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Semen quality, sperm variables, blood profile, immunity, and antioxidant capacity of Sinai cockers fed diet supplemented with vitamin E or/and pumpkin seed oil

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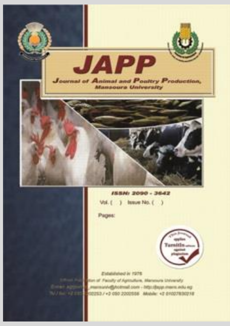

ABSTRACT

This study aimed to evaluate the antioxidant capacity of pumpkin seed oil (PSO), vitamin E (Vit. E), or their combination on semen quality, sperm parameters, health status, and immunity of cockers of Sinai local strain chicken. Total of 40 cockers (40 weeks old and 1946-1963.5 g LBW) were divided into four groups (10 in each). In the 1st group, birds were fed basal diet without any supplementation (Control). In the 2nd, 3rd, and 4th groups, the control diet was supplemented with 100 mg Vit. E/kg, 1% PSO, and 100 mg Vit. E+1% PSO/kg, respectively. At the end of the treatment period of 8 weeks, semen was collected twice a week for four weeks from 10 cockers in each group. All ejaculates were evaluated by CASA analyzerand blood samples were collected. All treatments improved ($P<0.05$) semen volume and concentration, the percentages of sperm motility, abnormality, and velocity parameters compare with control. Also, all treatments increased ($P<0.05$) plasma testosterone, total protein, , T_3, T_4, immunoglobulins, antioxidant capacity, and decreased AST and ALT activities. PSO alone or with Vit. E increased, hematology, glucose, total lipids, and hatchability, while decreased plasma cholesterol and creatinine levels. Egg fertility was increased only when chickens were mated only by males treated with Vit. E+PSO combination.Dietary supplementation with a combination of 100 mg Vit. E+1% PSO had beneficial effects on semen quality, sperm function, blood testosterone, liver, kidney, and thyroid functions, immunity, egg fertility and hatchability rates of cockers of Sinai local strain chicken.

Keywords: Sinai chicken, vitamin E, pumpkin, semen, blood profile.

INTRODUCTION

In poultry farms, the decrease in rooster fertility after 45-week of age is a main problem which could led to economic losses for breeders (Safari Asl *et al*., 2018). Therefore, improving the reproductive performance of aged roosters, in terms of increasing fertility rate is very important with aging (Safari Asl *et al*., 2018; Abbaspour *et al*., 2020). Poultry had no ability to produce omega-3 because it had not specific enzyme (Cerolini *et al*., 2005). In poultry, increasing male fertility rate was performed by using feeding strategies, such polyunsaturated fatty acids (PUFAs) addition can change the physiological situation of cookers (Feng *et al*., 2015). Recently, the addition of omega-3 to cocker diets had marked role in improve the quality of semen (Gulliver *et al*., 2012), by improving sperm motility, plasma membrane function, and spermatozoa viability (Alagawany *et al*., 2019).

Phytogenic had important role in animal spermatogenesis process (Jimoh *et al*., 2023) consequently improve their reproductive performance (Hassan *et al*., 2022). Pumpkin seeds (PS) contain about 40-45% oil, fatty acids, such as linolenic, palmitic, stearic, and oleic acid (Majid *et al*., 2020), 25-35% protein, including amino acids (phenylalanine, lysine, and alanine), beta-carotene, vitamin E, and minerals (Rohman, 2020; Lotfi *et al*., 2021). In poultry, PS were used to increase bird growth and production in association with improve immunity (Achilonu *et al*., 2018; Mathewos *et al*., 2019). In PS extract, there are diffeent phytochemicals sterols having the ability to modulate the immunity and reproduction, and also therapeutic impacts on several diseases (Glew *et al*., 2006; Fruhwirth and Hermetter, 2007; Stevenson *et al*., 2007).

