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EFFECTS OF DIETARY DEXAMETHASONE ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF PREMATURE JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

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ABSTRACT: The objective of this study was to examine the effects of dietary supplementation of various Dexamethasone (DEX) concentrations on productive and reproductive performance of premature male and female Japanese quails. Japanese quail of 3-weeks-old were received DEX at 0 (control group), 0.25 (low dose treated group) and 0.5 (high dose treated group) mg/kg diet, mixed in their mash, till the 42th day of age. As a result of this study, in high dose treated group, there were significant ($P \le$ 0.05) increases in the sex organs weight (g) and laying rate (%) of females meanwhile there was a significant decrease in egg weight and fertility percentage ($P \le 0.001$). No significant differences were observed in total testes weight (g) of males but cloacal gland area (mm²) was smaller in both low and high dose male groups compared to control. The serum corticosterone (CORT) level was significantly ($P \le 0.001$) higher in low dose treated males whereas no significant changes were recorded in high and low doses treated females. Conversely, a significant ($P \le 0.008$) increase in serum estradiol level was measured in treated females but there were no significant changes in serum testosterone level in treated males. The medullary tissues of the adrenal glands were increased on the expense of cortical tissues in birds treated with higher dose of DEX. The seminiferous tubules of the low and high dose treated males did not show all the stages of spermatogenesis and most of the till spermatid stage was inspected with morphologically abnormal cell. The present results concluded that sex differences exist in response to DEX administration prior to reproduction, which may be due to the different prevalence of certain sex steroids influences in specific periods of life.

Keywords: Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

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1. INTRODUCTION

Many environmental factors can lead to stress in animals in their natural habitats and consequently may cause elevations in stress hormones (Kang et al., 2020 and Tufarelli et al., 2021). The stress response is an adaptation process that is initiated when individuals experience a diversity of stressors, where stressors are stimuli that may form physical challenges or perceived as threats (DuRant et al., 2020). The main responses to stress are activation of the hypothalamic-pituitaryadrenal (HPA) axis and glucocorticoids secretion (GCs) hormones which can help animals coping with the changes in the environmental factors (Vitousek et al., 2019). However, long-term exposure to GCs can impair a variety of behaviors and physiological processes as well as suppress the immune functions. The activation of HPA axis by many stressors has been associated with down regulation hypothalamic-pituitary-gonad of the (HPG) axis which provides a reasonable mechanism for observation of stressinduced reproductive dysfunctions (Iwasa et al., 2017). Accordingly, prolonged GCs treatment prior to puberty can inhibit gonadal axis function and may in turn influence sexual maturation development (Shi et al., 2011).

Corticosterone (CORT) is the primary GCs in birds that secreted by the adrenocortical tissue and released into the blood circulation during stress to regulate carbohydrate and lipid metabolism (Jimeno et al., 2018). Treatment with exogenous CORT can mimic its elevated levels in plasma of birds that occur during acute or chronic stress, dependent on the duration of exposure. Treating birds with CORT in their food (Lin et al., 2004) or drinking water (Hull et al., 2007) over a period of days can be induce a

temporarily changes in plasma GCs concentrations that took place when animals exposed to chronic stress. Although treatment with CORT stimulates feed intake in some studies (Lõhmus et al., 2006), no effect or even decrease of feed intake was observed in other studies (Wall and Cockrem 2010). Chronic activation of the stress system may also interfere with the nutritionrelated hormones and often, but not always, lead to reduction of body weight gain of birds (Hull et al., 2007 and Busch al.. 2010). Furthermore, CORT et elevation can even act at the levels of reproductive hormones and consequently regress gonadal growth rate and egg laying rate in females (Salvante and Williams 2003) as well as testes weight and spermatozoa production in males (Hanafy and Khalil, 2015). The harmful effect of CORT elevation was observed in free-living birds during critical periods of nestling provisioning (Bonier et al., 2009). Despite the conduct of varied research related to the impact of elevating blood GCs level on the reproduction success, it is still unclear the casual relationship between sex steroids and development CORT on the of reproductive system in birds.

Dexamethasone (DEX), a synthetic analogue glococorticosteroid, is widely used in both human and animals medicine to treat several disease and mange medical condition. This compound has broad pharmacological activities, which reflects its significant role on physiological and biochemical pathways (Wyns et al., 2013). It is well known that DEX treatment mimics the adverse effects of elevated CORT level in the blood and precedes а decline in testosterone level of breeder males (Hanafy and Khalil, 2015). Most of this

Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

inhibitory effect on male reproductive function was related to suppress androgen synthesis and reduction in the number of Leydig cells (Hardy et al., 2005 and 2017). Semet et al., In contrast, administration of DEX to females improved ovarian responsiveness bv diminished effect of adrenal androgens on follicular growth (Ashrafi et al., 2007). However, it is still unclear what causes the variable effects of stress hormone releasing on reproductive success of male and female birds. Studies using animal models have clearly investigated that males and female's response contrastingly to stress, which may be in part, due to the diverse in prevalence of sex steroid during particular periods of life (Wang et al., 2019b).

Due to the little available knowledge about the variable effects of stressors during critical periods of life on reproduction of birds, immature Japanese quail was used to determine if there are sex differences in response to stress. So, this study aimed to examine the effects of ingesting diets having various concentrations of DEX on productive and reproductive performance, as well as adrenal gland and gonadal structure and function of premature male and female quail.

2. MATERIALS AND METHODS 2.1. Animal husbandry and treatment

The current work was conducted at the Poultry Farm, Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Fertile Japanese quail eggs were incubated under standard conditions. Newly hatched chicks were housed in floor pen under normal brooding conditions from hatching to 15th day age. When the chicks had developed their sexually dimorphic plumage, 144 birds were sexed and randomly distributed into 3 treatment groups (48 chicks each) of three replicates. Each replicate was included 16 birds with mating ratio of one male to one female quail.

