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Original Article

Influence of Temperature on the Behavior and Serum Proteins in Quail (*Coturnix coturnix*)

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ABSTRACT

In present living conditions various kinds of stressors are abundant in our environment. Flora and fauna on our planet face psychological, physical, and biological strains. The existing implications of destructive anthropogenic activities affect all organisms including Aves and that formed the base to conduct the study. The behavioral changes observed due to induced conditions of high temperature on the experimental Grey Quails (*Coturnix coturnix*) included slower breathing, sluggish movements and decreased food consumptions. The changes observed due to changes in temperature in serum protein after electrophoresis showed the absence of certain proteins, whose synthesis were checked when conditions were not favorable. There were also indications of proteins whose synthesis were initiated in unfavorable conditions while some proteins showed enhanced synthesis under conditions of stress. Such kind of study has not been documented in Aves very strongly and so this work has its importance in this aspect.

Key words: Coturnix coturnix**,** Electrophoresis, Temperature, Behavior, Serum proteins

1. INTRODUCTION

In present living conditions various kinds of stressors are abundant in our environment. Flora and fauna on our planet face psychological, physical, and biological strains. These strains can be present in our working and social environments, in air, soil, water, food, and medicines etc. Animals are frequently exposed to stressful conditions and various anthropogenic activities accentuate the existing stress factors. This prevents the animals from reaching their full genetic

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potential. Analysis of such stressful conditions may help us in the understanding of ecological adaptations and geographical distribution of a species.

Generally a population reacts and adapts to stress in two ways. It may develop capacity to make phenotypic changes through acclimatization or it may evolve macromolecules which are more resistant to functional discomforts or are better able to retain functional efficiency in the altered environment. [1-3]

The stress responses of an animal are described as the alarm reaction, followed by the phases of resistance and exhaustion. This response is dominated by the HPA (hypothalamic pituitary adrenal) system and it influences the diversion of resources away from non-critical functions, to those functions of the body which enable maintenance of homeostasis and in extreme cases its survival. [4, 5]

An animal's biological responses to stressors may be produced through behavioral, autonomic, neuroendocrine and immunological changes as discussed by researchers. [6]

A stress axis has been identified in animals and it is assumed to play an important role in the animal's activities. This axis is involved in normal day-to-day activities associated with the diurnal cycle of waking such as increased locomotion, exploratory behavior, increased appetite, and food-seeking behavior.[7] The stress axis also permits short-term adaptation to maintain survival in the face of acute, environmental stressors as well as provides long-term evolutionary adaptations to ecological and habitat pressures such as those encountered by the species inhabiting the cold regions in the north. [8] Stress is a big factor in determining the overall health of our birds. [9, 10]

In birds during trauma nutrients are depleted from the body, immune system becomes depressed, hormonal imbalances occur, respiratory infections, allergies, eating disorders, diarrhea, and skin and feather problems are a few of the outward symptoms of stress.

Aves have been long used as a model for vertebrate study and can be used for study as a reference to other vertebrates too. Quails are rapidly maturing as an avian model for experimental studies. So they were the first choice for the study.

To understand the population-level consequences of stressful events requires us to understand the effects of stress on an individual and on its wild populations. [11]

The tolerance of stress by a species can influence its distribution and abundance [12], the rate of population growth and the outcome of interactions with other species. [13]

Thermoregulation has been a matter of investigation since long and has drawn curiosity by workers in different groups, whereas histamine has been found to be involved in mammalian thermoregulation. [14]

Increase in temperature has produced climate-related long-term faunal changes in a California rocky intertidal community as found in studies.[15] Heat can also produce a heavy strain on organisms in humid environment. [16]

2. MATERIALS AND METHODS

2.1 Material

The present study deals with an analysis of temperature as a stress factor on the serum proteins of a species of Quails- *Coturnix coturnix*. As Quail is a seasonal, migratory bird its availability was an important factor to be assured. The species under observation i.e. *Coturnix coturnix* (Grey Quail) was available during winters (November-February) in the region of the study.

The choice of increasing temperature as a factor for the study was based on the fact that the effects of global warming though appearing gradual are steady and are producing a general rise in temperatures of earth and thus becoming a cause of discomfort to organisms.

2.2 Experimental Design

The study focused on two categories of birds, unstressed or the control group and stressed or experimental group.

The birds were purchased from the local dealers and checked for their health and activity before experimentation. The birds were kept in open aviary under natural conditions for about a fortnight to acclimatize them prior to experimentation.

