



## **Effect of Supplementation Different Levels of Vitamin E and Pumpkin Seed Oil to the Diet on Productive, Physiological and Reproductive Performance of Japanese Quail**

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Received 18 July 2017; Accepted 12 December 2017; Available online 25 December 2017

**Abstract:** The principal goal of this study was to evaluate whether dietary vitamin E and pumpkin seed oil would affect the productive, physiological and reproductive performance of Japanese quail by using 120 birds at 22 weeks of age. The birds were randomly distribution into five dietary groups 24 birds each group contain three replicated pens, as follow: First group: kept on basal diet without any addition as control group, second and third groups were fed the basal diet supplemented with 150 and 200 mg/ kg vitamin E / kg of diet, fourth and fifth groups were fed the basal diet supplemented with 15 and 30 ml/kg pumpkin seed oil /kg of diet respectively. The results showed a significant improvement in egg production (H.D%), egg number, egg weight and egg mass produced for the vitamin E (200mg/ kg of diet) compared with the other groups. Additionally, the results showed that vitamin E (200mg/ kg of diet) significantly increased gonads relative weight , testes development, egg characterizes and serum testosterone, estrogen, FSH and LH hormones compared with other groups. Also , the birds were fed on 15 and 30 ml seed pumpkin oil/ kg of diet were significantly increased in the most parameter compared with control group. It can be concluded that the addition of vitamin E (200 mg/kg of diet) and pumpkin seed oil (30ml of diet), had positive effects on gonads, testicular histology, egg production and serum testosterone, estrogen, FSH and LH hormones concentration.

**Keywords:** Vitamin E, Pumpkin seed oil, Reproductive parameter, Japanese quail.

### **Introduction**

Japanese quail is the one of avian give rise to zoological farmed and has been used as a source of animal protein recently. Quails are usually reared for egg and meat production in most Countries in Asian (Sultan *et al.*, 2017). Avian fertility depends on several factors such as spermatogenesis, gonads maturity and

function and survival of sperm in the oviduct (Froman *et al.*, 2011). Vitamin E (V.E) is found in most oil plants, and has many functions in animals and humans, as protect cells from oxidative damage, and promotes fertility in males and females in avian species (egg production, egg fertility and egg

hatchability) ((Rengaraj and Hong, 2015). In addition, V.E is essential for enhancing the cellular and humeral immunity (Sahin *et al.*, 2002). Pumpkin Seed Oil (PSO) is a food sources rich in antioxidants and polyunsaturated fatty acids, essential fatty acids,  $\beta$ -carotenes, lutein  $\gamma$  and selenium (AL-Zuhairu *et al.*, 2000). Pumpkin seed extract contain large amounts of common intense phytochemicals sterols which improve immune system and reproductive status and in addition being therapeutic benefits for many disease (Glew *et al.*, 2006; Fruhwirth and Hermetter, 2007 and Stevenson *et al.*, 2007). The antioxidant property of PSO could enhance male fertility (Murkovic, *et al.*, 1996). Sustaining PSO and vitamin E improve defensive and cancer prevention agent impacts against sodium valproate (SVP) and incited testicular harm in rats (Hashemi, 2013). Therefore, the objective of this study was to determine the effect of dietary supplementation with vitamin E and pumpkin seed oil on the productive, physiological and reproductive performance of Japanese quails.