After acclimatization for one week; on day 21 of age, birds were received DEX at dose 0 (control group), 0.25 and 0.5 mg/kg diet, mixed in their mash, till the 42th day of age. Birds were exposed to continuous light and fed on quail grower mash (2,800 kcal ME/kg diet; 24% CP) from hatch to 6-week of age. From day to day 63rd, the end of this $43^{\rm rd}$ experiment, quail breeder ration (2,900 kcal ME/kg diet; 20% CP) was provided and lighting time was decreased to 16L: 8D light/dark cycle. Diets and water were provided ad libitum throughout the experimental period.

2.2. Sampling and histological procedures

Live body weight (BW) and feed intake (FI) were estimated at 3 and 6 weeks of age. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated during the same experimental periods for each group. Since we could not identify which female laid an egg in group, we recorded the day at which we found the first egg in a group as the age at first egg (day).

The cloacal gland area (mm^2) of all males was measured by digital calliper on day 42. Also, 12 birds (6 males and 6 females) from each treatment group were weighed and slaughtered by slitting the jugular vein. Then after slaughtering, the abdominal cavity was incised to investigate the ovarian morphology, and count the number of follicles. We use the electronic analytical scale to weight the genital organs of male and female quails and also calculated as a percentage of live body weight. The blood samples were

collected from each experimental group at the 6th week of age in non-heparinized glass tubes. The samples were stored in refrigerator at 4 °C overnight then the serum was separated and stored at -20°C for further analysis. Serum testosterone, estradiol-17 β and CORT levels were measured by ELISA kits manufactured by DiaMetra, Spello-Perugia, Italy for sex hormones and IBL, Hamburg, Germany for adrenal hormone. The sensitivity of the assay was 1.631 ng/ml, 8.7 pg/ml and 70 pg/ml and the recovery percentage was 100-105, 95-103 and 95-100% for testosterone, estradiol-17ß and CORT, respectively. The intra- and inter-assay coefficients of variability were 4.08, 5.54 and 9% and 5.8, 10.5 and 10% for CORT, testosterone and estradiol-178. respectively.

In the remaining quail, laying rate (%) and egg weight (g) were estimated for all groups up to 9 weeks' age. During this period, the laid eggs were collected daily for 4 consecutive days and stored at 18°C 65% relative humidity and until incubation. The eggs then were incubated for 8 days at 37.5°C and 60% relative humidity. Fertility was ensured and verified by inspection eggs then calculated as ratio of the fertile eggs to total eggs number.

To assess the effect of DEX on adrenal gonadal structures, gland and the testicular and adrenal tissues were fixed in 10% formalin then left for at least one week in refrigerator and after that preserved in 70% ethyl alcohol in refrigerator. The preserved samples were dehydrated by graded ethyl alcohol series (75%, 80%, 90%, 95%, 3 changes of absolute ethyl alcohol), then subjected to three changes of xylene, and after that embedded in paraffin wax. The paraffin blocks containing the specimens were

sectioned at $5 - 7 \mu m$ thickness. The paraffin sections were exposed to Harris hematoxylin and eosin (H&E) and Masson's trichrome stains. The tissue sections were examined by light microscope and the photomicrographs Olympus BX41 were taken using microscope with an Olympus DP25 digital camera, Department of Cytology and Histology, Faculty of veterinary Medicine, Suez Canal University.

2.3. Statistical analysis

The differences between the treatments were statistically analysed by SPSS Statistics 22.0 using General Linear Model. The significance of differences among means of treatments were measured using Duncan's new multiplerange test (Duncan, 1955). $P \le 0.05$ was set as limit of significance.

3. RESULTS

3.1. Growth performance

A slight improvement of the growth performance in the DEX treatment groups compared with control group was observed (Table, 1) but there was no significant difference in the WG ($P \le 0.894$), FI ($P \le 0.993$) and FCR ($P \le 0.707$) among the three groups.

3.2. Laying rate and fertility

As shown in Table (2), the present results revealed that immature DEX exposure of female quail caused 2-4 days faster of produce eggs in treated groups, in pergroup-sexual-maturity (i.e. Age at first egg). While the per-group age at first egg in controls was at 43 days of age, DEX treated females were earlier (41 and 39 days) to begin egg production as group 0.25 and 0.5 (mg DEX/kg diet), respectively. The laying rate showed likewise dose liberated effect and in the controls was 42.0 %, and this was not significantly higher than the rate of egg production in the low DEX treatment

Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

group, except for the 0.5 mg DEX/kg diet (Table 2). While DEX treatment significantly ($P \le 0.001$) altered average egg weight in compare to control, average egg number per day was significantly higher in 0.5 DEX group. In contrast, fertility percentages of eggs produce from treated female quails was significantly $(P \le 0.001)$ lower than that eggs produce from control bird and the lowest percentage was recorded in 0.5 DEX treated birds.

3.3. Morphometric analysis

Significant increases in the absolute ovary weight ($P \le 0.024$), oviduct weight $(P \le 0.011)$ and average ovarian yellow follicle numbers ($P \leq$ 0.030) were recorded in high dose treated group (Table 3), but not with low dose treated group. In contrast, oviduct length (cm) and weight (g), relative oviduct weight (%), relative ovary weight (%) and average ovarian vellow follicle size (mm) were significantly increased in both low and high doses of treated females in compare to untreated bird. In male quails, no significant differences in weight (g) of right testis, lift testis and total testes weight were observed in both low and high dose groups, while cloacal gland area (mm²) was significantly ($P \le 0.042$) decreased in male birds exposed to 0.5 mg DEX/kg diet (Table 4).

