

Original Article

Noise-induced hormonal & morphological malformations in breeding pigeons

Malformações hormonais e morfológicas induzidas pelo ruído em pombos reprodutores

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Abstract

Environmental pollution has the potential to have a significant impact on animal's health especially on birds due to daily exposure and habitat. This experimental study was carried out for a 60 days period in which, a total of 24 pigeon birds with suitable weight (80-100 g) were kept in Animal house with suitable environmental conditions viz, controlled temperature, humidity & light source to minimize any other stress. Out of twenty-four, eighteen birds were divided into three treatment groups (6 birds in each group). Whole experiment was run in triplicate manner in breeding season. One served as Control (Group 1) and remaining three were experimental groups including Road traffic noise (Group 2), Military noise (Group 3) & Human activities noise (Group 4). Noise was applied as recorded high intensity music (1125 Hz/ 90 dB) through speakers for 5-6 hrs. daily. Blood sampling was done after 20, 40 and 60 days by sacrificing treatment birds. Noise stress significantly ($p < 0.05$) increase the serum levels of corticosterone and thyroid stimulating hormone (TSH) in Group 2 while significantly ($p < 0.05$) decrease the serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) of Group 3 birds. Moreover, major fault bars formation was seen both in Group 2 and Group 3. It was concluded as that Noise stress caused rise in serum levels of Corticosterone and TSH but fall in LH and FSH. Along with fault bars formation was also prominent in all treatment groups due to stress hormone.

Keywords: noise pollution, hormonal physiology, faults bars, breeding season, pigeons.

Resumo

A poluição ambiental tem o potencial de impactar significativamente a saúde animal, especialmente das aves, devido à exposição diária e ao habitat. Este estudo experimental foi realizado por um período de 60 dias em que, um total de 24 pombos com peso adequado (80-100 g) foram mantidos em biotério com condições ambientais adequadas, ou seja, temperatura, umidade e fonte de luz controladas para minimizar qualquer outro estresse. De 24, 18 aves foram divididas em 3 grupos de tratamento (6 aves em cada grupo). Todo o experimento foi executado em triplicado na época de reprodução. Um deles serviu como controle (Grupo 1) e os 3 restantes foram grupos experimentais, incluindo ruído de tráfego rodoviário (Grupo 2), ruído militar (Grupo 3) e ruído de atividades humanas (Grupo 4). O ruído foi aplicado como música gravada de alta intensidade (1125 Hz/90 dB) através de alto-falantes por 5-6 horas diárias. A coleta de sangue foi feita após 20, 40 e 60 dias sacrificando as aves do tratamento. O estresse sonoro aumentou significativamente ($p < 0,05$) os níveis séricos de corticosterona e hormônio estimulante da tireoide (TSH) no Grupo 2 enquanto diminuiu significativamente ($p < 0,05$) os níveis séricos de hormônio luteinizante (LH) e hormônio folicúlo estimulante (FSH) do Grupo 3. Além disso, a maior formação de barras de falha foi observada tanto no Grupo 2 quanto no Grupo 3. Concluiu-se que o estresse por ruído causou aumento nos níveis séricos de corticosterona e TSH, mas queda em LH e FSH. Junto com a formação de barras de falha também foi proeminente em todos os grupos de tratamento devido ao hormônio do estresse.

Palavras-chave: poluição sonora, fisiologia hormonal, barras de falhas, época reprodutiva, pombos.

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1. Introduction

Among all sorts of environmental stresses, noise stress is the most common and persistent causing negative morphological and physiological impacts (Akintoye et al., 2003). Noise pollution from building sites, transportation, and other sources has become an unavoidable part of avian lives (Kawada, 2004; Riley and McGregor, 2012). Noise exposure can affect bird reproductive success in diverse ways (Halfwerk et al., 2016). Small passerines, such as Great Tits (*Parus major*), European Robins (*Erithacus rubecula*), House Sparrows (*Passer domesticus*), and Pied Flycatchers (*Ficedula hypoleuca*) for instance, have been shown to exhibit altered reproductive behavior as a result of noise pollution (Carvalho et al., 2006; Francis et al., 2009; Kleist et al., 2018; Biard et al., 2017).

