninterrupted light throughout the experimental period. The birds under observation were provided with natural light during the day and by a halogen (Power Lamp, 500 W) during the night. Other environmental factors like temperature, humidity were dependent on the season.

Along with these a set of four birds was kept as a control group throughout the experimental period. They were given feed and water *ad. libitum*. Other environmental factors like photoperiod, temperature, humidity were dependent on the season.

After the completion of the experimentthe blood from the birds were collected in ampoules where it was left to clot and when serum separated from the blood sample it was collected in eppendorf tubes with a lysis buffer added to it to protect it from getting denaturated. (25µl sample + 5 µl lysis buffer) The samples of the serum proteins were marked and refrigerated in the deep freezer.Later electrophoresis (SDS- PAGE) was performed on the refrigerated samples to separate and/or identify proteins.

The gel surfaces were run with one sample from control birds and the three samples from each one of the experiment. Along with these samples a set of molecular markers were also run to have an idea of the range of the molecular weight of the proteins in the sample. The marker chosen was a common one (BSA-SHMT, range 66KD-45KD) and was chosen randomly too because the range of molecular weight of proteins in the sample was unknown. The gel surfaces obtained fromthe experiment were photographed and analyzed for the bandwidth of the serum proteins. This information was used to identify the change or the presence or absence of a band of protein in the sample as compared to the control. Along with this the bird behavior was monitored day to day.

Observations

The observation of the birds under experimentation was based on two criteria, one on their behavioral changes observed during experiment and second on the analysis of the gel surfaces obtained as a photograph regarding the presence and absence of bands or changes in their position or width when compared to the control induced due to the stress conditions.

Behavioral changes observed in *Coturnix coromandelica* with photoperiod as a stress factor:

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

After the first night of light exposure the birds were observed to centralize themselves in the cage. They showed no movement during early morning hours for some time and were very quiet. They did try to close their eyes but could not keep them closed for more than a few seconds. They refused food and water. After about two hours they resumed normal activity and started movement but there was marked restlessness. Later with the next night of exposure to light their water intake decreased significantly.

By forty eight hours of exposure during the daytime the birds were quite active and were running along in the cage and jumped a lot but signs of weariness were quite evident by the evening. On the last day they all sat crouched in the cage during early hours in the morning, later they showed vigorous activity running all around the cage and pecked at the cage boundaries to find points of escape. They even pecked at the entrance of the cage to run away. When nearing the exposure to seventy two hours it was observed that the feed consumption had decreased significantly with decrease in water intake.Birds settled down with weariness and also tried to close their eyes.

Analysis of the gel surfaces obtained after SDS PAGE in the different experimental groups in *Coturnix coromandelica* with photoperiod as a stress factor:

The experiment presents gel surface photograph of the birds in the experimental groups exposed to continuous light for seventy two hours. The left side of the surface shows roman numbers which denote the known marker with a limited range used along with the serum samples. The right side of the surface has numbering according to the major visible bands formed due to the control sample and they will be used to compare the experimental samples for the presence or absence of the bands or the changes observed in the band width.

I EXPERIMENT(Plate No. I)

The first band as seen in the control group has corresponding appearance in P2 and P3 whereas it cannot be seen in P1 due to loss of a part of the gel surface (the three experimental samples). The second, third and the fourth band observed in the control sample do not have any correspondence in all the experimental samples. The fifth band present in the control has similar corresponding presence in all the samples, but is found to be increasingly thicker in P1 followed by P2and P3. The sixth band observed in the control sample has correspondence in all the experimental samples. The seventh band in the control sample is a thin one and it shows similar appearance in P1, P2 and P3 like the sixth band. The eighth band formed is a thin band in the control sample and corresponding in position in all the samples but it is thicker in the experimental samples when compared with the control. Below the eighth band observed in the control sample there is a presence of a thin band in all the experimental samples which is not observed in the control sample.

Thus in the first experiment conducted on *Coturnix coromandelica* the control sample showed three bands whose absence was marked in the experimental samples. The experimental samples showed a band whose absence was marked in the control sample. Two protein bands were also found to be thicker in the experimental samples as when compared to the control sample.

II EXPERIMENT(Plate No. II)

The first band as seen in the control group has corresponding appearance in P1, P2 and P3 (the three experimental samples). There is a band observed above the first band of the control sample in all the experimental samples which is untraceable in the control sample. Before the second observable band of the control sample again a thin band is observed in all the experimental samples which isuntraceable in the control sample. The second band observed in the control sample has a similar correspondence in all the experimental samples. The third band observed in the control is also found in P1, P2 but not in P3. The fourth band in the control is found similar in P1 and P3 but is absent in P2. The fifth band observed in the control is found in P1 but not in P2 and P3. The sixth band observed in the control sample is a thick band but the corresponding bands in the experimental samples are thinner than the control. The seventh band observed in the control sample has similar incidence in all the experimental samples.

