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IMPACT OF MIXED DIETARY VITAMIN E-SELENIUM POWDER ON REPRODUCTIVE HORMONES' CONCENTRATION OF MALES AND FEMALES IN JAPANESE QUAIL BIRD (COTURNIX COTURNIX JAPONICA)

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ABSTRACT

The Study's goal was to show the effect of dietary supplementation of mixed vitamin E and Selenium powder (Sodium selenite) in Japanese quail on reproductive hormone levels. The study was conducted on a total number of (128) Japanese quail birds at 12 weeks old. The experimental birds were randomly divided into four groups (32birds /group), each group had eight replicates (1male/3 females /replicate). The four experimental diets were: T1= (Basal diet (control), T2= (vitamin E-Selenium powder 1.0 gm/kg of basal diet), T3= vitamin E-Selenium powder 1.5 gm/kg of basal diet) and T4 (vitamin E-Selenium powder 2.0 gm/kg of basal diet). The results indicated that adding vitamin E and selenium to diet (T4) significantly ($P \leq 0.05$) increased the level of plasma concentrations of Estradiol (E2), testosterone and progesterone as compared with the control group(T1) in male and female of Japanese quail. In concerned with the male group the T4 supplemented diet increased the Testosterone level significantly. There was no significant difference for the T2 and T3 in the level of testosterone and Estradiol compared to the control. Although the T2, T3 and T4 diet decreased the level of progesterone hormone significantly. There is a significant decrease of progesterone for the T3 and T4 compared to the control and T2, which lastly do not differ from the control in the female groups. It was clear that the supplemented diet with vitamin E and Selenium statistically ($P<0.05$) affected some reproductive hormones. So, it was recommended to perform further advanced, related, and specific nutritional studies through which accurate levels of minerals and trace minerals as supplementations will be added to the basal feed of various bird and animal species in order to increase both reproductive and productive aspects.

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INTRODUCTION

Nutritional impacts on reproduction are well-known and well-documented globally. In both males and females; feeding has an effect on every process of the reproduction from gametogenesis to puberty stage (Scaramuzzi *et al*., 2006). Vitamins, minerals, and trace minerals are body required elements for animal health, reproductively, and productivity, as they are engaged in both metabolic and physiological step processes (Adamnezhad and Ghalehkandi, 2018), for instance vitamin E (Tocopherol) improves sperm mitochondrial function and lowers lipid

peroxidation of the sperm membrane, developing sperm membrane integrity (Fouad *et al.*, 2020). Furthermore, dietary considerations are the most essential body requirement since nutrition influences all parts of the reproductive chain in females, including gametogenesis, follicular development, ovulation, and steroidogenesis (Daghigh Kia *et al*., 2019). One of the active natural antioxidants utilized in animal feed is vitamin E. at low concentrations, it has antioxidant function whereas at high concentrations, it has pro-oxidant activity. Tocopherol cannot be synthesized by animal bodies; so, they must be taken from various food sources. This vitamin supplement has been employed to improve the productive and reproductive futures' abilities of chickens as well as the semen of male Japanese quail (Abedi *et al*., 2016; Hezarjaribi *et al.*, 2016). In quails, lack of vitamin E made to slow the growth of the reproductive organs (cloacal gland and testes) and reduce foam production (Fouad *et al.*, 2020).

Selenium (Se) is a trace mineral that is required for a variety of physiological processes in different birds including quail, involving growth, fertilization, immunity, hormone metabolism, and protection against oxidative stress factor, even when vitamin E is available (El-kazaz *et al*., 2020). Increases in semen production and quality could be related to male reproductive organs development, which necessitates sufficient dietary selenium to increase the number of Sertoli cells and their survival, also reducing the induction of apoptosis in germ cells during the period of spermatogenesis by down regulating apoptosis-related genes and up regulating expression of the GSH-Px2 and GSH-Px4 genes (Fouad *et al*., 2020). Even in the presence of vitamin E, selenium is needed for quail growth. Se. deficiency in hens is linked to a reduction of body weight. Moreover Se. insufficiency has also been linked to reproductive issues, Se deprivation also causes spermatozoal mitochondria to become functionless (Altine *et al*., 2016; El-kazaz *et al.*, 2020). On the other hand, traditional kinds of selenium supplementation have low absorption and can cause toxicity (Ibrahim et al., 2020). In addition, supplementing feed with Se. and vitamin E improves sperm quality in broiler breeders and quails, as evidenced by enhanced sperm viability and fewer morphologically aberrant spermatozoa (El-kazaz *et al*., 2020).

