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Exogenous Estradiol: Blood Profile, Productive and Reproductive Performance of Female Japanese Quails at Different Stages of Production*

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Abstract: One hundred and eight, 3 weeks old female quails were distributed among 3 treatments to study the effect of estradiol administration, on their productive and reproductive performance. Birds of the first group were intramuscularly injected at 3 weeks of age daily with 100 μg E_2/bird/day for 7 consecutive days. Birds of the second group were treated in a like manner starting from 5 weeks of age and the third group served as control. Birds injected with E_2 either at 3 or 5 weeks of age had a significantly higher body weight at sexual maturity and females injected at 3 weeks of age matured significantly earlier compared to control. Egg number egg weight and egg mass had significantly increased due to E_2 injection at both ages with females injected at 3 weeks of age having the highest values. Estrogen-progesterone ratio fluctuated with reverse relation to egg production. Tibia relative weight, length and calcium content were significantly increased by estrogen injection with birds injected at 5 weeks of age having the highest values. It can be concluded that treating immature Japanese quails with E_2 can enhance their reproductive and productive functions and that applying the treatment earlier (3 weeks of age) has better results when compared to applying it at 5 weeks of age regarding egg production, feed conversion and calcium metabolism.

Key words: Egg production, feed conversion, calcium, total lipids, cholesterol

INTRODUCTION

Estrogens (estradiol-17β), which are synthesized and secreted by the gonads during avian embryonic development, regulate growth and differentiation of the sex accessory structures (Johnson, 1990). Steroid hormones have been implicated in the regulation of calcium metabolism in laying hens, throughout several modes of action. Shortly before sexual maturity, the formation of medullary bone and a parallel increase in calcium retention (Nys *et al*., 1989) are induced by the action of estrogen. Estrogens stimulate vitellogenesis, via its action on the liver, feed intake and the deposition of calcium within the medullary portion of long bones (Johnson, 1986; Bacon *et al*., 1980). In addition, gonadal hormones regulate the rapid development of the oviduct, which occurs before and during the sexual maturation. Treatment of immature Japanese quail and young female chickens with estradiol enhances growth of the oviduct and formation of tubular secretory glands and epithelial differentiation (Forgó *et al*., 1996). There is no sufficient information about some physiological parameters of a new developed Japanese quail strains in our country at different productive stages, also about the physiological effect of estrogens on productive and reproductive characteristics. Results showing the effect of estrogen hormone on regulation of metabolism in Japanese quail hens are limited, conflicting and inconclusive, therefore, the aim of this study was mainly to study the effect of estradiol administration at early ages, on the productive and reproductive performance of Japanese quail hens.

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MATERIALS AND METHODS

The present study was carried out at the Poultry Research Center, Faculty of Agriculture, Alexandria University, over the period from March 2002 to 2003. Using a new developed quail females (egg line) resulted from a 17 generations selection program through 10 successive years (1992-2001) (Ali *et al*., 2002) raised at the Poultry Research Center.

Preparation of E_2 Solution

Estradiol-17β (E_2) used in this study was a liquid in folone ampules (5 mg estradiol benzoate mL^{-1}) in oily solution purchased from Misr Co. for Pharm.Ind. S.A.A. Egypt. The injection solution was prepared by dissolving 2 mL of estradiol benzoate in 18 mL ethanol-sesame oil to give a concentration of 100 μg E_2 in 0.2 mL final solution.

Experimental Design

A total of one hundred and eight, 3 weeks old female quails were randomly and equally distributed among 3 treatment groups each of 6 sub groups (6 birds each) which were housed in one quail battery cages. Birds of the first group were individually intramuscularly injected at 3 weeks of age daily with 0.2 mL of the injection solution to provide 100 μg E_2/bird/day for 7 consecutive days. Birds of the second group were treated in a like manner starting from 5 weeks of age and the third group served as control and was injected with ethanol-sesame oil solution. Feed and water were provided *ad libitum* throughout the experimental period, birds were fed on starter ration (24.6% CP, 2902 kcal ME kg^{-1} and 0.96% Ca) from 3 to 6 weeks of age and on a breeder ration (20.1% CP, 2854 kcal ME kg^{-1} and 3.54% Ca) starting from 7 weeks of age.

Data Collected

Quail's age and body weight at sexual maturity (first egg laid) was recorded. Number of eggs laid in the period from sexual maturity till 90 days of production was recorded and weighed and egg mass was calculated. Feed consumption as (g/bird/day) was determined for the 90 days period and feed conversion was calculated as (g feed/g egg).