## Material and methods

This study was carried out at quail farm belonging to College of Agriculture, University of Basrah. A total of 120 Japanese quail (*Coturnix japonica*) at 22 weeks of age with an average initial body weight of (166.5 gm). A birds were randomly distributed in the five groups of three replicates each (4 males and 4 females) as follow First group: kept on basal diet without any addition as control group, second and third groups: were fed the basal diet supplemented with 150 and 200 mg/ kg vitamin E /kg of diets, while fourth and fifth groups : were fed the basal diet supplemented with 15 and 30ml/kg pumpkin seed oil /kg of diets respectively. Birds were housed in quail's batteries under the same condition and rearing system. Feed and water were allowed *ad libitum*. feed containing approximately 20.03% crude protein and

2904 kcal/kg metabolizable energy. Ingredients and chemical composition of experimental diet are shown in Table (1). Egg production was monitored for eight weeks after diet were treated with vitamin E and pumpkin seed oil (PSO). The following indices of number of eggs laid, average egg weight, egg mass was calculated as laying rate  $\times$  egg weight and Hen Day production (HDP) % which was calculated according by (Younis, 2014). Feed intake measured weekly and feed conversion ratio (calculated as kg feed intake per kg eggs laid) (Zduńczyk *et al.*, 2013). At the end of the experiment, the birds were slaughtered and blood samples (5 ml) were taken from each males and females. The blood sample was drawn and allowed to clot at room temperature (25 °C) for 2 h prior to serum collection. Serum was separated by centrifugation and stored at -20 °C for further analysis. A part of blood sample was prepared to measure the serum testosterone, estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) was also assayed by ELISA procedure. After slaughtering, the testes , ovaries and oviducts were weighed . The length and diameter of both testis were measured in millimeter using Vernier's caliper. The size of both testis were collocated by:  $(4/3 \times 3.5414 \times a^2 b)$  where a = half the longitudinal axis of the testicle, b = half transverse axis (Chaturvedi *et al.*, 1993; Moller, 1994). According Gridley, (1960), after weighing, the testes were fixed in 4% neutral formaldehyde, embedded in paraffin, and the sections were stained with haematoxylin and eosin at room temperature. The stained cells were analyzed using a light microscope. Then the measurements of different histological structures of the testes tissues were performed by the calibrated stage micrometer in  $\mu\text{m}$  (micrometer). Collected data were subjected to one way analysis of variance (ANOVA) and differences were considered to be significant if P was < 0.05 according to SPSS Statistics (2009).

Table (1): The ingredients and composition of experimental diets.

Ingredient	Percentage %
Yellow Corn	56
Wheat	4.0
Soybean meal (44%)	28
Protein Concentration	5.0
Oil plant	1.0
Limestone	4.4
Dicalcium Phosphate	1.0
Vitamin / mineral premix	0.3
Common Salt	0.3
total	100
Chemical analysis (%)	
<sup>1</sup> ME ( Kcal /Kg) diet	2904
Crude protein %	20.03
Ether extract %	3.93
Crude fiber %	3.49
Calorie : protein ratio	144.98
Calcium %	2.31
Phosphorus available %	0.46
Methionine %	0.38
Lysine %	1.06
Methionine + Cystine %	0.83

ME (Kcal /Kg) diet = Metabolized energy.

## Results and discussion

Data of some productive traits in Table (2) revealed highest ( $p < 0.05$ ) feed intake and better feed conversion ratio in birds fed diet supplemented with 200mg V.E/kg diet as compared with other groups. The improvement in feed efficiency by vitamin E supplementation might be due to the possible removal of free radicals. Free radicals are scoured by vitamin E containing enzyme glutathione peroxidase, which destroys any peroxides formed, that could damage the cell. This antioxidants protect cell membranes against oxidative damage, in addition to improving the utilization of nutrients (Chitra *et al.* 2014). The present study indicates a significant ( $p < 0.05$ ) increased in feed consumption and better feed conversion ratio in the birds fed 30 and 15 ml pumpkin seed oil / kg of diets compared with control group. Increasing feed intake with the addition of PSO could be due to the stimulation effect of essential oils toward appetizing and digestive process in birds (Hosna *et al.*, 2011). On the