For the experiment simultaneously three cages with three birds each were kept in neat disinfected cages. They were given feed and water *ad. libitum*. The temperature conditions were altered and increased. The birds were provided heat by a blower (2000 KWA/hr) continuously throughout the experimental period (which was seventy two hours) to provide acute stress. Along with these a set of three birds was kept as a control group throughout the experimental period. They were given feed and water *ad. libitum.* Other environmental factors like photoperiod, humidity etc. was dependent on the season for both the groups. Along with this the bird behavior was monitored day to day. After the completion of the experiments the blood serum of the birds 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 protein were then marked and refrigerated in the deep freezer. Later electrophoresis (SDS- PAGE) was performed on the refrigerated 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. 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.

2.3 Observations

2.3.1 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 uptake of food and water was normal. The temperature conditions during the starting of the experiment were in the range 23^{o} – 25^{o}C. The birds initially sat in the centre of the cage with their activity decreased in the evening. Slowly the temperature was raised to a range of 26^{o}- 32^{o}C.

After first twenty four hours of exposure it was observed that they consumed large quantities of water and feed consumption was decreased when compared to the control. It was also observed that the birds scattered the feed instead of consuming it. The excretory matter increased significantly. Their movements were restricted and they sat in one extreme corner of the cage crouched upon each other away from the heat source. By forty eight hours of exposure the birds became sluggish and sat nearly one upon the other in the cage on one extreme end. The excretory matter was same quantity when compared to the control. Nearing the exposure to seventy two hours it was observed that the water consumption increased largely. They all sat in the cage and showed very slow breathing movements. 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. This was the time when they consumed the maximum feed and water. During the rest of the day they showed normal movements in the cage and moved about effortlessly. They also showed play behavior. They were observed to crouch over one another during the evening hours and spread out during the day. The excretory matter produced was normal.

2.3.2 Gel Surfaces Changes Observed during the Experiment in Coturnix coturnix

The experiments present gel surface photographs of the birds in the experimental group exposed to continuous increased temperature for seventy two hours. 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 will be used to compare the experimental samples for the presence or absence of the bands or the changes observed in the band width.

2.4 I Experiment

The whole surface has major two portions due to a presence of a thick banding as seen (Plate No. 1). The first band as seen in the control group has corresponding appearance in T1, T2 and T3 (the three experimental samples). The second band observed in the control sample is a thick band and appears equally thick in T2, whereas in T1 and T3 it is thinner as compared to the control and T2. The third band in the control sample is present in all the experimental samples with similar thickness except in T2 where its presence cannot be observed. The fourth band in the control sample is similarly present in all the experimental samples except T3 where it is not found. The fifth band in the control sample is a thin band and is found in T1 but is absent in T2 and T3. The sixth band in the control sample is found uniformly in all the experimental samples. The seventh band observed in the control sample is found to be similar and corresponding in all the experimental samples as the sixth band. The eighth band found in the control sample is thinner as compared to T1, T2 and T3. The ninth band in the control sample is similar in all the experimental samples. The tenth band in the control sample has variations in position, that of the control sample and T3 are placed correspondingly but the band for T2 is placed a little below than them and T1 band is formed a bit higher in position than T2, T3.

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

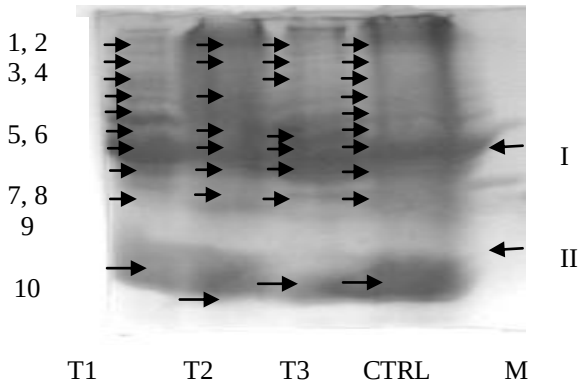

Fig 1: Temperature as a Stress Factor in Serum Protein (Plate I)

2.5 II Experiment

The whole surface has major two portions due to a presence of a thick banding as seen (Plate No. 2). The first band as seen in the control sample has corresponding appearance in T2 and T3 whereas its presence cannot be confirmed in T1 due to loss of a small part of the surface during handling (three experimental samples). The second and third band observed in the control sample has no incidence in the experimental samples. The fourth band present in the control sample is also observed in T2 and T3 but cannot be traced in T1. The fifth band present in the control sample can be seen in T3 though but not in T1 and T2. Before the sixth band observed in the control sample there is presence of a band in T1 and T2 but not in T3. The band observed in T1 is placed slightly higher than the one observed in T2. The sixth band

observed in the control sample is absent in T1, T2 and T3. The seventh band observed in the control sample has similar occurrence in all the three experimental samples but their positions are not same. The position of the control sample band corresponds with T3 but the position of T2 is slightly raised and that of T1 is still higher than T2.