Pumpkin seed oil (PSO) has antioxidant properties (Hashemi, 2013; Shaban and Sahu, 2017), because it contains antioxidants and poly-unsaturated and essential fatty acids, beta-carotenes, lutein γ and Se (Zuhair *et al*., 2000). In addition, it considered as an important plant which is used in improving male fertility (Gundidza *et al*., 2009), testosterone levels, semen quality, and antioxidant status in rabbit males (Ragab *et al*., 2016).

There are eight compounds soluble in fat that named vitamin E (vit. E), four of it are tocopherols in the forms α, β, γ, and δ (Górnaś, 2015), the most widespread component of these forms in nature is α-tocopherol. It is known as the major part of antioxidants in spermatozoa cell and it can reduce reactive oxygen species (ROS) production, one molecule of this component can balance the side effects of two peroxyl radicals which causes lipid peroxidation (Vincenzo and Vito, 2016). In chickens, Vit. E could regulate the transcription process of lipid metabolism and oxidation (Li *et al*., 2009; Zduńczyk *et al*., 2013). The first detection of Vit. E in avian semen was in turkey in 1981 and it recorded that sperm cells contain 85% while the seminal plasma had small amount (Surai and Ionov, 1992). This vitamin is important for poultry reproduction, and the reduction of Vit. E levels led to damage the reproductive system result in testes defection, seminiferous tubules degeneration, and sperm deformation (Todorovic *et al*., 2004; Surai *et al*., 2019). In avian, several

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authors (Traber and Atkinson, 2007; Tufarelli and Laudadio, 2016) indicated the importance of the requirements of Vit. E because of the lipid peroxidation process causes damage in cell membranes and it, as a natural antioxidant, had a guard role in against cell membrane. Vit. E is required for feeding poultry males to maintain the quality of sperm cells, in terms of reducing or preventing lipid peroxidation in semen (Khan, 2011; Rengaraj and Hong, 2015) and boosting the macrophagial and lymphocytic, proliferationally and functionally, against oxidative stress (Traber and Atkinson, 2007), consequently enhancing semen quality and fertility (Khan, 2011).

It has been shown that dietary intake of PSO and Vit. E improved reproductive function of male rats (Hashemi, 2013). Because of PSO contains Vit. E (Rohman, 2020; Lotfi *et al*., 2021), we assumed that Vit. E in PSO, as a natural antioxidant, may be sufficient to meet the Vit. E requirements in the diets of male birds, as an alternative to Vit. E. Therefore, the present study aimed to evaluate the antioxidant capacity of PSO, Vit. E, or their combinations to improve the reproductive performance, health status, and immunity of cockers of Sinai local strain chickens.

MATERIALS AND METHODS

The experimental work of this study was performed at El-Gimmizah research station belonging to Animal production research Institute, Agricultural Research Center, Egypt.

Birds and feeding system:

Total of 40 Sinai cockers at 40 weeks of age with (1946-1963.5 g LBW) were used in this study. The experimental birds were divided into four experimental groups (10 in each). Birds of all groups were housed on floor in one ventilated building under the same conditions and the daily light period (16 h L/8 h D) throughout experimental period of 15 weeks (3-wk as adaptation period, 8-wk as a treatment period, and 4-wk as a collection semen period. Birds in all groups were fed a basal diet *ad libtium,* while drinking water was available all daytime.

The basal diet was consisted of 62.41% yellow corn, 22.25% soybean (44%), 7.6% limestone ,4.43% wheat bran, 1.31% di-calcium phosphate, 0.15 methionine, 1.25% vegetable oil, 0.3% premix, and 0.3% common salt. Based on the chemical composition, the basal diet contained 16.52% CP, 3.84% CF, 2.91% ether extract, 3.37% calcium, 0.37% available phosphorus, 0.87% lysine, and 0.38% methionine. However, the calculated metabolizable energy was 2700 Kca/kg diet.