Three birds of each treatment were slaughtered at three intervals 6, 8 and 13 weeks of age resembling three stages of production ; before sexual maturity, BSM, at sexual maturity, SM and at peak of production, PP, respectively for blood and carcass analysis. Plasma total lipids concentration (mg dL^{-1}) was estimated according to Fringes *et al*. (1972). Plasma cholesterol concentration (mg dL^{-1}) was determined according to Richmond (1973) using commercial kits (Diamond diagnostics: El-Nasr Pharmaceutical Chemicals Co.). Triglycerides concentration (mg dL^{-1}) was determined according to Jacobs and VanDemark (1960) using commercial kits. Plasma glucose concentration (mg dL^{-1}) was estimated according to the method of Trinder (1969) using commercial kits. Serum calcium concentration (mg dL^{-1}) was measured according to the method of Tietz (1970) using commercial kits. The activities of serum aspartate amino transferase (AST) (U L^{-1}) and serum alanine amino transferase (ALT) (U L^{-1}) were assayed by the method of Reitman and Frankel (1957). Serum triiodothyronine hormone (T_3) concentration (ng mL^{-1}) was determined with enzyme immunoassay using commercial kits obtained from International Immuno-Diagnostics (1155 Chess Drives, Suite 121 Foster city, CA 94404 USA). Serum estradiol-17β hormone (E_2) concentration (pg mL^{-1}) was determined with enzyme immunoassay using commercial kits purchased from Biochem Immuno Systems. Serum progesterone hormone (P4) concentration (ng mL^{-1}) was determined with enzyme immunoassay using commercial kits obtained from International Immuno-Diagnostics. Estradiol-17β (E_2)/Progesterone (P4) ratio was calculated.

Carcasses were manually eviscerated and weighed. Livers, ovaries and oviducts were removed and weighed separately. The length of magnum, uterus and tibia bone were measured to the nearest millimeter (mm.) then weighed separately. Liver glycogen content (%) was determined as described by Allen and Ruff (1981). Liver lipids content (mg g^{-1}) was determined according to Fringes *et al*. (1972). Liver cholesterol content (mg g^{-1}) was determined as mentioned before by the method of Richmond (1973). Left tibia bones were dissected out, cleaned from muscles and connective tissues, weighed and stored frozen before oven drying at 60°C and burned at 600°C for 6 h in muffle furnace and ash content were weighed and prepared for calcium determination by atomic absorption spectrophotometer (Perkin, 1973).

Statistical Analysis

Means and standard errors were estimated for each studied trait. Data were analyzed using SAS program, using general linear model. Significant differences among treatments means were separated using Duncan's multiple range procedure (Duncan, 1955).

RESULTS AND DISCUSSION

Age and Body Weight at Sexual Maturity

Birds injected with E_2 either at 3 or 5 weeks of age had a significantly higher body weight at sexual maturity compared to the untreated females ($p \leq 0.05$), the difference were about 3% in the favor of the treated groups (Table 1).

The increase in body weight at sexual maturity may be due to the changes of carbohydrate metabolism induced by E_2 treatment which is intimately involved in glucose production, storage and metabolism (Bell and Freeman, 1971). Results here in are in agreement with that of Detwiler *et al*. (1950) and Almquist and Merritt (1952) who found that implanting chickens with stilbestrol improved carcass weight, quality and increased chickens body gain. Also Woody *et al*. (1969) and Herrick *et al*. (1970) reported that feeding 140 mg dienestrol diacetate kg^{-1} of diet to Leghorn type pullets (14-18 weeks of age) increased their body weight and improved broiler's weight gain. Douglas *et al*. (1989) recorded an increase in body weight gain of layers fed diets supplemented with synthetic estrogen (dienestrol diacetate). Moreover, Elghalid (2005) reported an increase in quails' body weight at sexual maturity as a result of estradiol treatment and related that to increased organs weight as liver's, ovaries' and oviduct's relative weights showed significant increases associated with E_2 treatment.