3.4. Hormonal assay

As shown in Figure (1), the CORT level in low dose treated males was increased significantly ($P \le 0.001$) but not changed significantly in high dose (0.5 mg DEX/kg diet) treated males. Also, there was no significant change in testosterone level in the blood of both low and high treated males in compare to control. In female quail, no significant difference of CORT level was reported in both low and high treated females. The level of serum estradiol-17 β was significantly ($P \le 0.008$) increased in both treated females' groups in compare to control group (Fig. 1).

3.5. Histological investigation

3.5.1 Adrenal gland

Generally, the adrenal gland of the quail was enclosed by thin connective tissue layer having blood vessels. The gland composed of two different endocrine areas; the cortex and medulla (Figs. 2A, 2D) which were intermingled together all over the gland. There are no observed differences in the adrenal corticomedullary percentages between males and females. The medullary tissue formed complete network of different islets while the cortical tissue consisted of solid, irregular, cylindrical cell cords.

Medullary cells were polyhedral in shape and larger in size than cortical cells, with basophilic cytoplasm and spherical, centrally located nuclei (Fig. 2A). The cortical cells were columnar with a small; spherical to slightly oval eccentrically located nucleus showing numerous mitotic figures, and the cortical tissue occupied a major part of the gland.

In low dose treated birds, there was an obvious increase of medullary tissues than in control group (Figs. 2B, 2E). Additionally, the medullary tissues were increased on the expense of cortical tissues in birds treated with higher dose of dexamethasone (Figs. 2C, 2F).

3.5.2 Testis

The seminiferous tubules of the control group showed all typical stages of spermatogenesis (spermatogonia, primary spermatocyte, secondary spermatocyte and spermatid), the cell layers were about 8-10 layers with a narrow lumen due to the high number of cell layers that occupies the interior of the tubule (Fig. 3A). Meanwhile the seminiferous tubules

of the low and high dose treated groups showed definite structural changes, the most marked effects are that all stages of spermatogenesis are not seen and only show the stages till primary spermatocyte or secondary spermatocyte or till spermatid with wide lumen filled with tissue debris and abnormal cells. (Figs. B, 23C).

4. DISCUSSION

This work was conducted during the growing period of male and female quails (3 to 6 weeks) since premature Japanese quail are more sensitive to stress hormones exposure than older birds. Thus, we investigated the effect of ingesting diets having various DEX concentrations on FCR and BWG, as well as adrenal gland and gonadal structure and function. Also, age at first egg, laying rate, egg weight and fertility percentages of eggs were calculated for exposed birds during pubertal period of life.

The present results showed no significant differences in BW, WG, FI and FCR in both low and high doses of male and female quails. In consistent with the present findings, Hanafy and Khalil (2015) found that DEX administration at 0.25 and 0.50 mg/kg diet did not decrease the BW and FI of mature male quail. The previous results might be due to less sensitivity of older birds to DEX administration (Breuner et al., 2008). Aengwanich (2007) stated that the BW of DEX treated broiler chicks decreased significantly control than group. Administration of DEX can increase the breakdown of protein and speed up energy consumption (Wang et al., 2017). Other researchers recorded that DEX administration could increase feed intake through inhibition of leptin-induced satiety and increasing the levels of

neuropeptide Y in hypothalamus (Liu et al., 2016). In addition, the difference between results might relate to the feeding state of birds and the severity of stress. Chronic stress is thought to occur long-term only (i.e. with few weeks/months) due to elevation of CORT hormone above normal levels, which suppresses the performance of individual and their immune system activity (Moore et al., 2005). Conversely, a relatively short-term (i.e. few hours/days) elevation baseline CORT might have a of stimulatory effect on animal fitness via reduce oxidative damage and enhance innate immune response (Vágási, et al., 2018).

The present results demonstrated that males and females' Japanese quails respond differently to DEX administration, which might be due to the different prevalence of certain sex steroid influences in specific periods of life. Whereas the high dose treated bird's significant increases revealed in productive and reproductive performance of female, no significant effects on development of testes weight was observed in treated male. These differences between the two sexes in the effects of stress on gonad development are due mainly to the influence on the gonadotrophic axis (Oyola and Handa, 2017). Nonetheless, administration of DEX reduced cloacal gland area of male quails, which has been reported previously by our previous study (Hanafy and Khalil, 2015), but no significant in testosterone level was increase measured in both high and low doses treated males. The cloacal gland development in males is generally considered to be regulated by adrenal androgens, which are, predominantly (but not totally), independent of the activation

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of testosterone. While most of serum testosterone is produced by the Leydig cells in the testes of mature male, the remainder small quantities are synthesized by the adrenal glands. Meanwhile, DEX administration has been shown to suppress not only cholesterol transportation and cAMP formation in the Leydig cells but also severely induce Leydig cell apoptosis (Hu et al., 2008 and Wang et al., 2019a). Alternatively, the oxidative stress hinders the function of Leydig cells causing malfunction testes especially steroid formation in rats (Metukuri et al., 2010), rabbits (Brecchia et al., 2010) and birds (Abolins-Abols et al., 2018). Other studies have also reported that DEX treatment can decrease the secretion of luteinizing hormone (LH), results in the suppression of androgen production (Deviche et al., 2010 and Dolatabadi and Zarchii, 2015). In fish, the cortisol inhibits the testicular androgen secretion independent of LH secretion (Milla et al., 2009). Long-term cortisol treatment of fish inhibits the spermatogenesis first waves which are associated with the beginning of puberty. In mammals, cortisol may directly affect Leydig cells, they the as have glucocorticoid receptors (Honda et al., 2008). Miller et al. (2019) stated that cortisol treatment decrease the plasma testosterone. The drop in testosterone level explain the impaired spermatogenesis in the treated groups, as the seminiferous tubules not show all stages of spermatogenesis and only show the stages till primary or secondary spermatocyte or till spermatid. It is well known that high levels of testosterone are usually accompanied by increased aggression during the male quail reproductive stage (Hanafy et al., 2018) and song sparrows (Moser-Purdy et al.,

2017). However, Mutzel et al. (2011) recorded that the levels of testosterone are not correlated to the exploratory behavior in the house sparrow. Also, the concentrations of androgen were not related to the nest defense behavior in western blue birds (Duckworth and Sockman, 2012).