Experimental design:

The four experimental groups were fed the basal diet, but differed in the supplemented materials of treatment. Birds of the first group received free basal-diet, while those in the second, third, and fourth groups were fed on basal diets supplemented with 100 mg Vit. E in form of dl-α-tocopherol acetate (Multivita Company, 6th October governorate, Egypt), 1% PSO (Al-Hedaia Group for Natural Oils Extraction and Herbal Cosmetics, El-Mahala El-Kobra, Egypt), and 100 mg Vit. E+1% PSO per kg diet.

Experimental procedures:

Live body weight:

Cockers were individually weighed at the beginning and end of experimental period, and then change in body weight was calculated.

Semen collection:

At the termination of the feeding period on different experimental diets (8 weeks), semen ejaculates were taken twice a week for four weeks (semen collection period) from 10 cockers in each group by the abdominal massage method. Ejaculate volume was recorded as a net semen volume. Also, sperm cell concentration in fresh semen was determined by hemocytometer (Cantwell, 1974).

All ejaculates in fresh forms were evaluated by CASA analyzer (SPERMOLAB®, Cairo, Egypt). The volume of semen (5 μL) was diluted and placed on a warmed slide (disposable Leja), then allow to settle on heating stage at 37 °C. According to CASA analyzer, semen was evaluated for progressive motility, non-progressive motility, total motility rate, rapid and slow progressive motilities, and immotile sperm percentages. In addition, normal forms (head, neck, and tail abnormalities as well as mono, dual, and tri deformation percentages of sperm cells) were measured. Sperm velocity parameters, including curve linear (VCL), straight linear (VSL), and average path (AVP) velocities were determined. Furthermore, linearity, straightness, and wobble indexes were calculated according to El-Hadad *et al.* (2024).

Blood sampling:

After 4-wk as a period of semen collection, samples of bloods were taken via the brachial vein of each cockers in each group. Each sample was collected in test tubes containing heparin, then divided into two sub-samples, one sample for hematological measurements in the whole blood and another sample for analytical assays in blood plasma. Blood plasma was obtained by blood centrifugation at 3000 rpm/15 min, then stored at -20°C.

Hematological parameters:

In samples of the whole blood, white blood cells (WBCs) and red blood cells (RBCs) counts, hemoglobin (Hb) content, packed cell volume (PCV), and fractionation of WBCs (distribution of basophils, eosinophils, heterophils, lymphocytes, and monocytes) were determined by Veterinary Hematology Analyzer (Exigo, Boule medical AB., Sweden) according to Wintrobe (1981).

Blood biochemical parameters:

In blood plasma of cockers, concentration of total protein, albumin, glucose, cholesterol, T_3, T_4, calcium, and phosphor were determined using spectrophotometer and commercial kits (Biodiagnostic Co. Giza, Egypt) according to the manufacturer procedure. Plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were measured using commercial kits (Bio-Merieux, Egypt). Globulin was obtained by calculation. According to the manufacturers' instructions, levels of total antioxidant capacity (TAC) and malondialdehyde (MDA) were assayed by chemical kits using a spectrophotometer (Shimadzu, Japan). Plasma of immunoglobulins type G (IgG) and M (IgM) were determined by ELISA procedure by specific chemical kits (Bethyl Laboratories, Montgomery, TX, USA). Testosterone concentration was determined by enzyme-immunoassay using commercial kit (Biosource-Europe S.A. 8, rue de L'Lndustrie. B-1400 Nivelles, Belgium), and thyroid hormones (T_3 and T_4) were also assayed in blood plasma by radioimmunoassay (RIA, Britton *et al*., 1975).

Fertility traits:

A total of 640 eggs were collected twice/week from 80 control hens (age of 37 wk and weight of 1954.62±7.24 g) lived with males at a ratio of 1 male: 8 females during four weeks as laying period. The broken and abnormal eggs were removed and only normal eggs (44.0-46.0 g/egg) were chosen for hatching. Eggs were incubated in automatic machine (REFORM®ZEDDAM-HOLLAND) with capacity of 16,000 eggs, temperature (101 ºF, 60% RH, 0.7-0.8% CO_2, while hatching conditions were 99 ºF, 70% relative humidity, and high ventilation.

