GESA
GLOCAL ENVIRONMENT & SOCIAL ASSOCIATION

International Journal of Biological Innovations
http://ijbi.org.in | http://www.gesa.org.in/journals.php
https://doi.org/10.46505/IJBI.2022.4108
IJBI 4(1): 77-82 **(2022)**
E-ISSN: 2582-1032

ALTERATIONS IN THE BEHAVIOR AND SERUM PROTEINS OF RAIN QUAIL (*COTURNIX COROMANDELICA*) DUE TO HYPOXIA

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Article Info:
Research Article
Received
20.12.2021
Reviewed
10.02.2022
Accepted
28.02.2022

Abstract: Nowadays air pollution is creating a general condition of stress and uneasiness for all flora and fauna including the Aves. The present study was focused on acute hypoxic stress and resultant effects in Rain Quails (*Coturnix coromandelica*). The changes in behaviour and serum proteins were observed due to experimentally induced conditions of hypoxia in Rain Quails. Quails showed swelling and slow breathing followed by signs of unconsciousness. The serum proteins were examined by using Gel Electrophoresis.

Keywords: Adaptation, Behavior, *Coturnix coromandelica*, Electrophoresis, Hypoxia.

Cite this article as: Pandey Eshita (2022). Alterations in the Behavior and Serum Proteins of Rain Quail (*Coturnix coromandelica*) due to Hypoxia. *International Journal of Biological Innovations*. 4(1): 77-82. https://doi.org/10.46505/IJBI.2022.4108.

INTRODUCTION

Animals within the natural environment, whether aquatic, aerial, or terrestrial, are confronted with adverse and challenging conditions during life. These adverse conditions act as a stress to them and can present a significant threat to their well-being or at worst, survival. The environment is full of stressors and changes of various types like psychological, physical, climatic and biological. These changes influence the ecological balance and sustainable development (Ashok, 2018; Verma, 2021).

Life-threatening environmental conditions and stresses affect animals negatively. The animals are subjected to face them frequently and prevent themselves from reaching their full genetic probability. Exploration of such stressful conditions helps in understanding ecological adaptations and distribution of a species.

A population tries to adapt and develop a capacity to make phenotypic changes or it may develop macromolecules resistant to external changes. It may help to be functionally active in an environment which has been altered (Hoffmann and Parsons, 1991; Bijlsma and Loeschcke, 1997).

General Adaptation Syndrome (GAS) is important in stress situations (Verma, 2017). Seyle (1946) identified three components of the stress response as being the alarm reaction, followed by the phases of resistance and exhaustion. The latter two stages (the acute stress response) with the first component, the alarm reaction, are dominated by the hypothalamic pituitary adrenal (HPA) system. It influences the departure of resources away from processes, which enable the animals to restore the normal homeostasis, internal balance and support the survival in life-threatening situations (Moberg, 1985; Storey, 1998).

Moberg (2000) outlined that an animal under stress will show different biological responses through changes in behavior, altered autonomic nervous system responses, changes in neuroendocrine and immunological responses.

In nature stress does not generally come in isolation and many stresses act hand in hand with each other. 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. A stress axis has been identified to play an important part in the animal's activities. It is found to be involved in activities associated with the diurnal cycle of waking such as increased locomotion, exploratory behavior, increased appetite, and food-seeking behavior (Wingfield and Romero, 2001). It allows short-term adaptation to preserve existence when under acute environmental stress. It also helps in long-term evolutionary adaptations to ecological and habitat pressures which can be exemplified with those species inhabiting the cold regions in the north (Wingfield and Hunt, 2002).

Stress is a big factor in determining the overall health of our birds (Hopkinson, 1926; Robbins, 1979). Stress also leads to hormonal imbalances of endocrine glands like adrenal, pituitary, thyroid, thymus etc. which disturb the immune function. Respiratory infections, allergies, eating disorders, diarrhea, and skin and feather problems are few of the outward symptoms of stress. In essence, when a bird is under stress, its whole body is affected.

Birds (Aves) also referred to as masters of air are homoiothermic or warm-blooded egg-laying vertebrates characterized by the presence of feathers and modification of forelimbs as wings for flight (Verma and Prakash, 2020). They have been long used as a model for vertebrate study as they can be easily handled and managed in laboratories, show similar responses to stimuli as mammals and so can be used for study as a reference to other vertebrates too.

The animals utilized for the present study were Quails because Quails are rapidly maturing as an avian model for experimental studies. For grasping the population-level concerns of stressful events, it requires an understanding of the effects of stress on an individual; it will help to comprehend problems of people dealing with study of conservation biology, stewardship of wild populations, and aquaculture (Schreck *et al.*, 2001).