Hormones interact with environmental and reproductive variables to influence reproduction and growth in bird species (Farner and Wingfield, 1980; Scanes et al., 1984). The effect of chronic noise stresses showed a significant decrease ($P < 0.01$) in serum estradiol, progesterone, LH and FSH hormones in female Albino Rats (Helal et al., 2014). During noise stress there is a decrease in releasing of gonadotropin releasing hormone (GnRH), which in turn leads to decrease of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary (Whirledge and Cidlowski, 2010). During moments of prolonged stress, birds may physically limit the activity of the hypothalamic-pituitary-adrenal (HPA) axis and increased the levels of glucocorticoid concentrations (Cyr and Romero, 2009).

Moreover, noise has been shown to have an effect on glucocorticoid signaling among various bird species, including adults and nestlings (Kleist et al., 2018). Corticosterone levels and chronic ambient noise have been linked in a number of bird species (Blickley et al., 2012; Crino et al., 2011; Kleist et al., 2018; Potvin and MacDougall-Shackleton, 2015; Wright et al., 2007a).

Furthermore, the short- and long-term effects of noise exposure on the levels of the thyroid stimulating hormone (TSH) are responsible for an increase in T3 levels and decrease in TSH levels (Ababzadeh et al., 2020). As a result of prolonged noise exposure, amount of thyrotropin-releasing hormone (TRH) increases, which in turn increase the production of thyroid stimulating hormone (TSH) (Mariotti and Beck-Peccoz, 2021).

Ptilochronology, a method for measuring growth bars, has been employed as a direct indicator of the environmental stress status of birds throughout the time of feather growth (Grubb Junior, 1989). Bird's feathers can develop more quickly in favorable conditions, resulting in broader growth bars, whereas feather growth can be slowed down in unfavorable conditions, resulting in narrower growth bars (Grubb Junior, 2006). Sluggish plumage growth in offspring in birds were caused by high corticosterone levels (Saino et al., 2005).

Pigeons are frequently employed for physiology, toxicology, and pathology research (Goodale, 1983). Pigeons have served as useful models in investigations on growth, metabolism, endocrinology, toxicity, and issues

with reproduction (Wagner and Clarkson, 1974; Elder, 1964; Sturtevant, 1970).

This study is important to understand the impact of selected level of noise stress on physiological/morphological changes in breeding birds. So, the present study is planned to know the possible effects of noise on serum luteinizing hormone (LH), follicle stimulating hormone (FSH) thyroid stimulating hormone (TSH), corticosterone (stress hormone) and fault bars formation in molted feathers of breeding season pigeons. The major objectives of this study were to determine the various hormonal and fault bar formation phenomenon under noise stress.

2. Methodology

2.1. Study animals

Twenty-four pigeon birds with suitable weight (80-100 g) were bought from market dealt with avian purchase/sale work under license and was kept in animal house of Department of Zoology, The Islamia university of Bahawalpur. The birds were housed in cage of standard size (**20 × 45 × 20 cm**) and was kept under observation for one week before the onset of the experiment to acclimatize to laboratory conditions and fed on water and *ad libitum*. Suitable environmental conditions especially controlled temperature, humidity & light source were adapted to minimize any other stress level on birds. Out of twenty-four, eighteen birds (18/24) were divided into three treatment groups (6 birds in each group). In each group 6 birds were kept. Daily observations were recorded in the morning and afternoon. The whole experiment was run in triplicate manner for a period of 60 days on breeding birds in breeding seasons. For identification purpose, each cage according to group and each bird was tagged by using different types of identification marks/ID using colored ribbons.