Rate of fertility on day 15 of incubation period was determined, then commercial and scientific hatchability rates were calculated on day 15 of incubation as the following:

Fertility rate = (Number of fertile eggs/ total number of eggs) × 100.

Commercial hatchability rate = (Number of hatched chicks/ total number of eggs) × 100.

Scientific hatchability rate = (Number of hatched chicks/ number of fertile eggs) × 100.

Statistical analysis:

Homogeneity and normality of distribution of all numerical data have been checked using Lieven's test and Shapiro-Wilk test, respectively. Data were statistically analyzed by one-way ANOVA using a computer program of SAS (2007) to study the effect of treatment (1….4), on all data. The model used was as the following: $Y_{ij}=\mu + T_i + e_{ij}$, where: Y_{ij} = observed item, μ = the overall mean, T_i = treatment effect, and e_{ij} = the random error. The significant differences among means were separated by Duncan's test (Duncan, 1955) at P<0.05. Data were presented as mean ± SE.

RESULTS AND DISCUSSION

Reproductive performance:

Semen characteristics,* and *sperm motility* and *morphological features:

Results in Table 1 show that all treatments increased (P<0.05) semen characteristics including net semen volume and sperm cell concentration as compared to control. Also, all treatments improved (P<0.05) sperm motility parameters, in terms of increasing progressive motility, total motility rate, and rapid progressive motility, and decreasing non-progressive motility, slow progressive motility, and immotility (P<0.05).

Concerning the morphological sperm features, sperm normality (normal forms) was increased (P<0.05), but abnormalities in head, neck, and tail as well as mono and dual deformations were decreased (P<0.05) by all treatments. However, deformity index was decreased (P<0.05) only by PSO or Vit. E+PSO combination (Table 1).

Table 1. Effect of pumpkin seed oil, Vit. E, and their combination on testosterone concentration and semen characteristics of Sinai cockers.

Item	G1(Control)	G2(Vit. E)	G3(PSO)	G4(Vit. E+PSO)	P-value
Semen characteristics:					
Net semen volume (ml)	0.310±0.008^d	0.358±0.010^c	0.403±0.006^b	0.435±0.009^a	0.000
Sperm concentration (10^6/ml)	2.143±0.028^b	2.483±0.082^a	2.536±0.043^a	2.608±0.056^a	0.000
Sperm motility parameters:					
Progressive motility (%)	51.43±1.59^d	69.75±1.63^c	78.70±1.85^b	84.48±1.91^a	0.002
Non-progressive motility (%)	22.86±0.21^a	8.40±0.26^b	5.40±0.32^c	5.17±0.29^c	0.000
Total motility rate	74.29±0.13^d	78.15±0.10^c	84.15±0.15^b	89.70±0.12^a	0.000
Rapid progressive motility (%)	28.60±0.17^d	50.45±0.15^c	63.60±0.19^b	66.38±0.12^a	0.001
Slow progressive motility (%)	22.86±0.22^a	19.33±0.19^b	15.10±0.20^b	18.10±0.18^b	0.004
Immotile sperm (%)	25.71±0.10^a	21.85±0.13^b	15.90±0.16^c	10.35±0.15^d	0.003
Morphological sperm features					
Normal forms	44.29±3.24^c	57.14±3.42^b	68.20±3.11^a	70.69±3.38^a	0.049
Abnormal head	30.14±1.87^a	22.51±1.61^b	18.27±1.70^c	17.94±1.54^c	0.041
Abnormal neck	15.39±1.11^a	11.28±1.03^b	08.23±1.09^b	07.34±1.05^b	0.037
Abnormal tail	10.18±1.12^a	09.07±1.09^a	05.30±1.06^b	04.03±1.24^b	0.046
Sperm deformation:					
Mono deformation	24.72±0.42^a	20.06±0.51^b	15.42±0.49^c	14.17±0.44^c	0.039
Dual deformation	20.12±0.32^a	18.48±0.29^b	12.18±0.35^c	11.01±0.30^c	0.044
Tri deformation	5.6±0.19	4.32±0.14	4.23±0.16	4.13±0.11	0.652
Deformity index	0.83±0.15^a	0.77±0.17^a	0.40±0.12^b	0.29±0.11^b	0.047