other hand, this improving, may be due to the possible removal of free radicals, where PSO has a rich antioxidant vitamins (Glew *et al.*, 2006 ). Results also showed that adding 200 mg V.E /kg to quail diets resulted in significant ( $P < 0.05$ ) increase in a hen day egg production (%) , egg mass, egg weight and number of eggs during the experimental periods. The results of this study were concurrence with the finding of Ciftci *et al.* (2005) who showed that supplemental vitamin E (125 mg/kg diet) significantly improved egg production in laying hens. The improvement in egg production may be due to vitamin E supplementation of diets contains many unsaturated fatty acids which prevent oxidation feed and help in the formation of egg , these beneficial protective effects of vitamins were evidenced by increase of egg production and qualities in supplemented laying hens in comparison to control birds (Gey, 1998). In addition, during eggshell formation excess amount of vitamin E (150

mg or 200 mg/kg of diets) restraint prostaglandin biosynthesis. Prostaglandins may regulate ovulation and are correlated with production performance (Mezes and Hides, 1992). The results indicates a significant ( $p<0.05$ ) increased in hen day production (HDP) % , number of egg , mass egg and egg weight in the hens fed 30 and 15 ml pumpkin seed oil / kg of diets compared with control. It can be predicted that the improvement in productive traits probably resulted from the highly unsaturated fatty acid

contents of PSO especially oleic (up to 46.9 %) and linoleic (up to 40.5 %) acid (Nakiae *et al.* 2006; Srbinoska *et al.*, 2012). Our findings are similar to the results of Abbas *et al.*, (2016) who revealed that, feeding laying quails during late period of egg production with diets containing pumpkin seeds oil (2 %) recorded the best results as concerns total hen-day egg production, cumulative egg number, egg mass and feed conversion ratio when compared with control group.

**Table (2): Effect of supplementation different level of vitamin E and pumpkin seed oil to the diet on some productive traits (Mean± SE).**

Groups	Feed intake (FI) (g/bird/8 weeks)	Feed conversion ratio (FI/EM*)	Hen day egg production (%)	Number of eggs (g/bird/8 weeks)	Egg mass (EM) (g/bird/8 weeks)	Egg weight (g)
T1: Control	957.36 <sup>c</sup> ± 15.96	2.77 <sup>c</sup> ± 0.04	71.82 <sup>e</sup> ± 0.01	30.16 <sup>c</sup> ± 0.04	345.7 <sup>d</sup> ± 0.94	11.45 <sup>d</sup> ± 0.02
T2: Vit. E (150mg/Kg)	1003.71 <sup>b</sup> ± 7.58	2.69 <sup>b</sup> ± 0.03	75.73 <sup>c</sup> ± 0.14	31.81 <sup>c</sup> ± 0.06	372.7 <sup>c</sup> ± 1.16	11.71 <sup>c</sup> ± 0.02
T3: Vit. E (200 mg/kg)	1067.02 <sup>a</sup> ± 1.77	2.53 <sup>a</sup> ± 0.01	80.50 <sup>a</sup> ± 0.12	33.81 <sup>a</sup> ± 0.05	421.7 <sup>a</sup> ± 1.01	12.48 <sup>a</sup> ± 0.05
T4: PSO (15ml /Kg)	1013.71 <sup>b</sup> ± 4.63	2.71 <sup>bc</sup> ± 0.01	76.29 <sup>b</sup> ± 0.04	32.04 <sup>b</sup> ± 0.02	373.7 <sup>c</sup> ± 0.18	11.66 <sup>c</sup> ± 0.01
T5: PSO (30 ml/Kg)	1011.36 <sup>b</sup> ± 8.13	2.67 <sup>b</sup> ± 0.01	73.62 <sup>d</sup> ± 0.12	30.92 <sup>d</sup> ± 0.05	379.4 <sup>b</sup> ± 1.32	12.28 <sup>b</sup> ± 0.02
Significance	*	*	*	*	*	*

Means ± SE with different superscripts in the same vertically are significant at  $p<0.05$ .

\*FI/EM : Feed intake / Egg mass.