2.2. Noise-source categories

There are three selected noise-source categories as: **Road traffic, military, and human activity sources.**

- 1. Road traffic sources** (both commercial and private vehicles, including road traffic (motorcycles, automobiles, buses etc., and industrial noise source).
- 2. Military sources** (gunfire, explosions, aircraft and entire military training operations).
- 3. Human activity sources** (machinery/equipment noise, alarms, loud speakers, tape recorders, DVD/ CD players etc.).

2.3. Application of noise

Noise will be applied through different sources of recorded high intensity music (1125 Hz/ 90 dB) for 5-6 hrs daily.

2.4. Animal groups

Twenty-four pigeon birds were divided into four groups (six birds in each group), one was served as Control

(Group 1) and three were served as treatment groups (Group 2, Group 3 and Group 4).

Group 1: This was served as control group with six untreated birds for a duration of 60 days.

Group 2: Six birds were exposed to loud traffic/industrial noise source recordings (90db, 5-6 hrs/per day) for a duration of 60 days.

Group 3: Six birds were exposed to military noise recordings (90db, 5-6 hrs/per day) for a duration of 60 days.

Group 4: Six birds were exposed to human activity sources noise recordings (90db, 5-6 hrs/per day) for a duration of 60 days.

2.5. Laboratory analysis

2.5.1. Blood collection

Sample collection from blood was done in three phases. During first phase (after 20 days of trial), blood sample was taken from bird's right juglar vein. During second phase (after 40 days of trial) and third phase (after 60 days of trial) again blood sample was taken from bird's right juglar vein. Blood samples were taken by a 23-gauge needle attached to a 5 mL syringe and sample were preserved in EDTA tubes.

2.5.2. Hormonal analysis

For hormonal analysis of TSH, Corticosterone (stress hormone) and gonadotropins (FSH & LH), the blood sample was centrifuged for 15 minutes at 3000rpm. Sera was collected and keep at -20 °C till analysis. The technique of Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the levels of hormones following method (Swami et al., 2007; Helal et al., 2014).

2.5.3. Fault bar formation phenomenon

Induced molting was performed in all three treatment groups given different noise sources to evaluate the effect of noise stress (1125 Hz) on feathers growth. Tail feathers was selected to study fault bar formation. Feathers were plucked in alternate manner. Feathers from tail were plucked one after another in order to induce forced molting (Farman, 2008).

2.6. Statistical analysis

Hormonal data from breeding birds was analyzed by IBM SPSS version 25.0 by One-Way ANOVA and Duncan Multiple Range Test with significance level at ($P \leq 0.05$).

3. Results

3.1. Serum hormonal physiology

An experimental trial was run on pigeons in breeding season to know the impact of noise stress on hormonal physiology (LH, FSH, TSH and corticosterone) and ptilochronology (fault bars that were made on bird's tail feather) of breeding birds. The serum concentrations of hormones and fault bars patterns were compared with that of respective Control groups.

According to findings, serum LH levels in the plasma of pigeons varied significantly within all groups. The highly significant ($p < 0.05$) low levels of LH were observed in Group 3 while Group 2 and Group 4 levels were less significantly ($p < 0.05$) low when compared to Control Group (As shown in Table 1 and Figure 1). Serum FSH concentrations in plasma levels of breeding pigeons were highly significant ($p < 0.05$) among all groups. The FSH levels of Group 3 were significantly ($p < 0.05$) lower when compared to Control group whereas Group 2 and Group 3 showed less significantly ($p < 0.05$) dropped levels of FSH when compared with Control Group (as shown in Table 1 and Figure 2).