Birds injected with E_2 at 5 weeks of age did not differ from the untreated birds regarding age at sexual maturity, While females injected at 3 weeks of age matured significantly ($p \leq 0.05$) earlier compared to the untreated ones. This comes in contrast with the findings of Schimke *et al*. (1975) and

Table 1: Effect of estradiol injection at 3 and 5 weeks of age on productive and reproductive traits of Japanese quail hens

Items	Body weight at sexual maturity (g)	Age at sexual maturity (day)	Egg No. at 90 days (egg)	Egg weight (g)	Egg mass (g)	Feed consumption (g/bird/day)	Feed conversion (g feed/g egg)
Control	197.66±0.933B	51.17±0.386A	31.15±0.540B	10.87±0.198C	337.89±6.53B	29.34±0.475	3.67±0.100A
3 week injection	203.90±1.334A	49.67±0.333B	34.08±0.607A	11.69±0.095A	398.37±6.99A	28.30±0.085	2.98±0.057B
5 week injection	203.98±1.030A	51.67±0.557A	33.29±0.765A	11.33±0.115B	377.36±11.6A	28.19±0.199	3.15±0.088B
Probability level	*	*	*	*	**	NS	**

Different letters(s) within a column denote significant differences between treatments. *, **, *** Probability levels $p \leq 0.05$, 0.001 and 0.0001, respectively. NS: Not Significant

Table 2: Effect of Estradiol injection at 3 and 5 weeks of age and different stages of production (before sexual maturity BSM, at sexual maturity SM and at peak of production PP) on serum transaminases activities and triiodothyronine and sex hormones concentrations of Japanese quail hens

Items	GOT (U L^{-1})	GPT (U L^{-1})	T_3 (ng mL^{-1})	E_2 (pg mL^{-1})	P_4 (ng mL^{-1})	E_2/P_4 ratio
Control	36.50±1.10B	13.22±0.83B	3.769±0.084A	114.43±4.83C	0.880±0.070C	134.65±8.37A
3 week injection	41.22±2.50A	15.39±0.44A	3.087±0.082B	159.76±10.74A	2.540±0.128A	64.03±5.29C
5 week injection	44.44±2.38A	15.56±0.73A	2.920±0.261B	124.29±5.16 B	2.206±0.348B	82.37±10.103B
Probability level	*	**	***	***	***	***
BSM	39.67±2.33	14.92±0.71	3.00±0.18C	140.46±11.13A	1.79±0.30	100.76±12.42A
SM	40.08±1.83	15.08±0.71	3.77±0.08A	127.51±5.82B	2.06±0.33	80.03±10.84B
PP	39.25±1.89	14.67±0.59	3.39±0.18B	124.12±6.89B	2.03±0.44	91.78±13.73A
Probability level	NS	NS	***	***	NS	*

Different letters(s) within a column denote significant differences between treatments. *, **, *** Probability levels $p \leq 0.05$, 0.001 and 0.0001, respectively. NS: Not Significant

Boogard and Finnengan (1976) who reported that treatment of immature Japanese quail and young female chickens with estradiol enhances growth of the oviduct and promotes the formation of tubular secretory glands and epithelial differentiation.

Egg Production

Egg number had significantly ($p \leq 0.05$) increased due to E_2 injection at both ages (3 and 5 weeks) with females injected at 3 weeks of age having the higher egg production. Increases reached 109 and 107% of the untreated females production with treatments at 3 and 5 weeks of age, respectively. The increase in egg production due to E_2 treatment was accompanied by a significant ($p \leq 0.05$) increase in egg weight, with the highest egg weight recorded to females injected at 3 weeks of age. Increases reached 108 and 104% of the untreated females egg weight with treatments at 3 and 5 weeks of age, respectively. Accordingly, egg mass showed a similar significant ($p \leq 0.001$) trend, as it increased by 18 and 12% compared to the untreated females egg mass with treatments at 3 and 5 weeks of age, respectively. These findings are in harmony with those of El-Afifi and Abu Table (2002), Hamdy *et al*. (2002) and Elghalid (2005) who reported that egg number and egg mass were significantly improved when Leghorn pullets and immature quail females were treated with estradiol and found a significant positive correlation between egg mass and plasma estrogen concentrations, which was also observed in this study (Table 2).

Feed Consumption and Feed Conversion

Results indicate that feed consumption did not differ significantly between the three groups studied, meanwhile, feed conversion ratio presented as (g feed/g egg) showed a significant ($p \leq 0.001$) improvement as a result of E_2 treatment compared to the untreated birds, with the group treated at 3 weeks of age being the best (Table 1). Improvement was by 19 and 14% compared to the untreated females feed conversion ratio with treatments at 3 and 5 weeks of age, respectively. Similar trend was observed by Elghalid (2005), who reported a non significant improvement of quail hens' feed conversion when they were treated with E_2.

Serum Transaminases Activities and Hormone Concentrations

Results indicate that serum transaminases (GOT and GPT) activities increased significantly ($p \leq 0.05$; 0.001) with E_2 treatment compared to control. Meanwhile, their activities were not affected with the different stages of production.