The effects of dietary supplementation with vitamin E(V.E) and pumpkin seed oil (PSO) on testes measurements are shown in Table (3). It was observed that treatment the Japanese quail with V.E and PSO have no significant effect on right testis width and length and width of left testis. Table (3) also showed that feeding 200 mg V.E /kg diet had a significantly ( $p<0.05$ ) higher in length of right testicle with no significant differences with group two and four. In addition, third group (200 mg V.E /kg) has achieved the highest ( $p<0.05$ ) values in volume of right and left testicle as compared to the other treatment. The improvement in volume of

testes with dietary vitamin E(150 and 200mg/Kg) and pumpkin seed oil (30 ml/kg) in this study, may be responsible for improving increase of serum testosterone, FSH and LH hormones (Table 5) as it has been established that testosterone is essential for spermatogenesis. Moreover, FSH and LH play an important role in germ cell progression and improved fertility in animal models (El-Boghdady, 2011). Also, pumpkin seed oil is food sources rich in antioxidants and polyunsaturated fatty acids, essential fatty acids,  $\beta$ -carotenes, lutein  $\gamma$  and selenium (El-Adawy and Taha 2001; Procida *et al.*, 2012). It's also contain very high concentration of

vitamin E which acts as a powerful antioxidant. Essential fatty acids are required constituents of health of cell membrane as

they maintain the fluidity of cell testes (Ryan *et al.*, 2007).

**Table (3): Effect of supplementation different level of vitamin E and pumpkin seed oil to the diet on some testes measurements (Mean± SE).**

Groups	Right Testis			Left Testis		
	Length (mm)	Width (mm)	Volume (mm <sup>3</sup> )	Length (mm)	Width (mm)	Volume (mm <sup>3</sup> )
T1: Control	20.60 <sup>b</sup> ± 1.13	13.30± 0.35	2140.90± 25.84 <sup>c</sup>	21.98± 1.02	13.11± 0.26	2229.70 <sup>c</sup> ± 21.44
T2: Vit. E (150mg/Kg)	22.19 <sup>ab</sup> ± 1.10	13.14± 0.35	2258.51± 4.12 <sup>b</sup>	21.33± 0.59	13.87± 0.12	2418.88 <sup>b</sup> ± 26.94
T3: Vit. E (200 mg/kg)	26.07 <sup>a</sup> ± 1.56	12.87 ± 0.34	2536.46 <sup>a</sup> ± 19.54	25.32± 0.26	13.44± 0.12	2697.34 <sup>a</sup> ± 23.59
T4: PSO (15ml /Kg)	23.64 <sup>ab</sup> ± 0.90	12.71± 0.26	2248.21± 20.37 <sup>b</sup>	22.90± 1.45	13.04± 0.40	2284.39 <sup>c</sup> ± 12.36
T5: PSO (30 ml/Kg)	22.14 <sup>b</sup> ± 1.36	13.34± 0.43	2311.79± 29.72 <sup>b</sup>	23.55± 2.78	13.33± 0.66	2424.32 <sup>b</sup> ± 31.03
Significance	*	N.S	*	N.S	N.S	*

Means ± SE with different superscripts in the same vertically are significant at  $p < 0.05$ . N.S : none significant).

Results of reproductive traits are presented in Table (4). The results showed a significant ( $p < 0.05$ ) increased in the seminal tubule diameter, thickness of spermatogenesis and the relative weights of gonads and oviduct in birds fed 200 mg vitamin E/kg of diet as compared with other groups. The improvement in these traits may be due to increased serum level of testosterone, FSH and LH hormones of male and estrogen in female (Tables 5). In addition, gonadotropins and steroid hormones are necessary in controlling the cyclical pattern of ovarian follicular development (Findlay *et al.*, 2009). LH/testosterone and FSH are the important endocrine factors controlling testicular development and its functions (Ramaswamy and Weinbauer, 2014). On other hands, estrogen stimulate hormone of reproductive organ development in Japanese quail hen (Mattsson and Brunstrom, 2010). This study indicates a significant ( $p < 0.05$ ) increasing in the seminal tubule diameter,