A number of studies on the behavioral, physiological, biochemical and molecular mechanisms of adaptation to life without oxygen have been conducted (Yang *et al.*, 1992; Storey and Storey, 2001; Hermes-Lima and Zenteno-Savín, 2002) but there is only a little documentation regarding consequences of hypoxia in birds (Richard and Sykes, 1967) and mammals. Keeping in mind, author attempted to examine the acute hypoxic stress and resultant effects in Rain Quails (*Coturnix coromandelica*) and observed that body temperature, heart rate, respiratory rate and blood pH are reduced after hypoxia.

MATERIALS AND METHODS

Author selected the Rain Quails, *Coturnix coromandelica* to analyze the acute hypoxia as a stress factor on the serum proteins. The research work was conducted in the Department of Zoology, Dayanand Girls Degree College, Kanpur, India. The Quail is a seasonal, migratory bird that remains available during rainy seasons (July -October) in this part of the Indian subcontinent.

During the research period, the birds were divided into two categories: non stressed and stressed. The non-stressed birds acted as the control group and the stressed as the experimental group. Prior to the beginning of the experiment, the lab was disinfected and entry of all kinds of possible predators was checked. A clean water supply and proper aeration were ensured. These birds were checked for their health and activity before experimentation. These 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 three glass boxes were taken and three birds were kept in each. They were given feed and water *ad. libitum*. The glass boxes were sealed from all sides so as to prevent the entry of any air inside the chamber to provide acute hypoxic conditions. When the birds started showing signs of unconsciousness (which was a few hours depending upon the condition of the birds) the experiment was terminated. Along with these a set of three birds was kept as a control group throughout the experimental period (which was a few hours depending upon the condition of the birds). They were given feed and water *ad. libitum.* Environmental factors like temperature, humidity etc. was dependent on the season for both the groups. Behavior of the birds was also monitored during the experimental periods.

After the termination of the experiments, the blood from the birds was collected in ampoules and was left to clot. The serum separated from the blood sample, was collected in eppendorf tubes and mixed with a lysis buffer to protect it from getting denaturated (25μl sample + 5 μl lysis buffer). The samples of the serum protein were marked, refrigerated in the deep freezer and later 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 experiments. The gel surfaces were later 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.

RESULTS

Author found that the birds in control group experienced normal conditions of temperature, humidity, photo cycle, and were given feed and water *ad. libitum*. They were observed behaving normally. Their maximum activity was seen during the early hours of the day and late hours of the afternoons. This was 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 with each other. They were observed to crouch over one another during the evening hours and spread out during the day. The excretory matter produced was normal. Their feeding and behavior patterns were used as parameter to check out the other birds under experiment.

The stressed or experimental bird behavior was also observed and monitored. During the initialization of the experiment, the birds were kept in a sealed chamber of glass. They ran all along the chamber and even tried to find escape routes and also fought with one another. After about one hour, the birds slowly settled down decreasing any activity for lack of air and the chamber was filled with moisture. After two hours, the birds were observed to be gaping with their beaks open towards the top of the chamber. The moisture that had collected on the walls of the chamber had trickled down as water by now. After the third hour the birds swelled up to the maximum and lay down on their sides or simply flopped down on the base. The birds showed no interest in either feeding or taking up any water. Their breathing was slow. After another half hour the birds started showing signs of unconsciousness and then the experiment was terminated for sampling. The birds under analysis showed little fecal matter during exposure to stress.

All the three experiments conducted were subjected to Gel Electrophoresis (SDS PAGE) on the serum proteins and then these surfaces were photographed for analysis. In the gel surface photograph of the birds 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 were used to compare the experimental samples for the presence or absence of the bands or the changes observed in the band width. Each gel surface was run with serum of a control bird and 3 experimental birds kept in that experiment.

Author found that the whole surface has two major portions due to a presence of a thick band (Plate No. 1) as a result of first experiment. The first band as seen in the control group has corresponding appearance in Hyp1, Hyp2 but not in Hyp3 (the three experimental samples). The second band observed in the control sample is a thick band, it is also observed in Hyp1 and Hyp2 but not in Hyp3, in the experimental samples its thickness decreases. The third and fourth band observed in the control sample are not observed in the experimental samples. After the fourth band in the control sample a band is observed in Hyp1 and Hyp2 but not in Hyp3. The fifth band observed in the control sample is a thick, prominent band and appears correspondingly in all the experimental samples. The sixth band observed in the control sample is a thick band and has similar correspondence in all the experimental samples. The seventh band observed in the control sample also appears in Hyp2 and Hyp3 but not in Hyp1 and the band is thicker in the control sample when compared with the experimental samples. The eighth band observed in the control sample is similar in correspondence and appearance in Hyp2 and Hyp3 but not in Hyp1. The band is thicker in the control sample when compared with the experimental samples.