The mean serum TSH levels in the plasma of breeding pigeons were significant ($p < 0.05$) among all groups. Group 4 levels of TSH were significantly ($p < 0.05$) increased when compared to control group while TSH levels of Group 2 were significantly ($p < 0.05$) increased as that of control group. On other hand, TSH levels of Group 4 were significantly ($p < 0.05$) lower when compared

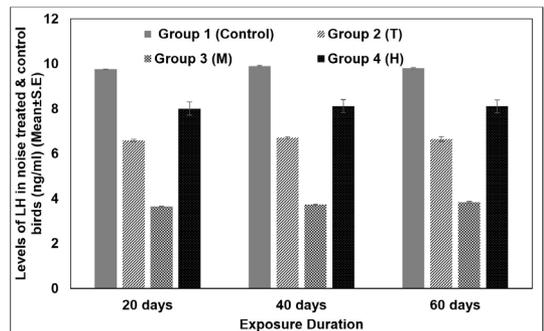


Figure 1. The Mean \pm S.E of LH levels of 4 groups at different levels of Noise stress in Breeding season.

Table 1. LH, FSH, TSH and Corticosterone levels of breeding pigeons after noise exposure.

Breeding Hormones	Noise Category (Mean \pm S.E)			
	G1 (Control)	G2 (Traffic)	G3 (Military)	G4 Human
LH (ng/mL)	9.82 \pm 0.044 ^d	6.65 \pm 0.26 ^b	3.74 \pm 0.067 ^a	8.07 \pm 0.06 ^c
FSH (ng/mL)	8.64 \pm 0.041 ^d	7.8 \pm 0.03 ^b	5.51 \pm 0.082 ^d	8.04 \pm 0.08 ^c
TSH (nmol/L)	37.84 \pm 0.29 ^c	55 \pm 0.58 ^b	28 \pm 0.56 ^d	69 \pm 71 ^a
Corticosterone (ng/mL)	0.545 \pm 0.01 ^c	0.68 \pm 0.01 ^b	0.34 \pm 0.01 ^d	0.77 \pm 0.02 ^a

Similar letters (a, b, c, d) in a single row represents non-significant difference within groups according to Duncan Multiple Range Test.

to control group (as shown in Table 1 and Figure 3). In the blood plasma of birds, mean serum concentration of corticosterone hormone varied significantly ($p < 0.05$) among all groups. Mean serum corticosterone levels of group 2 and group 4 showed significantly ($p < 0.05$) higher values when compared to control group. Whereas group 3 showed significantly ($p < 0.05$) dropped levels of corticosterone among all when compared to control group (as shown in Table 1 and Figure 4).

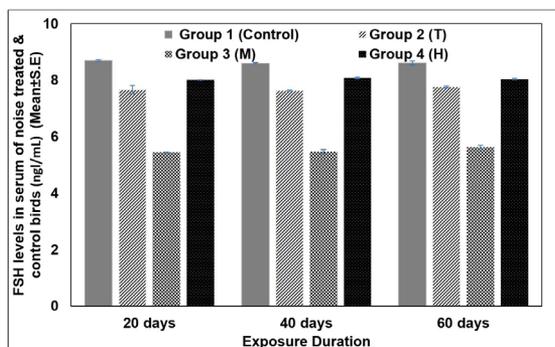


Figure 2. The Mean \pm S.E of FSH levels of 4 groups at different levels of Noise stress in Breeding season.

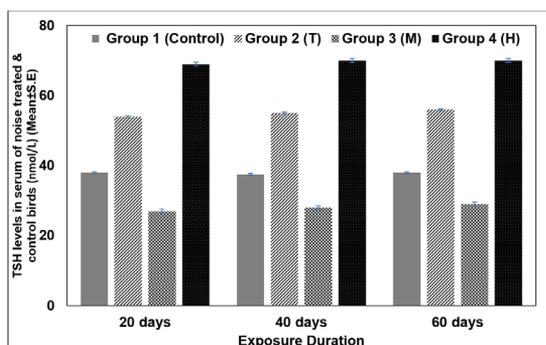


Figure 3. The Mean \pm S.E of TSH levels of 4 groups at different levels of Noise stress in Breeding season.