Contrary to the expected harmful effects of chronic stress on reproductive function of female quail (Alagawany et al., 2017), recent studies showed also positive effects. For instance, DEX administration led to markedly increasing in estradiol- 17β level and improves the development of reproductive organs of females. Similarly, previous studies have shown the effect of DEX on elaboration of ovarian response at the induction commencement of ovulation cvcles (Keay, 2002; Rockwell and Koos 2009). Within baseline levels as shown in Figure (1), CORT did not affect the reproductive of female quail, and effort can reproductive hypothetically enhance function (Angelier and Wingfield, 2013). Several studies in free-living birds have found that adequate elevations in baseline glucocorticoids can enhance reproductive effort of broody birds and encourage parental care (Patterson et al., 2014; Vitousek et al., 2018). Feeding CORT to wild songbird females before and during egg production increased the number of eggs, and the amount of egg volk (Bowers et al., 2016). The current results corroborate these findings, indicating that enhancing in GCs levels may improve fitness of female bird in an adaptive reproductive strategy facilitate to reproductive effort in the peak periods of energy demand (Love et al., 2004; Romero, 2002). Therefore, the development of female gonads reflects the role of estrogen increase on the ovary

and oviduct progress, which might rather be dependent on the overall adrenal androgen level and the local aromatase activity than on ovary estrogen production.

The present work showed increased adrenal medullary tissues on the expense of cortical tissues in birds treated with higher dose of dexamethasone. It has been reported that the adrenal glands tolerate well the treatment of DEX, and the cortical parenchyma exhibits only slight atrophy, occurring especially in the zona glomerulosa and zona fasciculata with a reduction in size and vacuolation (Stojanoski et al., 2005). Moreover, the testicular seminiferous tubules of the low and high dose treated quails did not show all stages of spermatogenesis and only show the stages till primary spermatocyte spermatocyte secondary or or till spermatid with wide lumen filled with morphologically tissue debris with abnormal cells. Similar result was reported by Sadeghzadeh et al. (2019) who stated that DEX treatment has a strong effect on the testis as it cause atrophy of the seminiferous tubules germinative epithelium. The present findings are in line with Hanafy et al. (2018) where low stressed male quail showed higher testes weights and less testicular injury than males selected for overstated stress response. Additionally, Khorsandi et al. (2013) demonstrated that

the mice exposed to DEX showed an epithelial vacuolization, sloughing and atrophy of seminiferous tubules and that may be due to enhancement of apoptosis of the testicular germ cells (Hardy et al., 2005).

It can be concluded that DEX treatment prior to reproduction have a negative effect on testicular structure and function of male quail while it enhances serum estrogen and reproductive performance of premature females. However, further research is needed to establish the direction of relationship between releasing stress hormones during critical period of life and reproductive success of male and female domesticated birds.

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CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest.

AUTHORS' CONTRIBUTIONS

Ahmed M. Hanafy designed the experimental design, statistical analysis and conducted the trial and composed the manuscript. Hassan S. A. performed the histological examination and prepared the manuscript. Both authors read and approved the final manuscript.

Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

Item	control	0.25	0.50	P-value
Initial body weight (g)	123.99±2.66	124.56±3.41	124.51±2.51	0.901
Final body weight (g)	222.42±12.18	221.12±12.67	224.52±10.58	0.814
Weight gain (g/bird)	98.53±5.64	97.06±7.64	100.42 ± 11.05	0.894
Feed intake (g/bird)	580.15±18.16	577.26±19.79	575.59±41.25	0.993
Feed conversion ratio (g feed/g	5.90±0.11	5.97±0.19	5.83±0.23	0.707
gain)				

Table (1): Body weight, weight gain, feed intake and conversion ratio of males and females Japanese quail with respect to dietary dexamethasone.

Table (2): Egg production parameters of Japanese quail as affected by dietary dexamethasone from 6 to 9 weeks of age (throughout 21 days from the first egg)

Item	control	0.25	0.50	P-value
Age at first egg (day)	43	41	39	-
Laying rate (%)	42.00±4.76 ^b	44.00±5.41 ^b	59.33±5.31 ^a	0.050
Average egg number/day	7.56 ± 0.86^{b}	7.92 ± 1.07^{b}	10.68±0.91 ^a	0.040
Average egg weight (g)	12.26±0.08 ^a	12.16±0.05 ^a	11.39±0.04 ^b	0.001
Fertility (%)	74.22±1.17 ^a	69.48±0.79 ^b	65.60±1.00 ^c	0.001

a,b,c Means in any row with no common superscript differ ($P \le 0.05$).

Table (3): Genitalia of female Japanese quail at 6 weeks of age with respect to dietary dexamethasone

	Treatments			
Item	control	0.25	0.50	P-value
Body weight (g)	255.60±12.41	237.80±17.12	247.21±11.45	0.672
Ovary weight (g)	3.16±0.52 ^b	4.74±0.46 ^{ab}	$5.50\pm0.59^{\text{a}}$	0.024
Ovary weight (%)	1.22 ± 0.17^{b}	2.03±0.26 ^a	2.23±0.24 ^a	0.018
Oviduct weight (g)	3.88±0.54 ^b	5.56±0.67 ^{ab}	6.79±0.45 ^a	0.011
Oviduct weight (%)	1.49±0.15 ^b	2.33±0.20 ^a	2.78±0.25 ^a	0.003
Oviduct length (cm)	17.20±3.35 b	27.10±2.44 ^a	30.30±3.16 ^a	0.024
Average ovarian yellow follicle number	2.60 ± 0.25^{b}	3.20±0.20 ab	3.60±0.25 ^a	0.030
Average ovarian yellow follicle size(mm)	5.20 ± 1.55^{b}	10.65±1.58 ^a	11.26±1.49 ^a	0.030

^{a,b} Means in any row with no common superscript differ ($P \le 0.05$).