Slower growth of birds is somehow caused by insufficient vitamin E quantities in the baseline diet (Abedi *et al.*, 2016), as well as caused male sterility, however this was an issues matter that solved by returning 40 I.U (Altine *et al*., 2016). The principal sex hormone in the avian testes is testosterone (El-kazaz *et al*., 2020). Testosterone affects several elements of male reproductive physiology, including reproductive organ development, spermatogenesis, secondary sexual traits, and sexual drive (Hanafy *et al.*, 2018). Additionally, depletion has been linked to a wide range of diseases (Kataoka *et al*., 2021).

Estradiol-17b is the most physiologically active kind of estrogen hormone, made by aromatase, which catalyzes an aromatic hydroxylation of the A ring of C19 androgens, from testosterone and androstenedione. When referring to E2, the name estrogen is most commonly used. Other organs and tissues of the quail, such as the liver and osteoblasts on the medullary bone surface, contain estrogen receptors (Ciftci and Yıldırım, 2013). However, there is insufficient data on the effects of selenium and vitamin E on reproduction, particularly in sheep.

In fact, selenium affects granulosa cells, boosts 17-estradiol synthesis, and enhances ovulation and the number of viable embryos in cattle, according to some researches, some blood metabolites can affect steroid hormones; for instance, cholesterol is employed as a precursor for progesterone synthesis by luteinized ovarian cells. Follicular growth and estradiol levels were both boosted in cattle with elevated cholesterol levels in a study (Daghigh Kia *et al*., 2019).

MATERIALS AND METHODS

Ethical Matter

The methods of handling protocol were in accordance with the Animal Use and Care Committee of University of Sulaimani Polytechnic University, Kalar Technical College. All quails were managed in accordance with the Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 2022).

Animals and Experimental Protocol

This experiment was conducted on a total number of 128 Japanese quail birds (32 bird's/treatment groups) each group was divided into eight replicates(1male/3female/replicate), at 12 weeks' age. The experimental birds were randomly divided into four groups, each group with eight replicates, 32 birds as a control group (8 males and 24 females= 1mal/3 females), and the other three groups were 96 birds, each was 24 birds (8 males and 24 females=1mal/3females). The four experimental diets (for males and females) were: T1 = the basic diet (control) without any additions (Vit. E-Selenium), T2 = 1.0 gm. Vit. E-Selenium (Sodium selenite) / kg. feed, T3= 1.5 gm Vit. E-Selenium/kg. feed, T4 = 2.0 gm. Vit. E-Selenium/ kg. feed.

Source of Vitamin E-Selenium

VAPCO vitamin E-Selenium powder was used in the experiment, the imported Vit. E - Selenium was obtained from veterinary and agricultural products company (VAPCO), manufactured in Jordan, a package containing powder weighing 1kg. and each gram composition contains: Vitamin E 20 IU and Sodium Selenite 2 mg.

Sampling

Sampling process was conducted after six weeks of supplementation of vitamin E–Selenium powder to the basal diet, during the experiment blood samples were collected from the jugular vein four interval times, so for measuring concentrations of testosterone, estrogen, and progesterone hormones in blood plasma of the birds, the experimented birds were selected randomly and the blood from male and female birds were drawn three times each 30 minutes in order to achieve the mean level of the hormones. Using a venoject syringe and tubes containing EDTA, Then, blood was centrifuged in 2500 rpm/12 minutes at 18 ˚C to separate the plasma from whole blood, after that all separated plasma samples were stored in special open dorf tubes, finally they were frozen at -20 ˚C until the time of analysis.

Analysis

The studied hormones, Testosterone, progesterone, and estrogen (E2) were measured accurately by Monobind Microplate Enzyme Immunoassay, Colorimetric

assay (ELISA) kit No 3725-300 and 4825-300 and 4925-300A manufactured by Monobind Inc., USA.