a, b, …. d: Significant differences among means in the same row at P< 0.05.

In general, the combination of Vit. E+ PSO showed the highest impact on improving semen characteristic, sperm motility parameters, sperm abnormality, and deformity index. In accordance with the obtained results, Ragab *et al*. (2016) reported that PSO with black seed oil improved semen quality in rabbits. PSO increased sperm count and decreased sperm DNA fragmentation in human (Elfiky *et al*., 2012), improved semen parameters (Aghaie *et al*., 2016) and sperm count (Akang *et al*., 2010) in rats, and increased percentages of sperm motility, livability, and concentration of spermatozoa, while decreased sperm abnormality in rabbits (Ragab *et al*., 2016). Further, it was reported that PSO has a protective effect on sperm abnormality against ROS (Bakeer *et al*., 2021) normally generated during sperm production process (Sikka, 1996). In this respect, PS powder in the diet had a significant improvement in ejaculate volume, sperm concentration, total sperm output, advanced motility, and livability, while decreased abnormal sperm percentage compared with the control in rabbits (Shahba *et al*., 2023). *Cucurbita moschata* treatment improved semen quality and increased sperm motility, while decreased abnormal sperm in rooster under heat stress condition (Rochmi and Pertiwi, 2020).

Regarding the positive impacts of Vit. E, the dietary addition of 150 IU/kg enhanced reproductive efficiency of males (Biswas *et al*., 2007). Dietary Vit. E a levels from 100 to 400 mg/kg can improve the quality of rooster's semen

(Keskes-Ammar *et al*., 2003; Khan *et al*., 2012; Alm El-Dein *et al*., 2013). In 32-week-old roosters of Mandarah chicken fed α-tocopherol acetate-diet at a level of 150 mg/kg under heat stress, Attia *et al.* (2020) reported a significant increase in ejaculate volume, sperm concentration/ejaculate, and sperm livability compared with control. Asrol and Abdul Rashid (2017) reported that the semen quality of roosters (i.e., motility and viability) was increased with the dietary supplementation of 400 IU Vit. E for 4 weeks. Asrol and Abdul Rashid (2017) found that the percentages of sperm motility and livability were higher in local kampong roosters fed diet with 400 than 200 IU Vit. E, but no significant effect was found on abnormal sperm. Feeding poultry males diet supplemented with Vit. E can maintain the quality of sperm cells by reducing the semen lipid peroxidation (Rengaraj and Hong, 2015). In Muscovy drakes, Vit. E in the diet showed an improvement in semen volume, sperm cell concentration, and sperm motility percentage, as well as a reduction in sperm abnormality and dead sperm percentages of Taiwan native male chicks fed diets supplemented with Vit. E at levels of 20, 40, 80, 160 mg/kg (Abdel-Fattah *et al*., 2013). Sperm motility and viability percentages as well as sperm cell concentration were higher in cockerels (35-wk of age) receiving diets with Vit. E as compared to those fed the control diet (Lin *et al*., 2005).