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

Author noticed that the whole surface has two major portions due to a presence of a thick band (Plate No. 2) as a result of second experiment. The first band seen in the control group has corresponding appearance in Hyp1, Hyp2 and Hyp3 (the three experimental samples). The second and third band appearing in the control sample has no correspondence in the experimental samples. The fourth band observed in the control sample is similar in appearance and correspondence in all the experimental samples. The fifth band observed in the control sample is found corresponding in Hyp3 but is not found to be present in Hyp1 and Hyp2. The sixth band observed in the control sample has equal correspondence in all the experimental samples. The seventh band observed in the control sample shows a similar appearance in Hyp1, Hyp2 and in Hyp3 although the band is thicker in the experimental samples when compared with that of the control sample. The eighth band observed in the control sample is not observed in the experimental samples. The ninth band observed in the control sample is a thick and distinct band and is similar in correspondence and appearance in all the experimental samples. The tenth band observed in the control sample is a thin band and is observed to appear correspondingly in all the experimental samples. Below the tenth band observed in the control sample there is a band observed in all the experimental samples which cannot be traced in the control sample. The eleventh band observed in the control sample is also seen present in all the experimental samples.

In the second experiment conducted on *Coturnix coromandelica,* the experimental samples showed a band whose absence was marked in the control sample. The control sample showed four bands whose absence was marked in the experimental samples. A protein band was found to be thicker in the experimental samples as when compared to the control sample.

Author saw that the whole surface has two major portions due to a presence of a thick band (Plate No. 3) as a result of third experiment. The first band as seen in the control group has corresponding appearance in Hyp1 but not in Hyp2 and Hyp3 (in the three experimental samples) and so the related protein marks its absence in the experimental samples. The second band observed in the control sample is a thin band and although can be seen in Hyp1 but cannot be identified in Hyp2 and Hyp3. The third band observed in the control sample and Hyp1 does not correspond in the remaining two experimental samples. The fourth band observed in the control sample lacks any correspondence to the experimental samples. The fifth band observed in the control sample is a thick prominent band and has equal correspondence in all the experimental samples. The sixth band observed in the control sample is a thin band and has similar correspondence in all the experimental samples.

In the third experiment conducted on *Coturnix coromandelica,* the control sample showed four bands whose absence was marked in the experimental samples.

DISCUSSION

Hypoxia was chosen as a parameter for the study because of the fact that there was an increasing problem of pollution and resulting in decrease of available oxygen content in the air. An acute stress was thus provided to understand the repercussions. The hypoxia, even for brief periods, can be detrimental or fatal to humans and most mammals and birds.

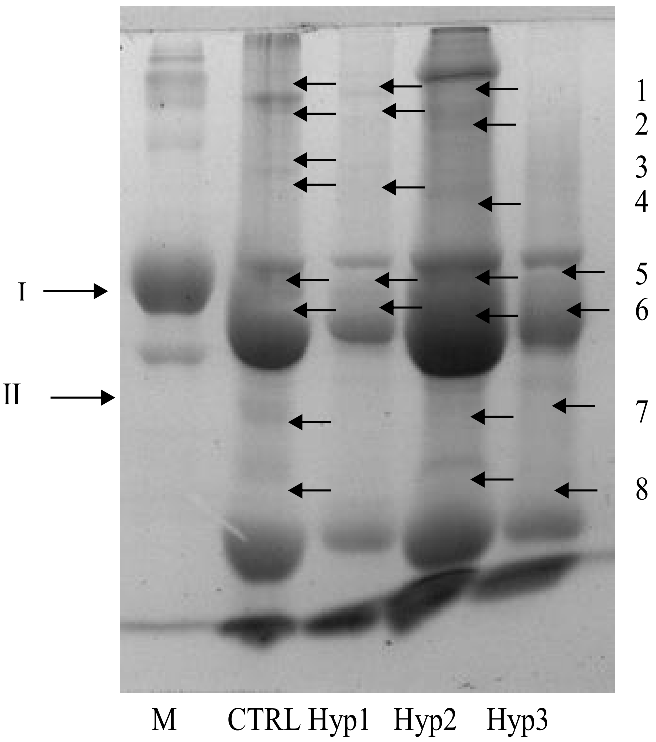

Plate No. 1: Hypoxia as a stress factor in serum protein (expt.1).