Statistical Analysis

One-way T-test was used to statistical analysis of the experimental data of the study. The data are presented as Mean Standard Error of the mean, with a significance level of ($P < 0.05$). Multiple Range Comparisons with the Liner Recreation test were also used to analyze significant treatments and replicate effects(IBM SPSS (2016), Statistics for Windows, version 23.0).

RESULTS AND DISCUSSION

The effect of the additions of supplemented dietary vitamin E-Selenium powder to the basal diet on male and female reproduction hormones, testosterone, progesterone, and estradiol (E2) in Japanese quail were showed in both Tables (1) and Tables (2).

Male sex hormones: Table (1) showed that the level of plasma estrogen (Estradiol) hormone was elevated significantly ($P<0.05$) by the effect of supplementation of diet with T4, as well as T4 had a significant effect on the Testosterone level when compared with a control group(T1), T2 and T3 in the same male group. Also, the plasma progesterone concentration in birds of the experimental groups ((T2, T3 and T4) was significantly higher ($P<0.05$) than control group (T1) throughout the period of this study Table (1). However, no significant differences in plasma testosterone and Estradiol (estrogen E2) were observed among treatments (T1, T2 and T3) throughout the period. Moreover, T4 group had the values as regards estradiol E2 and testosterone concentration Table (1) of the study. While, significant differences in plasma progesterone were observed between T2 and T3.

Female sex hormones: For those related to the female group (T2), the result also revealed the superiority ($P<0.05$) of most of the treated birds supplemented diet with vitamin E and selenium in comparison to a control group in plasma testosterone concentration. Significant differences were found among vitamin E treatment groups with respect to this trait. Moreover, birds fed diets containing different levels of vitamin E and selenium recorded higher testosterone concentration when compared with birds fed a control diet(T1). Whereas, no significant differences between T1, T2, as well as T3, T4 regarding progesterone parameter. On the other hands, no significant differences between T1, T4, and T2, T3 regarding estradiol trait. It was clear that the supplemented diet with Vitamin E and Selenium had a positive statistically significant effect on some reproductive and productive features.

Table (1): Effect of mixed dietary vitamin E-Selenium powder on reproduction of male Testosterone, Progesterone, and Estradiol (E2) hormones in Japanese quails (Mean ± SE).

Experimental Feed / Variables	Testosterone ng/ml	Progesterone ng/ml	Estradiol (E2) pg/ml
T1	3.599 ±1.166 b	1.956 ± 1.257 d	539.038 ±183.889 a
T2	2.726 ±0.746 b	4.090 ± 1.459 c	525.805 ±56.812 a
T3	2.616 ±1.322 b	8.583 ± 1.472 a	592.445 ±117.660 a
T4	5.376 ±0.851 a	6.115 ± 2.194 b	1673.276 ±657.049 d

Means in columns that do not have the same letters differ significantly (P < 0.05). T1=control, T2=1.0 gm vitamin E-Selenium (Sodium Selenite)/kg feed, T3=1.5 gm vitamin E-Selenium/kg feed, T4=2.0 gm vitamin E-Selenium/kg feed.

The results presented that supplementation of the diet had statistically no significant effect on the concentration of plasma estradiol (E2) among (T1, T2, and T3) of the male group in Japanese quail (P<0.05) Table (1), whereas (T2, T3 and T4) had significant deference on the E2 (P<0.05), and the Table (1) showed that there were statistically significant differences among (T1, T2, T3 and T4) in progesterone concentration of female group (P<0.05). Significant elevations were observed in plasma concentration of both male and female testosterone and progesterone hormones in the quail birds when compared to control groups Table (1) and Table (2), additionally, the testosterone in Table (2) from (T1 to T4) of females and the progesterone in Table (1) from (T1 to T4) of male appeared to be increased.

Table (2)**:** Effect of mixed dietary vitamin E-Selenium powder on reproduction of female Testosterone, Progesterone, and Estradiol (E2) hormones in Japanese quails (Mean ± SE).