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

III EXPERIMENT(Plate No. III)

The first band as seen in the control group has corresponding appearance in P1, P2 but not in P3 (the three experimental samples). The second band observed in the control is a thick band and is also observed in P1 and P2 but not in P3. The third band and the fourth band observed only in the control sample have no correspondence in the experimental samples. After the fourth observable band in the control sample a band is observed in P1 and P2 but not in the control sample and in P3. The fifth band observed in the control sample is a thick and prominent band and has similar appearance and correspondence in all the experimental samples. The sixth band in the control sample is similar to the fifth band in appearance in the control as well as the experimental samples. The seventh band observed in the control sample shows a similar appearance in P2 and P3 but not in P1 although the band is thicker in the control sample when compared with the experimental samples. The eighth band observable in the control sample is observed to be similar in correspondence and appearance to the seventh band in the experimental sample.

Thus in the third experiment conducted on *Coturnix coromandelica* the experimental samples showed a band whose absence was marked in the control sample. The control sample showed two bands whose absence was marked in the experimental samples. Three protein bands was also found to have grown thinner in the experimental samples as when compared to the control sample.

Gel surface photographs obtained after SDS PAGE in the different experimental groups in *Coturnix coromandelica*with photoperiod as a stress factor:

I Experiment

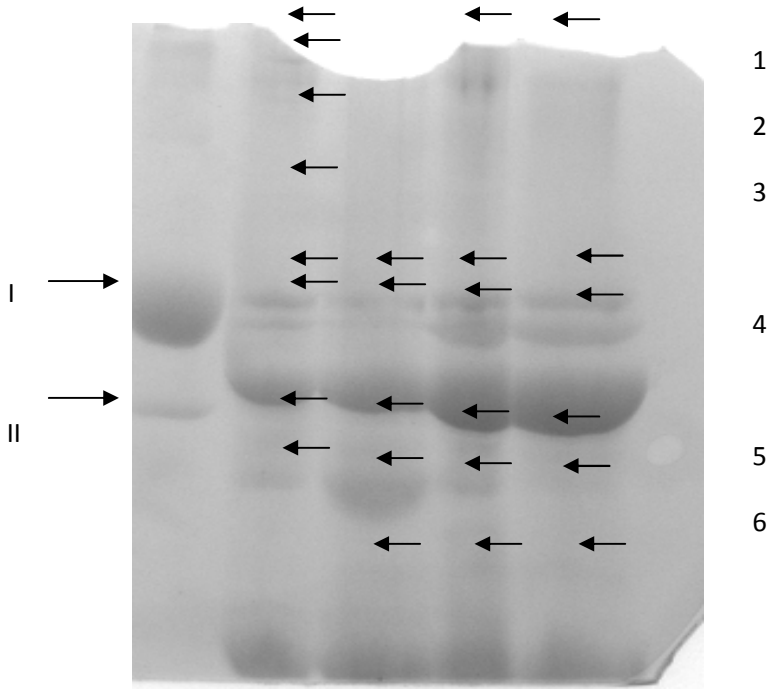

Plate Number I

ii Experiment

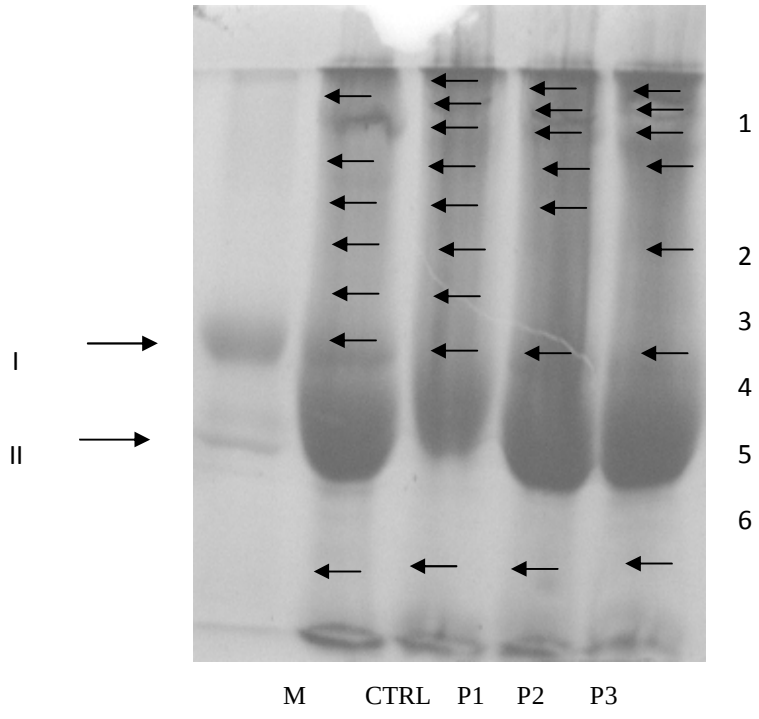

Plate Number ii

iii Experiment

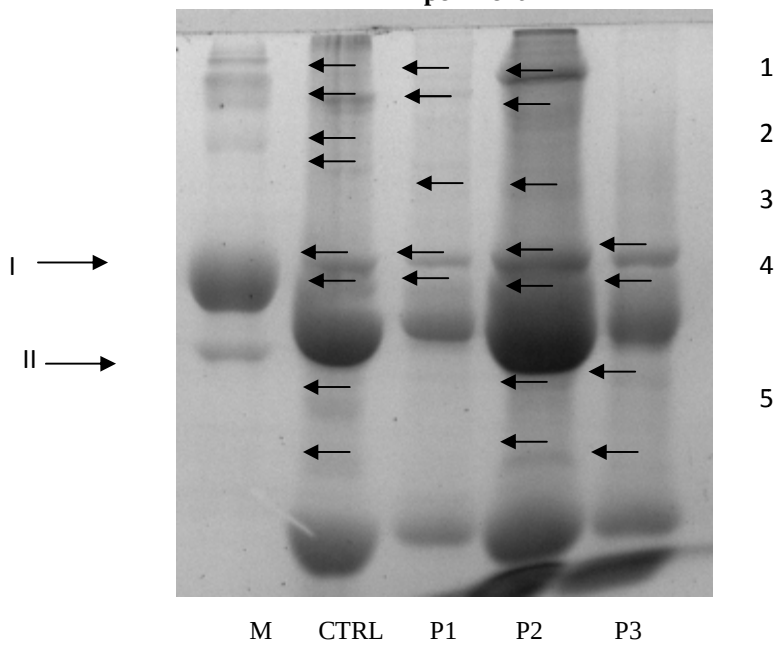

Plate Number iii

DISCUSSION

The choice of photoperiod as a stress factor for this study was simply based on an observation that in present era the nights are not naturally dark. The exposure to light has increased in all animals due to artificial lights in urban as well as rural areas. The natural light dark cycle of photoperiod has been significantly altered and this is bound to produce stress in animals.This has altered the daily circadian rhythm and activity in the animals and so can be assumed to be inducing some sort of changes.