The obtained results in our study indicated a synergetic effect of Vit. E with PSO on semen quality and sperm characteristics of cockers. In this respect, Lotfi *et al.* (2021) found that Vit. E+ PSO treatment showed the greatest value of sperm viability, membrane integrity, total motility and progressive motility as compared to control, in Ross breeder roosters at 45-wk-old on Day 60 of treatment. Hashemi (2013) reported that combination of PSO+Vit. E significantly increased semen quality, while decreased sperm cell abnormalities in rats. According to the obtained results, Vit. E or/and PSO protect the testicles from the impaired effects of lipid peroxidation, then stimulate sperm production (spermatogenesis) and improve the epithelium of the epididymis as did Vit. A, according to Al-Salhie (2014). Dietary supplementation of 150 and 200 mg Vit. E or 30 ml PSO per kg diet improved the volume of testes (Al-Salhie *et al*., 2017), diameter and cavity of seminiferous tubules, thickness of spermatocytes layer, and the relative weight of gonads in the birds compared with control. PSO contains α-tocopherol and Vit. A antioxidant activity (Bairy and Rao, 2010).

On the other hand, several authors found that Vit. E can increase the quality of chicken's semen (Cerolini *et al*., 2006; Tabatabaei *et al*., 2011) when it is provided at a level of 500 times greater than 15 IU/kg diet according to the NRC requirements (Tabatabaei *et al*., 2011), therefore, Hayanti *et al.* (2022) observed no effect of Vit. E at a low level on chicken semen volume. Also, a longer time was required for Vit. E to decrease the morphological abnormality in sperm cells (Min *et al*., 2016). Supplementation of Vit. E (200 mg/kg diet) to Egyptian local cross males at 42-weeks-old had no significant effect on sperm count and motility, while increased sperm livability as compared to control (Eid *et al*., 2006).

Sperm kinematics:

Different kinetic variables studied, including curvilinear (VCL), straight linear (VSL), and average path (AVP) velocities, and linearity, straightness, and wobble indexes in semen of Sinai cockers were affected significantly ($P<0.05$) by treatment (Table 2). In comparing with the control group, Vit. E treatment increased ($P<0.05$) only VCL value; POS treatment increased ($P<0.05$) VCL and AVP values as well as wobble percentage; while Vit. E+PSO combination increased all sperm kinetic parameters, showing the highest benefits.

Table 2. Effect of pumpkin seed oil, Vit. E, and their combination on sperm motion parameters and kinetic indexes of Sinai cockers.

Item	G1(Control)	G2(Vit. E)	G3(PSO)	G4(Vit.E+PSO)	P-value
VCL(μm/s)	30.69±4.16^{b}	32.62±4.78^{ab}	34.40±4.62^{ab}	36.94±4.92^{a}	0.030
VSL (μm/s)	12.91±1.25^{b}	15.14±1.34^{ab}	17.28±1.21^{ab}	20.18±1.28^{a}	0.041
AVP(μm/s)	25.54±2.40^{b}	27.29±2.37^{b}	30.03±2.25^{a}	32.94±2.19^{a}	0.027
Linearity(%)	42.07±2.51^{b}	46.41±2.29^{ab}	50.23±2.46^{ab}	54.63±2.34^{a}	0.039
Straightness(%)	50.55±2.63^{b}	55.47±2.91^{ab}	57.54±2.77^{ab}	61.26±2.52^{a}	0.037
Wobble (%)	83.21±10.29^{b}	83.66±10.64^{b}	87.29±11.02^{a}	89.17±11.19^{a}	0.040

a, and b : Significant differences among means in the same row at $P< 0.05$.

In line with these findings, Lotfi *et al.* (2021) found that diet supplemented with PSO+Vit. E combination improved sperm velocity parameters and kinetic indexes in Ross breeder roosters (45-wk old), in terms of increasing VAP, VCL, and VSL, and kinetic index such as LIN compared with control group. In this concern, El-Hadad *et al.* (2024) reported that sperm velocity parameters (VCL, VSL, and VAP) were higher ($P<0.05$) for cockers treated with 4-ml thyme than in control one.

