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Research Article

Influence of Starvation on the Behavior and Serum Proteins in Rain Quail (*Coturnix coromandelica*)

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Abstract: The environmental changes due to human activities have been a constant cause of concern for all of us. They are the reason for creating a general condition of stress for all organisms including the Aves. The base for this study was starvation stress being observed due to lack of natural food available for Aves. The study focusses on behavioral changes observed as a result of induced conditions of acute starvation in Rain Quails (*Coturnix coromandelica*). Later their serum protein were observed using Gel Electrophoresis and the banding pattern in the gel surfaces revealed changes which showed definite presence of proteins being formed or inhibited due to stress conditions. The results were later discussed.

Keywords: *Coturnix coromandelica*, Starvation, Electrophoresis, Behavior.

INTRODUCTION

Humans have the ability to destroy the environment and biodiversity drastically. We are highly manipulative and most of our activities lack consideration, are indiscriminate and focus on self-interest. Our planning towards the improvement and welfare of our race and biodiversity are less in comparison to the harm we are producing to important ecosystems which maintain our biosphere.

Our working and social working environments, air, soil, water, food etc. have abundant Stressors of various types, psychological, physical, and biological. Animals are frequently exposed to an excess of stress conditions. Anthropogenic activities have also accentuated the existing stress factors. These all stress factors are a menace for animals and 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.

Analysis of such stressful conditions is a major focus in the development of the understanding of ecological adaptations and biogeographical distribution of a species.

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 (Hoffmann and Parsons, 1991, 1997; Bijlsma and Loeschcke, 1997).

Much of stress research is founded on Seyle's, 1946 description of the General Adaptation Syndrome. Distress 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 (Moberg, 1985; Storey, 1998).

An animal's biological responses to stressors may be expressed through one or more of four channels: behavioral, autonomic, neuroendocrine and immunological as discussed by Moberg (2000).

In response to the stress factors various genes are up regulated in the body, they help to mitigate the effect of stress and lead to adjustment of the cellular environment and animal tolerance. In response to stress signals, nature has developed diverse pathways, for their combat and tolerance.

A stress axis in organisms has been identified and is assumed to play an important part in the animal's activities. The stress axis is supposed to be a critical one, 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 (Wingfield and Romero, 2001). Secondly, the stress axis also permits short-term adaptation to maintain survival in the face of acute, environmental stressors. Thirdly, the axis is important to manage long-term evolutionary adaptations to particular ecological and habitat pressures such as those encountered by the 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).

Comprehending the population-level consequences of stressful events requires understanding the effects of stress on an individual; such understanding is critical for conservation biology, maintaining wild populations, and aquaculture (Schreck *et al*., 2001).

The tolerance of stress by a species can influence its distribution and abundance (Sousa, 1984), the rate of population growth and the outcome of interactions with other species (Sagasti *et al*., 2001).

Prolonged or severe food shortages may evoke secretion of glucocorticoid hormones, the glucocorticosteroids and they actively compromise aspects of immune function (Kelly, 1985). Eisermann *et al.*, (1993) studied physiological responses due to over winter food shortage food in wild European rabbits.

Effects of starvation on the larval development of *Hyas areaneus* larvae (Decapoda) have been studied by Anger and Dawirs (1981) in invertebrates. Paul and Paul, (1980) studied the effects of early starvation on later feeding success of king crab zoeae.

A hypothesis was advanced by Murray and Murray (1977) that starvation suppresses and refeeding activates certain infections as an essential part of an ecological balance between men, his animals, and his environment.

Poultry are prey and their genetic code predisposes them to the flight instinct, even when they're hand-reared and tame. During stress, nutrients are depleted from the body rapidly, immune system becomes depressed, hormonal imbalances of adrenal, pituitary, thyroid, thymus is observed and they interfere with the immune function.

Aves have been long used as a model for vertebrate study. They are warm blooded like mammals, 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 they are an avian model for experimental studies.

MATERIAL AND METHOD

Material:

The present study deals with an analysis of acute **starvation** as a stress factor on the serum proteins of a species of Quails- *Coturnix coromandelica.*

As Quail is a seasonal, migratory bird its availability was an important factor to be assured. The species under observation i.e. *Coturnix coromandelica* (Rain Quail) was available during rains in this part of India (July -October).

Work Place:

The study involved a series of experiments followed by analysis. The experiments were conducted at Department of Zoology, Dayanand Girls Degree College, Kanpur, India.

Method:

For the study the birds were divided into two categories, one which were unstressed (Control group) and the other which were stressed (Experimental group). Prior to the initialization of the experiment the lab was disinfected. The entry of all kinds of possible predators was also checked. Clean water supply and proper aeration was ensured.

The birds were purchased from the local dealers. Birds 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 according to the experimental design.

Experimental Design:

For the experiment simultaneously three cages with three birds each were kept in neat disinfected cages. They were provided with water but no feed. They were deprived of food throughout the experimental period (which was seventy two hours) to provide them acute starvation conditions leading to 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, temperature, 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 of the birds was in collected in ampoules where it was left to clot. Slowly when the serum separated from the blood sample it was collected in eppendorf tubes with a lysis buffer already 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.