Table (4): Genitalia of male Japanese quail at 6 weeks of age with respect to dietary dexamethasone

Itana		Duralara			
Item	control	0.25	0.50	P-value	
Body weight (g)	213.81±7.72	217.00±4.38	207.75 ± 8.45	0.596	
Right testis weight (g)	3.15±0.28	2.95 ± 0.28	3.46 ± 0.54	0.389	
Lift testis weight (g)	3.44±0.41	3.27±0.42	3.42 ± 0.33	0.777	
Total testes weight (g)	6.59±0.63	6.22 ± 0.68	6.88 ± 0.85	0.556	
Total testes weight (%)	3.06±0.19	2.89 ± 0.37	3.29 ± 0.32	0.663	
Cloacal gland area $(mm)^3$	521.82±19.37 ^a	481.00±33.45 ^{ab}	413.60±29.80 ^b	0.042	

^{a,b} Means in any row with no common superscript differ ($P \le 0.05$).

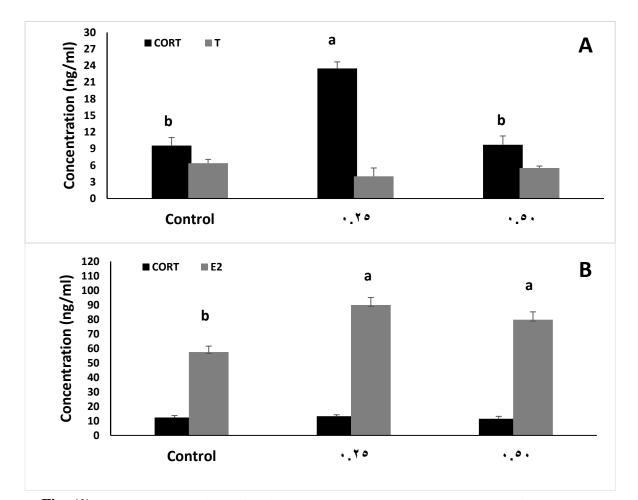


Fig. (1): Serum concentration of corticosterone (CORT) and testosterone (T) of male (A) and corticosterone (CORT) and estradiol- 17β (E2) of female (B) quails at 6 weeks of age as affected by different levels of dexamethasone treatments. Values represent the means \pm S.E.M.

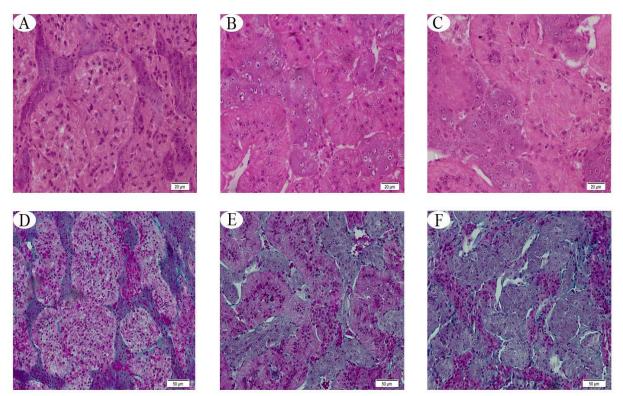


Fig. (2): Representative photomicrograph of quail adrenal gland; A, control stained with H&E; B, low dose treatment stained with H&E; C, high dose treatment stained with H&E; D, control stained with Masson's trichrome stain; E, low dose treatment stained with Masson's trichrome stain; F, high dose treatment stained with Masson's trichrome stain.

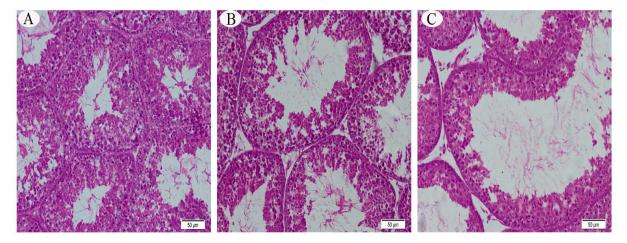


Fig. (3): Representative photomicrograph of quail testis as affected by different levels of dexamethasone treatments; A, control group; B, low dose treatment; C, high dose treatment. H&E stain.

REFERENCES

- Abolins-Abols, M., Hanauer, R. E., Rosvall, K. A., Peterson, M. P., and Ketterson, E. D., 2018. The effect of chronic and acute stressors, and their interaction, on testes function: an experimental test during testicular recrudescence. J. Exp. Biol., 221 (17). jeb180869 DOI: 10.1242/JEB.180869
- Aengwanich W. 2007. Effects of dexamethasone on physiological changes and productive performance in broilers. AJAVA., 2 (3): 157-161. https://scialert.net/abstract/?doi=ajava. 2007.157.161
- Angelier F., and Wingfield J.C. 2013. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. Gen. Comp. Endocr., 190 (1): 118–128. DOI: 10.1016/j.ygcen.2013.05.022
- Alagawany M., Farag M.R., Abd El-Hack M., and Patra A. 2017. Heat stress: Effects on productive and reproductive performance of quail.

Worlds Poult. Sci. J., 73 (4): 747-756. https://doi.org/10.1017/S00439339170 00782

- Ashrafi M., Zafarani F., Nejad S. E., Baghestani A. R., and Amirchaghmaghi E., 2007. Dexamethasone as a supplement for exogenous gonadotropin to improve ovarian response of women over 35 years undergoing IVF/ICSI cycles. Iran J. Fertil. Steril., 1 (2): 69-74. DOI:10.22074/ijfs.2007.45635
- Bonier F., Moore I.T., Martin P., and Robertson R.J. 2009. The relationship between fitness and baseline glucocorticoids in a passerine bird. Gen. Comp. Endocr., 163 (1-2): 208-213.