Experimental Feed/ Variables	Testosterone ng/ml	Progesterone ng/ml	Estradiol (E2) pg/ml
T1	0.710 ±0.264 d	9.535 ±2.127 a	2527.2667 ±338.299 b
T2	1.525 ±0.513 c	10.230 ±3.823 a	3115.11 ±383.168 a
T3	3.557 ±0.756 b	7.455 ±2.560 b	3066.25 ±308.066 a
T4	4.445 ±0. 839 a	6.580 ±1.243 b	2395.107 ±492.145 b

Means in columns that do not have the same letters differ significantly (P < 0.05). T1=control, T2=1.0 gm vitamin E-Selenium (Sodium Selenite)/kg feed, T3=1.5 gm vitamin E-Selenium/kg feed, T4=2.0 gm vitamin E-Selenium/kg feed.

The elimination of free radicals could explain why vitamin E and selenium administration improves development and feed efficiency. Vitamin E and the selenium-containing enzyme glutathione peroxidase scavenge free radicals, destroying any peroxides that could harm the cell, these two antioxidants protect cell membranes from oxidative damage while also enhancing nutrient utilization (Chitra et al., 2014and Madkour et al., 2015). A considerable increase in estrogen, testosterone, and progesterone yolk concentrations in Leghorn hens exposed to moderate heat stress was documented in a prior study (Bertin *et al*., 2015).

Effect of sodium selenium and vitamin E supplementation on testosterone, progesterone, and estradiol reproduction hormones in male and female Japanese

quail, different dosages throughout the period of the study were shown to be significantly connected to increase the concentration of reproduction hormones stated above, with the exception of Estradiol (E2) concentration of (T1and T4) in female group, where found to be significantly enhanced estradiol in (T2and T3) of female Table (2) and all treatment groups of female groups in Japanese quail as shown in Table (1). This result disagrees with that's founded by (Abedi *et al.,* 2016 and Skřivan *et al*., 2010), as well as agree with that's showed by (Egbuniwe et al., 2021; El-kazaz *et al*., 2020; El-Sheshtawy et al., 2014 and Al-Salhie *et al*., 2017). This significant decrease in estrogen and progesterone concentration in blood plasma of control group birds may be attributed to the decrease of LH and FSH release from the anterior pituitary gland and also may be due to progesterone acts as the level of the ovary and hypothalamus to stimulate the LH surge for ovulation, and triggers gonadotropin-releasing hormone(GnRH) release by the hypothalamus(Amen and Al-Daraji,2011).The LH stimulates an even output of progesterone by the granulose cells of the hierarchical follicles(F1) (Etches,2008). These significant increments ($P<0.05$) and amelioration in plasma testosterone concentration may be attributed to vitamin E and selenium participation in regulating the function and protection of the sexual gland system.

The nutrient requirements of Japanese quails are highly depended on the birds' age, growth, egg production, and production purposes (Altine *et al*., 2016). When it is compared to a non-supplemented diet, dietary supplementation with different vitamins had no significant effect on fertility percentages (El-Mekawyp et al., 2016). Feed intake had no effect significantly between the control (basal diet) and 250 mg kg-1 vitamin E supplementation treatments in a study of vitamin E's influence on reproductive and productive performance in turkeys (Abedi *et al*., 2016). In birds, testosterone is a significant male steroid hormone belonging to the androgen group, changes in the Gonadotropin-releasing hormone (GnRH) which releases from the hypothalamus, testosterone production in the testes, or other steroidogenic tissues could all affect testosterone concentrations in the blood. Although minor amounts are secreted by the adrenal glands, it is principally generated and secreted by Leydig cells in males' testicles and females' ovaries (Hanafy et al., 2018). Selenium is a vital trace element for all living things, so selenium supplementation raises testosterone levels in goats.

Selenium deficiency makes a reduction in testosterone levels in rats, whereas selenium supplementation raised testosterone through modulating LH levels, in mice, selenium protected against aflatoxin-induced testicular damage and decreased testosterone depletion. it was also found to preserve testosterone levels in animals by reducing testicular toxicity in several experiments (Kataoka *et al*., 2021). Vitamin E, when combined with flaxseed oil, raises testosterone levels in roosters (Kataoka *et al.*, 2021). In Taiwan native roosters, dietary supplementation of vitamin E at 0, 20, 40, 80, and 160 mg/kg had no effect on plasma testosterone concentration, which was similar to these findings in vitamin E levels less than 240 mg/kg (Abedi *et al*., 2016). In animals and birds, the favorable benefits were seen as an increase in testes weight, semen quality parameters, antioxidant status, and testosterone. (Zubair M., 2017). The present result is in line with that's presented in the study conducted by (Abedi *et al*., 2016; Daghigh Kia et al., 2019 and Skřivan *et al*., 2010). Female quails had higher

serum estrogen concentrations in 120 mg/kg vitamin E than controls. (Abedi et al., 2016). The concentration of E2 also increased at doses of 20, 40, 80 and 160 mg/kg compared with the control group (Yin *et al*., 2019). Also, the findings of this study declared its similarity with the results of (Abedi *et al*., 2016 and Al-Salhie *et al*., 2017).