After the 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.

OBSERVATIONS

Behavioral Changes Observed during the Experiment in *Coturnix* coromandelica

These birds were available during rainy season and it was observed that they seemed to be fascinated by rains. A heavy downpour caused these birds to align behind one another silently to observe the falling rain.

This behavior in the **Control** Group was observed in almost all the three experiments conducted in this species. The birds were provided normal conditions of temperature, humidity, photo cycle, and were given feed and water *ad.libitum*.

They behaved in a normal way by showing maximum activity during the early hours of the day and during late hours of the afternoons. During this time 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 amongst 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 a parameter to check out the other birds under experiment.

This behavior was observed in the **Experimental group** consistently in all the three experiments conducted for starvation as a stress factor in this species.

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

After first twenty four hours of exposure they were observed to take up almost all the water provided to them. The amount of excreta produced had also increased significantly. They moved about in the cage vigorously. Their water intake increased significantly. The birds were also observed to peck at the newspaper laid at the base of the cage.

By forty eight hours of exposure the birds became quite active. They kept running along in the cage, jumped a lot and there was marked restlessness. By the evening birds showed serious signs of weariness and settled down. The excretory matter decreased.

When nearing the exposure to seventy two hours it was observed that the water consumption by the birds had also decreased. They were not interested in drinking water also. They did run in the cage, all around, and their heads stretched to the maximum limit. The birds were also observed to be quite puffed up and swollen by the end of the experiment.

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

All the three experiments conducted presented a gel surface photograph each of the blood serum of the birds in the experimental group exposed to continuous starvation 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.

Each gel surface was run with serum of a control bird and 3 experimental birds kept in that experiment.

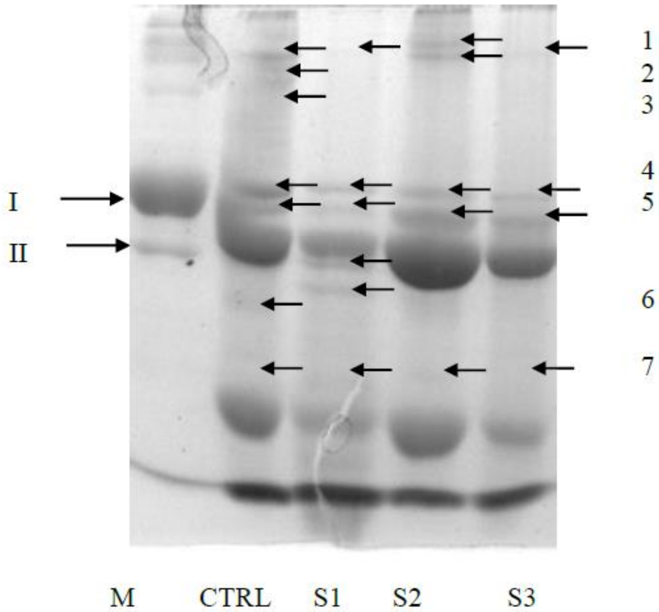
I EXPERIMENT

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 S1, S2 and S3 (the three experimental samples). The second band in the control is a moderate band but does not appear in the experimental samples except S2. The third band is observed only in the control sample and is not found in S1, S2 and S3. The fourth band observed in the control is a thick band and finds equal correspondence in all the experimental samples. The fifth band in the control is also a thick one and is similar in correspondence in all the samples like the fourth band. Below the fifth band observed in the control sample two distinct bands are observed in the experimental sample S1. These bands are absent in the control sample. The sixth band observed in the control sample finds its presence only in the control and is absent in the experimental samples and it is a thin band. The seventh band in the control is found to be similar and corresponding in all the samples.

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 also showed two bands whose absence was marked in the control sample.

I EXPERIMENT



Starvation as a Stress Factor in Serum Protein

PLATE NUMBER 1

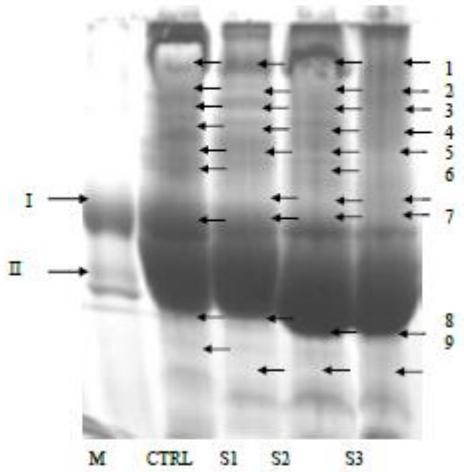
II EXPERIMENT

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 group has corresponding appearance in S1, S2 and S3 (the three experimental samples). The second band observed in the control sample is a thin band, has equal correspondence in all the experimental samples although the size of the band appears increased and thicker in all the experimental samples. The third band observed in the control sample is again similar in correspondence in all the experimental samples. The fourth band observed in the in the control sample is also same in width and appearance in all the experimental samples. The fifth band of the control sample also appears similar in all the experimental samples. The sixth band can be seen in the control sample and in S2 but not in S1 and S2. After the sixth band in the control sample a band is observed in all the experimental samples but cannot be traced in the control sample. The seventh band in the control sample is a thick band indicating its prominence, is similar and corresponding in all the experimental samples. The eighth band in the control sample is also present in all the experimental samples but they are not placed at the same level i.e. the control sample depicts the band at the highest level when compared to the others, followed by S1 which is placed higher in position to S2 and S2 is placed higher than S3. The ninth band in the control sample is found only in the control sample and cannot be traced in the experimental samples. Below the ninth band observed in the control sample a thick band is observed in all the experimental samples but is observed to be absent in the control sample.