DOI: 10.1016/j.ygcen.2008.12.013

Bowers E.K., BowdenR.M., Thompson C.F., and Sakaluk S.K. 2016. Elevated corticosterone during egg production elicits increased maternal investment and promotes nestling growth in a wild songbird. Horm.

Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

Behav., 83: 6–13. https://doi.org/10.1016/j.yhbeh.2016.0 5.010

Brecchia G., Cardinali R., Mourvaki E., Collodel G., Moretti E., Dal Bosco A., and Castellini C. 2010. Short- and long-term effects of lipopolysaccharide-induced inflammation on rabbit sperm quality. Anim. Reprod. Sci., 118 (2-4): 310-316. DOI: 10.1016/j.anjreprosci.2009.06.01

DOI: 10.1016/j.anireprosci.2009.06.01 6

- Breuner C.W., Patterson S.H., and Hahn T.P. 2008. In search of relationships between the acute adrenocortical response and fitness. Gen. Comp. Endocr., 157 (3): 288-295. DOI: 10.1016/j.ygcen.2008.05.0 17
- Busch D.S., Addis A.A., Clark A.D., and Wingfield J.C. 2010. Disentangling the effects of environment and life history stage on corticosterone modulation in Costa rufous-collared Rican sparrows, Zonotrichia capensis costaricensis. Physiol. Biochem. Zool., 83 (10): 87-96. https://doi.org/10.1086/648488
- Deviche, P., Hurley, L. L., Fokidis, H. Lerbour. **B.**, Silverin. **B.**. **B.**. Silverin, B., Sabo, J., and Sharp, P. **J. 2010.** Acute stress rapidly decreases plasma testosterone in a free-ranging male songbird: Potential site of action and mechanism. Gen. Comp. Endocr., 169 (1): 82-90. https://doi.org/10.1016/j.ygcen.2010.0 7.009
- **Dolatabadi A. A., and Zarchii S. R.** 2015. The effect of prescription of different Dexamethasone doses on reproductive system. Biomed. Res., 26, (4): 656-660

- Duckworth R.A.,and Sockman K.W. 2012. Proximate mechanisms of behavioural inflexibility: implications for the evolution of personality traits. Funct. Ecol., 26 (3): 559-566. https://doi.org/10.1111/j.1365-2435.2012.01966.x
- **Duncan D.B. 1955.** Multiple Range and Multiple F-Test. Biometrics, 11: 1-5.
- DuRant, S., Love, A. C., Belin, B., Tamayo-Sanchez, D., Pacheco, M. S., Dickens, M. J., and Calisi, R. M. 2020. Captivity alters neuroendocrine regulators of stress and reproduction in the hypothalamus in response to acute stress. Gen, Comp. Endocr. 295: 113519. DOI: 10.1016/J.YGCEN.202 0.113519
- Hanafy A.M., and Khalil H.A. 2015. Influence of chronic dexamethasone administration on reproductive parameters and semen traits in male of Japanese quail. Asian J. Poult. Sci., 9 (4): 223-232. https://scialert.net/abstract/?doi=ajpsaj. 2015.223.232
- Hanafy A.M., Khalil H.A., and Hegab I.M. 2018. Endogenous testosterone hormone and agonistic behavior in male Japanese quail (*Coturnix japonica*). J. Anim. Health and Prod., 6 (2): 51-56. http://dx.doi.org/10.17582/journal.jahp /2018/6.2.51.56
- Hardy, M. P., Gao, H. B., Dong, Q., Ge, R., Wang, Q., Chai, W. R., Feng, X., and Sottas, C. 2005. Stress hormone and male reproductive function. Cell Tissue Res., 322 (1): 147–153. https://doi.org/10.1007/s00441-005-0006-2
- Honda, Y., Ohno, S., and Nakajin, S. 2008. Leydig cells from neonatal pig testis abundantly express 11βhydroxysteroid dehydrogenase (11β-

HSD) type 2 and effectively inactivate cortisol to cortisone. J. Steroid Biochem., 108 (1): 91–101. DOI: 10.1016/J.JSBMB.2007.07.003

- Hu G.X., LianQ.Q., Lin H., Latif S.A., Morris D.J., Hardy M.P., and Ge R.S. 2008. Rapid mechanisms of glucocorticoid signaling in the Leydig cell. Steroids, 73 (9–10): 1018–1024. https://doi.org/10.1016/j.steroids.2007. 12.020
- Hull K., Cockrem J.F., Bridges J.P., Candy E.J., and Davidson C.M. 2007. Effects of corticosterone treatment on growth, development, and the corticosterone response to handling in young Japanese quail (*Coturnix coturnix japonica*). Comp. Bioch. Physiol. A., 148 (3): 531–543. https://doi.org/10.1016/j.cbpa.2007.06. 423
- Iwasa T., Matsuzaki T., Yano K. and Irahara M. 2017. Gonadotropininhibitory hormone plays roles in stress-induced reproductive dysfunction. Front. Endocrinol., 8: 62-62. DOI: 10.3389/fendo.2017.00062
- Jimeno, B., Hau, M., and Verhulst, S. 2018. Corticosterone levels reflect variation in metabolic rate, independent of 'stress'. Sci. Rep., 8 (1): 13020. https://doi.org/10.1038/s41598-018-31258-z
- Kang S., Kim D-H, Lee S., Lee T., Lee
 K-W, Chang H-H, Moon B., Ayasan
 T., and Choi Y-H. 2020. An acute, rather than progressive, increase in temperature-humidity index has severe effects on mortality in laying hens.
 Front. Sci., 7: 568093, DOI: 10.3389/fvets.2020.568093
- Keay S.D. 2002. Poor ovarian response to gonadotropin stimulation the role of adjuvant treatment. Hum. Fertil., (Cambridge, England), 5 (1 Suppl):

46-52.