CONCLUSIONS

The addition of different quantities of a combination of dietary vitamin E and Selenium powder in the Japanese quail basal diet in this experiment made the bird's various reproductive hormones and productive features alterations positively. So, this study declared that the addition of various levels of dietary vitamin E-Selenium powder (1gm, 1.5 gm and2 gm) to each kilogram of basal food somehow had a positive significant effect on the plasma Testosterone, Progesterone, and Estrogen hormones concentration of male and female quails ($P<0.05$) and made these hormones be increased in levels. Further related and specific nutritional studies should be performed on adding accurate and specific levels of most supplemented materials, especially the vitamin E and Selenium to basal food of different bird and animal species in order to make the reproductive and productive futures be increased, then directly applying these studies to achieve the best goals.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

أثر خليط الفيتامين E والسيلينيوم في الغذاء على تركيز هرمونات التناسل في بلازما الدم لذكور واناث طائر السمان الياباني (COTURNIX COTURNIX JAPONICA)

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الخلاصة

هدفت الدراسة الى تقييم تأثير اضافة مخلوط فيتامين E والسيلينيوم (سيلينيت الصوديوم) في غذاء السمان الياباني على مستويات الهرمونات التناسلية. أجريت الدراسة على 128 طائراً من السمان الياباني بعمر 12 أسبوعًا. قسمت طيور التجربة عشوائياً إلى أربع مجموعات (32طائراً / مكرر)، كل مجموعة مكونة من ثماني مكررات (ذكر واحد / 3اناث / مكرر). المعاملات الغذائية التجريبية الأربعة هي T1 = النظام الغذائي

الأساسي (التحكم) بدون إضافة، T2 = مخلوط فيتامين +E السيلينيوم 1.0 جم / كجم علف، T3 = فيتامين +E السيلينيوم 1.5 جم / كجم علف، T4 = فيتامين +E السيلينيوم 2.0 جم / كجم علف. أشارت النتائج إلى أن إضافة فيتامين E والسيلينيوم إلى العلف في المعاملة (T4) أدى إلى زيادة معنوية ($P < 0.05$) في مستوى هرمون الإستراديول (E2) والتستوستيرون والبروجسترون مقارنة بمجموعة التحكم (T1) في الذكور واناث السمان الياباني. فيما يتعلق بمجموعة الذكور، أظهرت النتائج أن المعاملتين T2 و T3 لم يكن بينهما فرق معنوي في مستوى هرمون التستوستيرون والاستروجين مقارنة بمجوعة المقارنة. أدى النظام الغذائي المضاف إليه T4 إلى زيادة مستوى هرمون التستوستيرون بشكل معنوي. بينما لم يكن لجميع المعاملات T2 و T3 و T4 تأثيراً معنوياً على تركيز هرموني البروجستيرون فيما يخص مجموعة الذكور. هناك انخفاض معنوي للمعاملتين الثالثة والرابعة مقارنة بالسيطرة والمعاملة الثانية والتي لم تختلف بدورها عن السيطرة في مجاميع الاناث. اتضح أن العلف المعامل بفيتامين E والسيلينيوم إحصائياً ($P < 0.05$) يؤثر على بعض هرمونات التكاثر. لذلك، تمت التوصية بإجراء المزيد من الدراسات الغذائية المتقدمة التي يتم من خلالها إضافة مستويات دقيقة من المعادن والمعادن النادرة كمكملات إلى العلف الأساسي لمختلف أنواع الطيور والحيوانات من أجل تحسين الكفاءة التناسلية والإنتاجية.

الكلمات المفتاحية: فيتامين E، السيلينيوم، الهرمونات، السمان.

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