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 two bands whose absence was marked in the experimental samples. The experimental samples showed a band whose position was a little lower than the corresponding control sample’s band. A protein band was also found to be thicker in the experimental samples as when compared to the control sample.

II EXPERIMENT



Starvation as a Stress Factor in Serum Protein

PLATE NUMBER 2

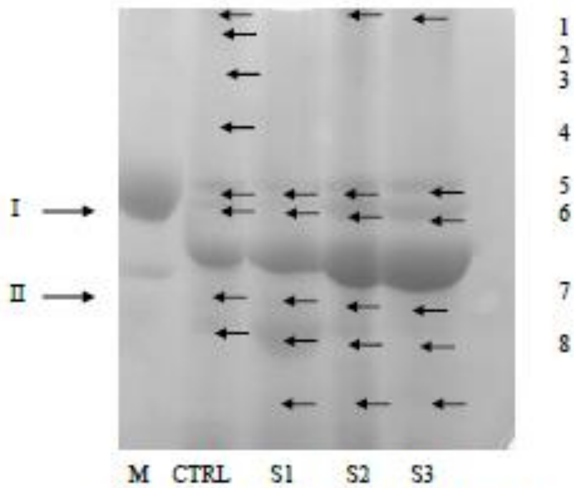
III EXPERIMENT

The whole surface has major two portions due to a presence of a thick banding as seen. (Plate No. 3)

The first band as seen in the control group has corresponding appearance in S2 and S3 whereas it cannot be seen in S1 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 sample has similar corresponding presence in all the samples, but is found to be thicker in S1 than the control sample, in S2 it is thicker than S1 and in S3 it is found to be the thickest. The sixth band observed in the control sample has correspondence in all the experimental samples similar to the fifth band. The seventh band in the control sample is a thin one and it shows similar appearance in S1, S2 and S3. The eighth band formed in the control sample is a thin band and corresponding in position in all the experimental samples although the band shows increased thickness in the experimental samples when compared with the control. Below the eighth band in the control sample there is a presence of a thin band in all the experimental samples not observed in the control sample.

Thus in the third experiment conducted on *Coturnix coromandelica* the control sample showed three bands whose absence was marked in the experimental samples. The experimental samples also 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.

III EXPERIMENT



Starvation as a Stress Factor in Serum Protein

PLATE NUMBER 3

DISCUSSION

The choice of starvation as a factor was based on the fact that anthropogenic factors have reduced both feeding sites and food for animals forcing them to starve unnaturally.

All animals face the possibility of limitations in food resources that could ultimately lead to starvation-induced mortality as studied by Marshall D (2010).

It has been found that starvation suppresses and refeeding activates certain infections as an essential part of an ecological balance between man, his animals, and his environment (Murray and Murray, 1977).

When faced with stress, an organism calls upon several mechanisms to maintain biological homeostasis. One of the manifestations of these central and peripheral noradrenergic changes is the change in blood pressure during the starvation-feeding cycles. El Fazaa *et al*., (1999) concluded that starvation should be considered as an unusual state of stress.

In fish chronic stress, like food deprivation, can compromise the ability of the animal to compensate for additional stressors, such as acute handling (Vijayan and Moon, 1992; Blom *et al.*, 2000).

Reductions in food supplies caused by human activities along with chemical contaminants in the environment can be expected to adversely influence reproductive success and pose serious restrictions on avian populations. Treatments affect females more than males as observed by Keith and Mitchell (1993).

Food deprivation decreases water intake in many species, including humans as seen in studies (Wolf, 1958; Olsson *et al.*, 1997) however an opposite behavior was seen in the experimental birds *Coturnix coromandelica*, where by the time of nearing the exposure to seventy two hours it was observed that the water consumption had increased.

Starvation tolerance is very variable among species. Genetic variation in stress tolerance will result in adaptive changes that depend on the environmental conditions faced by organisms (Dev and David, 2000).

It has been established by Berg *et. al.,* (2002) that the metabolic changes observed on the first day of starvation are like those after an overnight fast. After about 3 days of starvation the liver produces large quantities of ketone bodies, which are released into the blood. The heart also uses ketone bodies as fuel.

Between October 2016 and January 2017, inhabitants of St Paul Island in the Bering Sea found the starved bodies of more than 350 seabirds, primarily tufted puffins. Collected specimens were severely emaciated, suggesting starvation as the ultimate cause of mortality as reported by Ruby Prosser (2019).

Four bands on the whole in *Coturnix coromandelica* 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 was initiated when conditions are 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 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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