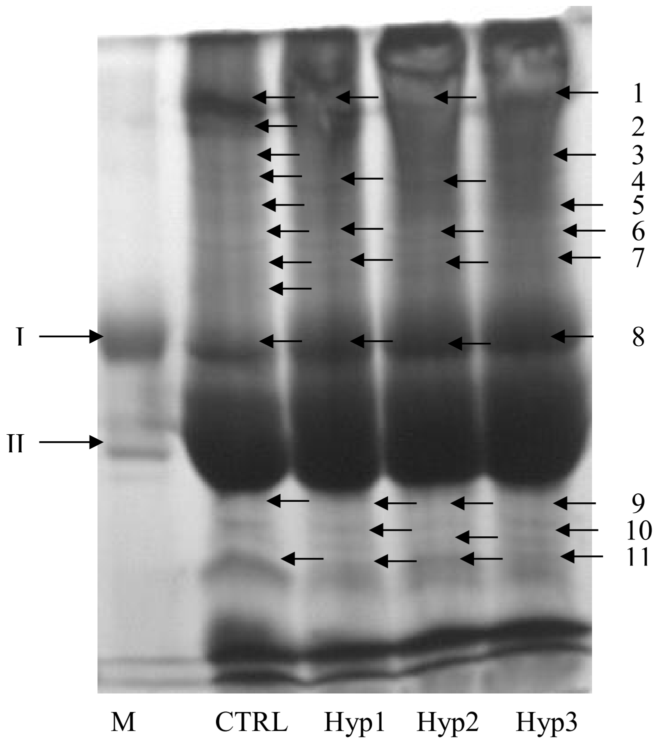

Plate No. 2: Hypoxia as a stress factor in serum protein (expt.2).

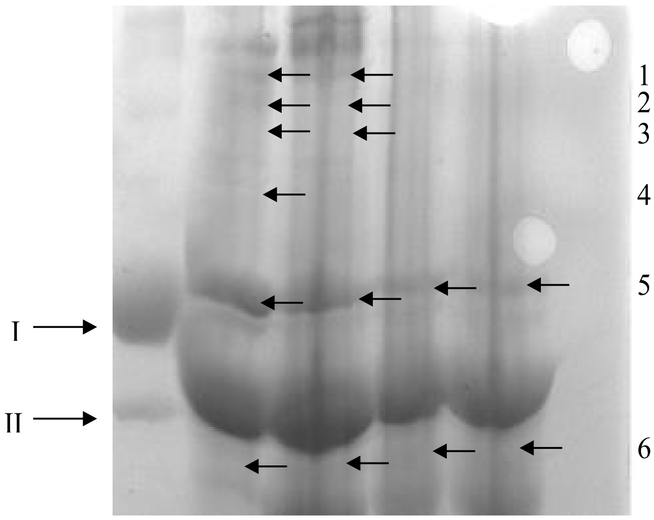

Plate No. 3: Hypoxia as a stress factor in serum protein (expt.3).

Hypoxia represents a major challenge for cells. Short-term reduction of O_2 supply is followed by the modification of existing proteins through phosphorylation or other post translational changes (Langen *et al.,* 1997). Longer reduction of O_2 can lead to cell death when the adaptive mechanisms are exhausted (Garbis *et al.,* 2005). However, many animal species are adapted to endure hypoxia or anoxia exposure for long durations. Genes and their products have a great potential to serve as indicators of hypoxic stress (Fountoulakis, 2001).

The condition of reduced tissue oxygen availability also causes a number of life-threatening clinical manifestations including shortness of breath, tachycardia, headache, dizziness, mental confusion and memory deficit, ophthalmological disturbances, cerebral haemorrhages, and sleep disturbances (Seillier-Moiseiwitsch *et al.,* 2002; Jochen *et al.,* 2017). Much has been elucidated on the biochemical and physiological adaptation mechanisms that make these animals endure oxygen deprivation (Fritsche and Nilsson, 1993; Jackson, 2000; Hochachka and Lutz, 2001; Hermes-Lima and Zenteno-Savín, 2002). The effects of hypoxia on the avian cardiovascular system were reviewed and it was found that the avian cardiovascular system seems well adapted to deal with the stress of hypoxia. Tolerance to hypoxia varies among taxonomic groups and habitats. Hypoxia depresses feeding in some species (Sobral and Widdows, 1997) while increasing it in others (Breitburg *et al.,* 1994). The starvation also affects the behavior and serum proteins in certain birds (Pandey, 2020). It is reported that more efficient gas exchange in the parabronchial lungs of birds provides an advantage over alveolar gas exchange at altitude (Powell, 1993). This is the reason why birds tolerate hypoxia so much better than mammals.

CONCLUSION

All the four bands observed in *Coturnix coromandelica* in control samples are not to be traced in the experimental samples indicating towards proteins whose synthesis are checked when conditions are not favorable. Similarly, two bands can be traced in the experimental samples which could not be seen to be present in the control sample thus indicating towards protein whose synthesis were initiated when conditions were not favorable. Four bands are found to be thicker in the experimental samples as when compared to the control sample which indicates towards proteins whose synthesis was 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 a protein whose synthesis was checked under conditions of stress. One band 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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