Data reflect the normal reverse relationship between T_3 and E_2 where, T_3 significantly ($p \leq 0.0001$) decreased with the E_2 treatment at both ages, which comes in harmony with the findings of Maiti and Sahu (1982) who reported an antithyroidal activity of estrogen in juvenile ducks (Table 2).

Table 3: Effect of estradiol injection at 3 and 5 weeks of age and different stages of production (before sexual maturity BSM, at sexual maturity SM and at peak of production PP) on reproductive system of Japanese quail hens

Items	Ovary (%)	Oviduct (%)	Oviduct length (cm)	Magnum length (cm)	Shell gland length (cm)
Control	0.547±0.026	3.10±0.090	20.59±1.171	15.37±1.011	2.43±0.218
3week injection	0.612±0.052	4.09±0.250	23.84±1.058	16.82±0.754	3.35±0.275
5 week injection	0.571±0.072	4.39±0.976	22.40±2.215	18.06±1.114	3.56±0.465
Probability level	NS	NS	NS	NS	NS
BSM	0.528±0.059	3.60±0.602	19.17±1.185C	15.11±0.946B	3.64±0.485
SM	0.550±0.054	3.83±0.491	22.13±1.112B	16.13±0.654B	3.13±0.320
PP	0.680±0.047	4.33±0.323	26.72±1.111A	19.07±0.842A	4.24±0.305
Probability level	NS	NS	*	*	NS

Different letters(s) within a column denote significant differences between treatments. *, **, *** Probability levels p≤=0.05, 0.001 and 0.0001, respectively. NS: Not Significant

Table 4: Effect of estradiol injection at 3 and 5 weeks of age and different stages of production (before sexual maturity BSM, at sexual maturity SM and at peak of production PP) on liver and tibia analysis of Japanese quail hens

Items	Liver glycogen (%)	Liver total lipids (mg g^{-1})	Liver cholesterol (mg g^{-1})	Tibia weight (%)	Tibia length (mm)	Tibia calcium (%)
Control	1.57±0.079C	31.49±0.190B	19.83±0.640B	0.687±0.036C	4.78±0.085B	17.35±0.274C
3week injection	1.72±0.094B	31.64±0.144B	21.67±0.607A	0.875±0.025B	4.90±0.069A	17.93±0.310B
5week injection	2.02±0.043A	31.88±0.110A	22.22±0.713A	0.957±0.047A	4.96±0.098A	18.91±0.285A
Probability level	***	**	***	***	*	**
BSM	1.67±0.092B	31.26±0.045C	20.29±0.886B	0.813±0.043B	4.83±0.113	16.86±0.247B
SM	1.79±0.070B	31.61±0.083B	21.08±0.460B	0.826±0.044B	4.84±0.133	17.79±0.172A
PP	2.03±0.055A	32.31±0.077A	22.67±0.562A	0.908±0.042A	4.79±0.095	18.11±0.340A
Probability level	***	***	***	**	NS	**

Different letters(s) within a column denote significant differences between treatments. *, **, *** Probability levels p≤0.05, 0.001 and 0.0001, respectively. NS: Not Significant

Estrogen-progesterone ratio fluctuated with reverse relation to egg production (p≤0.0001), as it decreased when egg production increased (Table 2). The lowest estrogen-progesterone ratio was associated with the early (3 week) injection of estradiol where the highest was associated with the untreated birds. Whereas regarding phase of production, the lowest E_2/P_4 ratio was observed at sexual maturity (p≤0.05). This supports the findings of Nagwa *et al*. (1998), who found that the higher E_2/P_4 ratio in Fayoumi hens was associated with their lower egg production compared to the lower E_2/P_4 ratio found in LSL hens which was associated with their higher egg production.

Reproductive System

Ovaries and oviducts relative weight, oviducts length, magnum length and shell gland length were non significantly increased by the estradiol injection at either ages (Table 3). Whereas, oviduct length and magnum length differed significantly (p≤0.05) between different stages of production, showing a normal development with being longest at peak of production.

Slaughter Traits

Injecting immature quail hens either at 3 or 5 weeks of age with estradiol, significantly (p≤0.0001) increased their liver glycogen content by 9.5 and 28.7% when compared with the untreated control birds (Table 4). The increase in liver glycogen due to E_2 treatments was associated with decreased blood glucose concentrations (Table 5) indicating a stimulated pancreatic activity. Liver glycogen content was also significantly affected by stage of production (p≤0.0001) and reached 121.5% at peak of production compared to its value before sexual maturity.