thickness of spermatogenesis, cavity diameter of seminiferous tubular and the relative weights of gonads in the birds fed 30 ml pumpkin seed oil / kg of diet compared with control and group 4, may be due to pumpkin seed oil content of  $\alpha$ -tocopherol and vitamin A antioxidant activity (Bairy and Rao, 2010). According to Sato, (2004), vitamin A protects the testis against lipid peroxidation, therefore, stimulate spermatogenesis and improves structural differentiation of epithelial cells of the epididymis. Besides the fact that vitamin A increase the productive performance (Al-Salhie, 2014). This explains the increased in spermatogenesis at 30ml /kg of PSO levels. Also, linoleic acid, a polyunsaturated fatty acid present in PSO is known to increase membrane fluidity and allows for osmosis, intracellular and extracellular gaseous exchange (Lovejoy, 2002). Also, the presence of oleic acid, a monounsaturated fatty acid also decrease the susceptibility of the testis to lipid peroxidation (Bourre *et al.*, 2004;

Lovejoy, 2002). This probably explains the better seminal tubule diameter, thickness of spermatogenesis, cavity diameter of

seminiferous tubular and the relative weights in the testis at the lower dose.

**Table (4): Effect of supplementation different level of vitamin E and pumpkin seed oil to the diet on some reproductive traits (mean± SE).**

Groups	Diameter of seminiferous tubular (µm)	Thickness of spermatogenesis (µm)	Relative Weight of right testis	Relative Weight of left testes	Relative Weight of ovary	Relative Weight of oviduct
T1: Control	336.67 <sup>b</sup> ± 8.82	107.13 <sup>d</sup> ± 3.13	1.62 <sup>c</sup> ± 0.01	1.68 <sup>cd</sup> ± 0.05	4.99 <sup>b</sup> ± 0.06	4.85 <sup>c</sup> ± 0.06
T2: Vit. E (150mg/Kg)	353.33 <sup>b</sup> ± 17.64	107.22 <sup>c</sup> ± 3.23	1.66 <sup>c</sup> ± 0.01	1.73 <sup>b</sup> ± 0.02	5.25 <sup>a</sup> ± 0.02	5.09 <sup>b</sup> ± 0.02
T3: Vit. E (200 mg/kg)	403.33 <sup>a</sup> ± 14.53	140.30 <sup>a</sup> ± 0.19	1.71 <sup>a</sup> ± 0.01	1.78 <sup>a</sup> ± 0.02	5.32 <sup>a</sup> ± 0.01	5.31 <sup>a</sup> ± 0.05
T4: PSO (15ml /Kg)	340.00 <sup>b</sup> ± 11.55	110.50 <sup>cd</sup> ± 0.14	1.64 <sup>d</sup> ± 0.01	1.67 <sup>d</sup> ± 0.02	5.03 <sup>b</sup> ± 0.04	4.69 <sup>d</sup> ± 0.07
T5: PSO (30 ml/Kg)	383.33 <sup>a</sup> ± 8.82	127.03 <sup>b</sup> ± 3.17	1.68 <sup>b</sup> ± 0.01	1.72 <sup>bc</sup> ± 0.02	5.24 <sup>a</sup> ± 0.02	4.92 <sup>c</sup> ± 0.08
Significance	*	*	*	*	*	*

Means ± SE with different superscripts in the same vertically are significant at p<0.05.