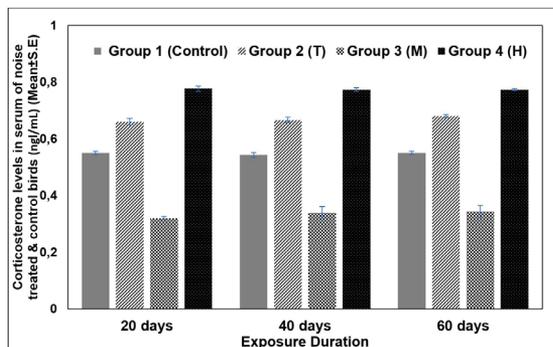


Figure 4. The Mean \pm S.E of Corticosterone levels of 4 groups at different levels of Noise stress in Breeding season.

3.2. Ptilochronology

Results of this noise induced experimental trial showed major fault bars formations in molted feathers of birds. Plucking of normal patterned feathers were done after 15 days of starting experiment then a keen observation was recorded for the molted new feathers. Newly formed feathers had a clear growth fault bars which are showing in the given pictures according to different noise groups.

Feathers of breeding pigeons from Group 1 (Control) had a normal barbule structure (Figure 5). In Group 2 (Traffic noise) clear fault bars were formed near the mid rib of left side and on the margins of right and top side of feather (Figure 6). Whereas fault bars present on feathers of breeding pigeons in Group 3 (military noise) also showed clear and visible deformation across mid rib and on margins (Figure 7). In the feathers of Group 4 (anthropogenic noise) birds, a less patterned fault bars were seen (Figure 8).

4. Discussion

As revealed by our results, serum hormonal levels of LH and FSH in breeding pigeons were decreased while TSH and corticosterone levels were increased by sound stress. This might be due to chronic stress faced by birds. Chronic stress can have a variety of negative effects on animals, including birds. When animals are exposed to long-term stressors, it can result in changes to their physiology and behavior, including alterations to their endocrine system. As early documented by a study that environmental sound stress can induce increased secretions of adrenal gland and can decrease the levels of pituitary gonadotropin hormones



Figure 5. Group 1 (Control Group) showing normal patterns in the feather of breeding pigeon.



Figure 6. Group 2 (Traffic noise source) showing fault bars in the feather of breeding pigeon.



Figure 8. Group 4 (Human activities noise source) showing less fault bars in the feather of breeding pigeon.



Figure 7. Group 3 (Military noise source) showing intense fault bars in the feather of breeding pigeon.

and eventually caused disturbed reproductive behaviours and functions in animals (Hafez and Hafez, 2004).

Serum hormonal results obtained from this experiment are compatible with an earlier study in which they found that a decrease in the levels of gonadotropin-releasing

hormone (GnRH) is possible after exposure to stress and can further lead to a decrease in the levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) after exposure to stress as investigated by (Whirledge and Cidlowski, 2010).

In harmony of our results, a study concluded that various types of stress can trigger the increase in glucocorticoid secretion from the adrenal gland which in turn reduces the levels of gonadotropins from pituitary gland (Whirledge and Cidlowski, 2010; Rabin et al., 2008; Collu et al., 1984). When glucocorticoid secretions are increased, they can reach at hypothalamus level and inhibit the release of gonadotropin releasing hormone (GnRH) (Dubey and Plant, 1985; Kamel and Kubajak, 1987). Effect of these glucocorticoids on pituitary will result in decline in serum LH levels (Briski and Sylvester, 1991).