Seasonal changes in the onset and severity of stressors may influence survival in animals[16]. Vertebrates have been found to have ancient stress-regulating peptides as observed in their study by Chang *et al.*,[5].

Studies in rodent species have indicated that immune changes in response to photoperiod occur in the absence of elevated glucocorticoids[7]. Such unpredictable events are generally thought to exert their effects through the hypothalamo–pituitary–adrenal (or the stress) axis[21,27.]

As some of the most highly mobile organisms on earth, birds have access to diverse options for avoiding problem situations. Seasonal migrations between breeding and wintering sites can permit individuals to use geographic areas that would be unacceptable if they had to remain year-round[8].

Changes in day length alter several indices of immunity in seasonally breeding rodents[16].

Deer mice housed in short days had smaller lesions, and their wounds healed significantly faster than did those of mice housed in long days[10]. 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 where it took a longer time than 24 hours to heal the wound.

Corticosterone levels are routinely used as an indicator of stressful conditions[26] as although short-term elevations in corticosterone may be protective and adaptive, chronically elevated levels of glucocorticoids have several detrimental physiological effects if the animal is unable to remove itself from the source of stress[14].

Five bands on the whole in *Coturnix coromandelica* are found to be thicker in the experimental samples as when compared to the control sample which reflect towards protein whose synthesis were enhanced under conditions of stress. Six bands on the whole are such which are seen to be present in the control samples but are not to be traced in the experimental samples denoting towards proteins whose synthesis are checked when conditions are not favorable. Similarly four bands can be traced in the experimental

samples which cannot be seen to be present in the control sample thus indicating towards those proteins whose synthesis was initiated when conditions are not favorable. Two bands are found to be thicker in the experimental samples as when compared to the control sample which indicate towards protein whose synthesis were enhanced under conditions of stress. Four bands are found to be thinner in the experimental samples as when compared to the control sample which indicate towards protein whose synthesis were reduced under conditions of stress.

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