DOI: 10.1080/1464727022000199921

- Khorsandi L., Mirhoseini M., Mohamadpour M., Orazizadeh M., and Khaghani S. 2013. Effect of curcumin on dexamethasone-induced testicular toxicity in mice. Pharm. Biol., 51 (2): 206–212. https://doi.org/10.3109/13880209.2012 .716854
- Lin H., Decuypere E., and Buyse J. 2004. Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*): 2. Short-term effect. Biochem. Physiol. B. Biochem. Mol. Biol., 139 (4): 745–751. https://doi.org/10.1016/j.cbpc.2004.09. 014
- Liu L., Xu S., Wang X., Jiao H., Zhao J., and Lin H. 2016. Effect of dexamethasone on hypothalamic expression of appetite-related genes in chickens under different diet and feeding conditions. J. Anim. Sci. Biotechnol., 7 (23): 2-7. https://doi.org/10.1186/s40104-016-0084-x
- Lõhmus M., Fredrik Sundström L., and Moore F.R. 2006. Non-invasive corticosterone treatment changes foraging intensity in red eyed vireos *Vireo olivaceus*. J. Avian Biol., 37 (5): 523 – 526. https://doi.org/10.1111/j.0908-8857.2006.03733.x
- Love O.P., Breuner C.W., Vezina F., and Williams T.D. 2004. Mediation of a corticosterone-induced reproductive conflict. Horm. Behav., 46 (1): 59–65. https://doi.org/10.1016/j.yhbeh.2004.0 2.001
- Metukuri M.R., Reddy C.M.T., Reddy P.R.K., and Reddanna P. 2010.

Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

Bacterial LPS mediated acute inflammation-induced spermatogenic failure in rats: role of stress response proteins and mitochondrial dysfunction. Inflammation, 33 (4):235-243:https://doi.org/10.1007/s1 0753-009-9177-4

- Milla, S., Wang, N., Mandiki, S. N. M., and Kestemont, P., 2009. Corticosteroids: Friends or foes of teleost fish reproduction? Comp. Biochem. Physiol. A Mol. Integr. Physiol., 153 (3): 242–251. DOI: 10.1016/J.CBPA.2009.02.027
- Miller, K. A., Kenter, L. W., Breton, T. S., and Berlinsky, D. L., 2019. The effects of stress. cortisol administration and cortisol inhibition on black sea bass (Centropristis striata) differentiation. Comp. sex Biochem. Physiol. A Mol. Integr. Physiol., 227: 154–160. https://doi.org/10.1016/j.cbpa.2018.10. 009
- Moore F.L., Boyd S., and Boyd D.B. 2005. Historical perspective: Hormonal regulation of behaviors in amphibians. Horm. Behav., 48 (4): 373–383.

https://doi.org/10.1016/j.yhbeh.2005.0 5.011

- Moser-Purdy, **C.**, MacDougall-Shackleton, S. A., Bonier, F., Graham, B. A., Bover, A. C., and Mennill, D. J. 2017. Male song sparrows have elevated testosterone in neighbors response to versus strangers. Horm. Behave., 93: 47-52. https://doi.org/10.1016/j.yhbeh.2017.0 4.006.
- Mutzel A., Kempenaers B., Laucht S., Dingemanse N.J., and Dale J. 2011. Circulating testosterone levels do not affect exploration in house sparrows: observational and

experimental tests. Anim. Behav., 81 (4):731-739.https://doi.org/10.1016/j.a nbehav.2011.01.001

- **Oyola M. G. and Handa R. J., 2017.** Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. Stress, 20 (5): 476-494. doi: 10.1080/10253890.2017.1369523.
- Patterson S.H., Hahn T.P., Cornelius J.M., and Breuner C.W. 2014. Natural selection and glucocorticoid physiology. J. Evol. Biol., 27 (2): 259-274.https://doi.org/10.1111/jeb.12 286
- Rockwell, L. C., and Koos, R. D., 2009. Dexamethasone enhances fertility and preovulatory serum prolactin levels in eCG/hCG primed immature rats. J. Reprod. Dev., 55 (3): 247–251. DOI: 10.1262/JRD.20108
- Romero L.M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocr., 128 (1): 1–24. https://doi.org/10.1016/s0016-6480(02)00064-3
- Sadeghzadeh F., Mehranjani M.S., and Mahmoodi M. 2019. Vitamin C ameliorates the adverse effects of dexamethasone on sperm motility, testosterone level, and spermatogenesis indexes in mice. Hum. Exp. Toxicol., 38 (4): 409-418.https://doi.org/10.1177/09603 27118816137.
- Salvante K. G and Williams T. D., 2003. Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. Gen. Comp. Endocr., 130 (3): 205-214.https://doi.org/10.1016/S0016 -6480(02)00637-8
- Semet, M., Paci, M., Saïas-Magnan, J., Metzler-Guillemain, C., Boissier, R.,