As indicated by our results serum corticosterone levels in the plasma of breeding pigeons were increased after exposure to noise stress. It seems to suggest that noise stress can cause an increase in plasma stress hormones in rats. These finding could have potential implications for understanding how stress affects different physiological systems and how it might contribute to other negative outcomes (Glover et al., 2012). Corticosterone is a hormone that is produced by the adrenal gland in response to stress, and it plays an important role in regulating the body's stress response. Our present results are in favor with the finding of a study in which results reported that stress can increase plasma corticosterone levels in animals, which in turn can affect their gonadal functions and reproductive behaviors (Li et al., 2013). Our results are also supported by a study in which they demonstrated that noise stress may be attribute to rise in the levels of adreno-corticotropin

and cortico-releasing hormones in rats (Thorsell, 2010). This rise in cortico-releasing hormones can inhibit the serum levels of LH and FSH as documented by in a study (Novaes et al., 2001).

In birds, regulation of thyroid hormones is not controlled by anterior pituitary gland but its regulation is controlled by the T3 levels, that is somewhat different from mammals (Merryman and Buckles, 1998). There are two forms of thyroid hormone i.e., T3 & T4. T4 is biologically active form (Harr, 2002; Hudelson and Hudelson, 2009; Kaneko, 1997) which majorly regulates the metabolism, plumage and fertility in birds (Merryman and Buckles, 1998). When talk about any type of stress, its effect is inhibitory for thyroid functioning in birds. Levels of plasma cortisol also becomes high with increasing stress. Increased levels of cortisol can lead to decrease in the levels of T3 & T4 or thyroid hormones (Chastain and Panciera, 1995; Palme et al., 2005). As our results revealed and was documented in a study that high cortisol levels may result in decreased levels of T3 & T4 due to which negative feedback will occur that would increase the secretion of TSH in serum of birds (Baos et al., 2006).

In relation to feather growth, bird plumage is unusual because feathers may be grown during a specific molt season, but can then remain influential throughout a bird's life, generally a full season past the molt. It has been demonstrated in many studies that the condition of a bird during molt season influences its ability to produce feathers that are of superior quality since energy and nutrients are expended to maintain a complete complement of body and flight feathers. The compensatory mechanism of individuals under nutritional stress was to exhibit reduced feather growth and plumage abnormalities (Grubb Junior, 1989; Jenkins et al., 2001; King and Murphy, 1985; Yosef and Grubb, 1992) to compensate for nutritional demands of other activities.

Corticosterone is a hormone that is produced in response to stress in birds and other animals, and it can have negative effects on feather growth and quality. Studies have shown that when birds are exposed to high levels of corticosterone, either through natural stressors such as food shortage or through experimental manipulation, their feather growth can be slowed down or even halted altogether. Additionally, the feathers that do grow under these conditions may be of lower quality, with reduced structural integrity and lower levels of pigmentation. These effects are thought to be due to the way that corticosterone affects the bird's metabolism and immune system, diverting resources away from feather growth and maintenance and making the birds more vulnerable to pathogens and other stressors (DesRochers et al., 2009; Müller et al., 2009; Almasi et al., 2012).

We can say that in the light of our results that fault bars are formed in the feathers of breeding pigeons are due to ecological stress that is in our case is noise. Our findings are in the favor of a study in which it is summarized that ptilochronology is identified as a biomarker, it has been used to address a variety of ecological issues and, in most cases, has produced excellent findings as a gauge of habitat quality (Brown et al., 2002; Strong and Sherry, 2000; Vangestel and Lens, 2011).

4.1. Limitations of study

We revealed some limitations for this study while reviewing the literature. Birds have a strong affinity for light duration. Long days are common during breeding season. More light hours can have a direct impact on bird reproduction and thyroid function. As a result, the daylight hours were strictly controlled. On a daily basis, humidity was managed alongside light. Moving on to methodology, taking samples of blood from jugular veins of birds was extremely difficult because birds do not provide enough blood to accomplish all laboratory tests. As a result, blood samples were collected rapidly.

5. Conclusion

It is concluded that Noise stress caused rise in serum levels of Corticosterone and TSH but fall in LH and FSH. Fault bars formation was also prominent in all treatment groups of breeding pigeons.

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