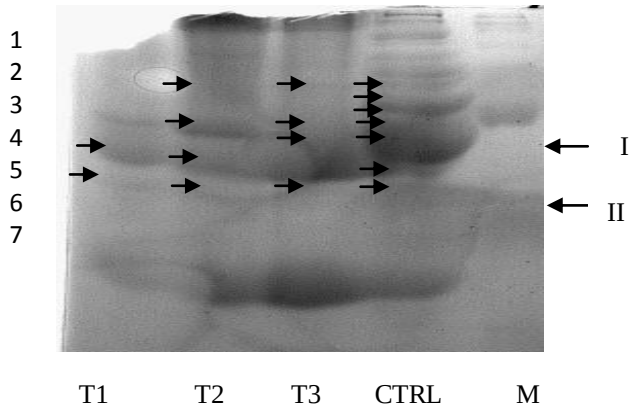

Fig 2: Temperature as a Stress Factor in Serum Protein (Plate II)

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 sample showed three bands whose absence was marked in the experimental samples.

2.6 III Experiment

The whole surface has major two portions due to a presence of a thick banding as seen (Plate No. 3).The first and the second band as seen in the control group have corresponding appearance in T1, T2 and T3 (the three experimental samples). The third band observed in the control sample also has a presence in T1, T2 and T3 and is comparatively thicker in the experimental samples. The fourth band observed in the control sample has similar correspondence in all the experimental samples. The fifth band observed in the control sample is a thick band and is found to be similar in thickness in all the experimental samples. The sixth band observed in the control sample is observed to be thick; it is thinner than the control in T3, thicker than the control in T2 and it is the thickest of all in T1. The last major observable band in the control sample is the seventh band which is similar in all the experimental samples i.e. T1, T2 and T3.

Thus in the third experiment conducted on *Coturnix coturnix* two protein bands were observed to be thicker in the experimental samples when compared to the control sample.

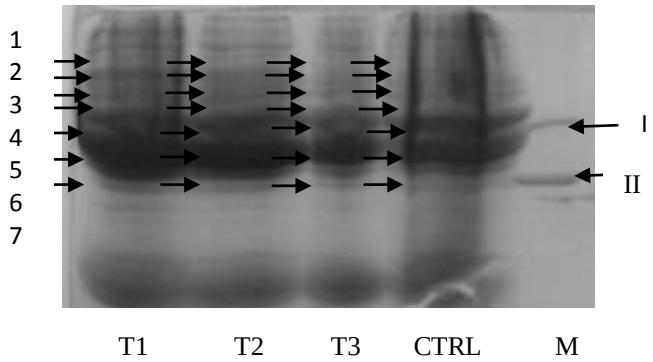

Fig 3: Temperature as a Stress Factor in Serum Protein (Plate III)

3. DISCUSSION

The choice of increased temperature as a factor was based on the fact that the effects of global warming though appearing gradual are steady and are producing a general change in temperature conditions, increasing it and thus becoming a cause of discomfort to organisms.

Among all homeostatic systems, thermoregulation is considered to be one of the most precise and stable. Body temperature (Tb) has a marked effect on oxygen uptake ($V0_2$) of resting animals. [17] Stocking density affects poultry and leads to variations in heat balance maintenance in domestic fowl. [18] High environmental temperatures produce variations in fertility of Holstein cattle. [19]

4. CONCLUSIONS

Energy intake and amino acid balance is of extreme importance in heat stress. Broilers under heat stress have to make critical life sustaining physiological adjustments. Feed intake is depressed and water intake is increased.

Along with fasting it was found that the water intake of experimental birds decreased. They showed a decrease in food and water intake. When nearing the exposure to seventy two hours it was observed that the water and feed consumption by the birds had decreased significantly.

It has been observed by workers that during periods of heat stress the broiler has to make major thermo-regulatory adaptations in order to prevent death from heat exhaustion. Heat stress interferes with the broilers comfort, suppresses productive efficiency and the full genetic potential of the broiler is often not achieved.

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 protein whose synthesis was initiated when conditions were not favorable. Three bands are found to be thicker in the experimental samples as when compared to the control sample which indicate towards proteins whose synthesis were enhanced under conditions of stress. One band was found to be thinner in the experimental samples as when compared to the control sample which indicated towards protein whose synthesis was checked under conditions of stress. Two bands in the experimental sample showed a change in position with the corresponding control sample indicating towards a change in molecular weight of the protein concerned due to stress.

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