Results of some sex hormones concentration are presented in Table (5). The results showed the highest testosterone, estrogen, LH and FSH hormones concentration a significant (p<0.05) increase in the males and females birds fed 200 mg vitamin E/ kg and followed by 150 mg vitamin E/ kg and 15 or 30 ml pumpkin seed oil/kg of diets as compared with control. While the males fed 15 ml pumpkin seed oil/kg of diet were not significantly affect the testosterone and LH hormones concentration as compared to control group. The increase of serum testosterone, estrogen, FSH and LH hormones caused by administration of vitamin E and Pumpkin seed oil to quail, in this study, might be increased of gonads development testes and ovaries (Table 4) in the birds. Moreover, the serum testosterone hormone was increased significantly in males birds treated with vitamin E might be due to the positive effect of vitamin E on testosterone secretion may be associated with testes better utilization of selenium and vitamin E (Jerysz and

Lukaszewicz, 2013). In addition, administration of pumpkin oil and vitamin E attributed to their antioxidant activity (Bairy and Rao, 2010). The significant increase in LH, FSH and testosterone hormones levels were concurred with (Al-Attar, 2011, Muthu and Krishnamoorthy, 2012). LH and FSH hormones activity depends on both the levels of these hormones and the quantity of particular receptors in the testicles. It has been demonstrated that Leydig cells of the testis are in charge of then biosynthesis and secretion of androgens, critical for developmental and reproductive function in the males. In any case, FSH influences sterol's cells, in that it triggers the development of a germ cells testosterone restricting protein. Additionally, the got results might be clarified by activities of vitamin E and pumpkin seed oil. These activities were reflected by the expansion of serum testosterone, LH and FSH levels. Vitamin E is an important antioxidant, as protect cells from oxidative damage

(Deivendran and Yeong, 2015) . The serum estrogen hormone was increased significantly in females birds treated with vitamin E and

fed 30 or 15 ml pumpkin seed oil/ kg of diets may be due to increase in size of the ovary in the hens (Table 4).

**Table (5): Effect of supplementation different level of vitamin E and pumpkin seed oil to the diet on some sex hormone (mean± SE).**

sex Groups	Male			female		
	Testosterone (ng/ml)	FSH (IU)	LH (IU)	Estrogen (pg/ml)	FSH (IU)	LH (IU)
T1: Control	2.15 <sup>d</sup> ± 0.11	1.54 <sup>d</sup> ± 0.05	1.75 <sup>c</sup> ± 0.04	115.74 <sup>e</sup> ± 2.15	4.45 <sup>d</sup> ± 0.03	2.82 <sup>c</sup> ± 0.07
T2: 150mg Vit.E /kg	3.06 <sup>b</sup> ± 0.10	1.63 <sup>b</sup> ± 0.05	1.89 <sup>c</sup> ± 0.03	156.49 <sup>b</sup> ± 2.79	5.08 <sup>b</sup> ± 0.08	3.93 <sup>b</sup> ± 0.08
T3: 200mg Vit.E /kg	3.79 <sup>a</sup> ± 0.11	1.85 <sup>a</sup> ± 0.07	2.29 <sup>a</sup> ± 0.04	182.85 <sup>a</sup> ± 1.81	5.56 <sup>a</sup> ± 0.04	4.29 <sup>a</sup> ± 0.08
T4: 15ml oil /Kg	2.49 <sup>cd</sup> ± 0.09	1.51 <sup>b</sup> ± 0.06	1.80 <sup>c</sup> ± 0.08	125.13 <sup>d</sup> ± 1.50	4.83 <sup>c</sup> ± 0.06	3.73 <sup>b</sup> ± 0.05
T5: 30 ml oil /Kg	2.60 <sup>c</sup> ± 0.13	1.69 <sup>ab</sup> ± 0.05	2.05 <sup>b</sup> ± 0.04	140.48 <sup>c</sup> ± 2.59	5.00 <sup>bc</sup> ± 0.10	3.87 <sup>b</sup> ± 0.07
Significance	*	*	*	*	*	*

Means ± SE with different superscripts in the same vertically are significant at p<0.05.

## Conclusion

The study concluded that the addition of vitamin E (200 mg/kg of diet) and pumpkin seed oil (30ml), had positive effects on gonads, testicular histology, egg production and serum testosterone, estrogen, FSH and LH hormones concentration in Japanese quail.

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