Data shown in Table 4 indicates that liver total lipids and cholesterol contents increased significantly by 0.5 and 1.2% and by 9 and 12% with E_2 injections either at 3 or 5 weeks of age, respectively (p≤0.001 and 0.0001). Development of reproductive stage also had an increasing effect

Table 5: Effect of Estradiol injection at 3 and 5 weeks of age and different stages of production (before sexual maturity BSM, at sexual maturity SM and at peak of production PP) on blood biochemical characteristics of Japanese quail hens

Items	Total lipids	Cholesterol	Triglycerides	Glucose	Calcium
	----------(mg dL^{-1})----------				
Control	304.44±5.9B	116.46±4.84C	128.24±4.42	154.82±5.86A	11.66±0.46B
3week injection	307.50±3.5B	128.26±4.70B	111.39±2.73	141.10±3.46B	12.09±0.48B
5week injection	329.31±2.1A	140.10±3.44A	107.53±2.74	124.28±5.34C	14.29±0.36A
Probability level	***	*	NS	**	*
BSM	312.71±5.1B	127.07±5.66B	120.44±4.13	158.51±9.54A	12.35±0.62B
SM	314.79±5.1B	129.40±4.96B	124.44±4.49	138.26±2.98B	13.46±0.44A
PP	325.42±3.7A	137.22±4.09A	111.68±5.76	134.50±3.79B	13.44±0.55A
Probability level	**	*	NS	*	*

Different letters(s) within a column denote significant differences between treatments. *, **, *** Probability levels $p \leq 0.05$, 0.001 and 0.0001, respectively. NS: Not Significant

on liver total lipids and cholesterol contents, both reaching their heist values at peak of production ($p \leq 0.0001$), as they reached 103 and 112% of their values before sexual maturity, respectively. These effects of estradiol on liver total lipids and cholesterol contents are in good agreement with the findings of Walzem (1996) who stated that yolk lipids are synthesized in the liver under the influence of estrogen.

Tibia relative weight, length and calcium content were significantly affected by estrogen injection (Table 4), as they increased with E_2 injection at both ages, with birds injected at 5 weeks of age having the highest values. Tibia relative weight increased by 27 and 39%, while tibia calcium content increased by 2.5 and 3.8% and tibia calcium content increased by 3.3 and 9% with E_2 injections at 3 and 5 weeks of age, respectively.

Blood Biochemical Characteristics

Plasma total lipids increased by 1% with E_2 injection at 3 weeks of age and increased by 8% ($p \leq 0.0001$) when females were injected at 5 weeks of age (Table 5). Similar trend was observed with plasma cholesterol as it increased by E_2 injections at 3 and 5 weeks of age by 10 and 20%, respectively ($p \leq 0.05$). Whereas plasma triglycerides were not affected by E_2 injections. Development of reproductive stage also caused increases in plasma total lipids and cholesterol where they reached their highest values at peak of production (104 and 108% of control, respectively). These increases in plasma total lipids and cholesterol can be attributed to the fact that estradiol activates lipids metabolism during vitellogensis Walzem (1996) and comes in agreement with the findings of Johnson (1986) who reported that laying hens with short-term administration had significantly higher plasma total lipids and that development of sexual maturity of the fowl increases lipid metabolism to provide yolk lipids.

Plasma glucose decreased ($p \leq 0.001$) with E_2 treatments and was 91 and 80% of control with injections at 3 and 5 weeks of age, respectively (Table 5). Also, development of reproductive stage caused a decrease in plasma glucose in compare to before sexual maturity, as it decreased by 13 and 15% at sexual maturity and at peak of production, respectively. These decreasing trends was accompanied by increased liver glycogen (Table 4) indicating a stimulated pancreatic activity, which comes in agreement with the findings of Schulz (1940) who reported that in pigeons, the pancreatic islets of Langerhans increase in size and number during the laying period of the female.

Serum calcium increased by E_2 injections and reached 104 and 123% of control with injections at 3 and 5 weeks of age, respectively. Also with development of reproductive stage (Table 5). Which was also accompanied with increased Tibia calcium content (Table 4). This can be attributed to estrogen increasing total blood calcium, primarily by stimulating the production of blood-calcium binding proteins (Bacon *et al*., 1980) and that in the fowl, the medullary bone develops under the influence of ovarian hormones and that estrogen stimulates deposition of calcium within the medullary portion of long bones (Redshow and Follet, 1972).

It can be concluded that treating immature Japanese quails daily (for a week) with E_2 can enhance their reproductive and productive functions and that applying the treatment earlier (3 weeks of age) has better results when compared to applying it at 5 weeks of age regarding age and weight at sexual maturity, egg production, feed conversion and calcium metabolism.

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