- Lejeune, H. and Perrin, J. 2017. The impact of drugs on male fertility: a review.Andrology,5:640663. https://do i.org/10.1111/andr.12366
- Shi L., Wudy S.A., Buyken A.E., Christiane Maser-Gluth, Hartmann M.F., and Remer T. 2011. Prepubertal glucocorticoid status and pubertal timing. J. Clin. Endocr. Metab., 96 (6): E891–E898. https://doi.org/10.1210/jc.2010-2935
- Stojanoski, M. M., Nestorović, N., Negić, N., Filipović, B., Šošić-Jurjević, B., Milošević, V., and Sekulić, M., 2005. The pituitaryadrenal axis of fetal rats after maternal dexamethasone treatment. Anat. Embryol., 211 (1): 61–69. DOI: 10.1007/S00429-005-0057-X
- Tufarelli V., Baghban-Kanani **P.**, Azimi-Youvalari S., Hosseintabar-Ghasemabad B., Slozhenkina M., Gorlov I., Seidavi A., Avasan T., and Laudadio V. 2021. Effects of Horsetail (Equisetum arvense) and Spirulina (Spirulina platensis) dietary supplementation on laying hens productivity and oxidative status. Animals, 11 (2): 335-348. https://doi.org/10.3390/ani11020335.
- Vágási C.I.. Pătras L., Pap P.L., Vincze O., Mureșan C., Németh J., and Lendvai A. 2018. Experimental increase in baseline corticosterone level reduces oxidative damage and enhances innate immune response. PloS One, 13 (2): e0192701.https://doi.org/10.1371/jour nal.pone.0192701
- Vitousek M.N., Taff C.C., Hallinger K.K., Zimme R.C., and Winkler D.W. 2018. Hormones and fitness: evidence for trade-offs in glucocorticoid regulation across

contexts. Front. Ecol. Evol., 6: 42. https://doi.org/10.3389/fevo.2018.00042

- Vitousek M.N., Taff C.C., Ryan T.A., and Zimme R.C. 2019. Stress resilience and the dynamic regulation of glucocorticoids. Integr. Comp. Biol., 59 (2): 251–263. https://doi.org/10.1093/icb/icz087
- Wall J. P., and Cockrem J. F., 2010. Effects of corticosterone treatment in laying Japanese quail. Br. Poult. Sci., 51(2):278-288.https://doi.org/10.1080/ 00071661003745828
- Wang X., Liu L., Zhao J., Jiao H., and Lin H. 2017. Stress impairs the reproduction of laying hens: An involvement of energy. Worlds Poult. Sci. J., 73 (4): 845-856. https://doi.org/10.1017/S0043933917 000794
- Wang Y., Chen Y., Ni C., Fang Y., Wu K., Zheng W., Li X., Lin H., Fan L., and Ge R. S. 2019a. Effects of dexmedetomidine on the steroidogenesis of rat immature Leydig cells. Steroids, 149:108423. doi: 10.1016/j.steroids.2019.05.015.
- Wang, Y., Guo, J., Wang, L., Tian H., and Sui J., 2019b. Transcriptome potential analysis revealed mechanisms of differences in physiological stress responses between caged male and female magpies. BMC Genom., 20 (1): 447. https://doi.org/10.1186/s12864-019-5804-0
- Wyns H., Meyer E., Watteyn A., Plessers E., De Baere S., De Backer P., and Croubels S. 2013. Pharmacokinetics of dexamethasone after intravenous and intramuscular administration in pigs. Vet. J., 198 (1): 286-288https://doi.org/10.1016/j.tvjl.2 013.06.015

Dexamethasone, egg laying, histochemistry, Japanese quail, reproduction.

الملخص العربى

تأثير المعاملة بالديكساميثازون في فترة ما قبل البلوغ الجنسى على الأداء الإنتاجي والتناسلي للمعاملة بالديكساميثازون في فترة ما قبل البلوغ الجنسي

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تم استخدام عدد ١٤٤ (٧٢ ذكر + ٢٢ انثى) طائر عمر ٣ اسابيع لدر اسة تأثير اضافة الديكساميثازون (DEX) الٰي العلائقُ بتركيزات مُختلفة على الأداء الإِنتَاجي والتناسلي في السمان الياباني. قسمت الطيور عشوائيا الُي ثلاث مجموعات (٢٤ ذكر و ٢٤ انثى للمجموعة) وغذيت المجاميع لمدة ٣ اسابيع على النحو التالي: المجموعة الاولى تم تغذيتها على العليقة الاساسية (كنترول) دون اي اضافات، المجموعة الثانية والثالثة تم تغذيتها على العليقة الأساسية مضاف اليها DEX بمعدل ٢٥. • و ٠٠. • ملجم //كجم عليقة على التوالي. أظهرت النتائج تبكير في وضع البيض وزيادة معنوية في وزن المبيض وطول قناة البيض ومعدل وضع البيض للاناث مصحوباً بانخفاض معنوى في وزن البيض ونسبة الخصوبة في المجموعة الأعلى في مستوى الديكساميثازون (DEX). لم يتم تسجيل اي اختلافات معنوية في وزن الخصية للذكور بين المجمو عات ولكن تم تسجيل انخفاض في مساحة غدة المجمع للذكور المعاملة مقارنة بالكنترول. المعاملة بالمستوى الاقل من DEX ادى الى ارتفاع معنوى (P≤ 0.001) في مستوى هرمون قشرة غدة فوق الكلية (CORT) في دم الاناث ولكن لم يتم تسجيل اي تغيرات معنوية في دم الذكور المعاملة مقارنة بالكنترول. على العكس من ذلك، تم تسجيل زيادة معنوية (P< 0.008) في مستوى الهرمون الأنثوى (الأستروجين) في دم الإناث المعاملة ، لكن لم تكن هناك تغييرات كبيرة في مستوى هرمون الذكور (تستستيرون) في دم الطيور المعاملة. ازدادت مساحة الأنسجة النخاعية في الغدة الكظرية على حساب انسجة القشرة في الطيور المعاملة بجرعة أعلى من DEX . لم تظهر في الأنابيب المنوية جميع مراحل تكوين الحيوانات المنوية في الذكور المعاملة مقارنة بالكنترول. خلصت النتائج إلى أن الأستجابة للمعاملة بالديكساميثازون في مرحلة ما قبل البلوغ الجنسى تختلف حسب الجنس والتي قد تكون بسبب ارتفاع مستويات الهرمونات المسببة للبلوغ الجنسي خلال تلك الفترة في الذكور والأناث