Testosterone profile:

The effect of treatment on testosterone concentration in plasma of cockers illustrated in figure 1 was significant ($P<0.001$). Plasma concentration of testosterone was higher ($P<0.05$) in all treatment groups than in control one, being higher ($P<0.05$) in a combination and PSO groups than in Vit. E group.

These results indicated a positive impact of Vit. E, PSO, or their combination on plasma testosterone concentration of cockers, being higher for cockers fed PSO or Vit. E+PSO combination than those fed Vit. E-diet alone. It is of interest to not that all improvements in semen quality and sperm characteristics are in association with increasing plasma testosterone levels in treatment groups. It is well known that, testosterone hormone is important for sperm production (spermatogenesis) and dietary supplementation of PSO (30 ml/kg) may be responsible for increasing serum testosterone (Hamdi, 2020). Hashemi (2013) reported that Vit. E+PSO combination significantly improved serum level of testosterone in rats. Hashemi (2013) and Shaban and Sahu (2017) demonstrated that the presence of natural antioxidants in PSO and the supplemented Vit. E increased plasma testosterone level. In rabbits, Ragab *et al.* (2016) found that PSO in a mixture with *Negilla sativa* oil increased the level of testosterone as compared to control. Moreover, intramuscular Vit. E injection with CnRH analogue improved the level of testosterone in blood serum of chicken broiler breeders (Hezarjaribi *et al*., 2016). On the other hand, Hayanti *et al.* (2022) observed that Vit. E does not affect chicken testosterone concentration. Also, Bakeer *et al.* (2021) found that PSO had no effect on serum testosterone level.

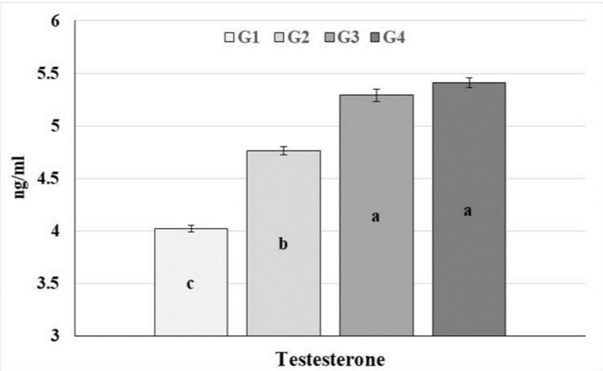

Fig. 1. Effect of pumpkin seed oil, Vit. E, and their combination on plasma testosterone concentration and semen characteristics of Sinai cockers.

Hematological parameters:

Results in Table 3 reveal that PSO treatment increased (P<0.05) hematological parameters including Ht percentage and WBCs count as compared to control group. Also a combination treatment improved (P<0.05) Hb concentration, Ht percentage, and WBCs count. However, Vit, E treatment failed to change all hematological parameters, and RBCs count was not affected by treatments (Table 3).

In our study, the present results indicated a pronounced increase in Hb concentration by a combination of Vit. E+PSO, although the effect of Vit. E or PSO alone was not significant on Hb concentration. This finding revealed a synergetic impact of both Vit. and PSO on Hb concentration. In cockers, El-Hadad *et al*. (2024) found that Hb concentration and Ht percentage were increased by dietary antioxidant (thyme) addition as compared to control cockers. Abdelnour *et al*. (2023) found that 2 ml of PSO/kg diet improved most hematological parameters of growing rabbits which is in line with increasing Ht percentage and WBCs count in PSO or Vit. E+PSO groups in our study. In line with the present results, presence of linoleic acid (omega-6 fatty acid) in PSO was reported to improve different parameters of hematology in birds (Diwakar *et al*., 2010; Abbas *et al*., 2016; Bardaa *et al*., 2016).

Table 3. Effect of pumpkin seed oil, Vit. E, and their combination on hematological parameters of Sinai cockers.

Item	G1(control)	G2(Vit. E)	G3(PSO)	G4(Vit. E +PSO)	P-value
Hemoglobin (g/dl)	10.77 ± 0.69^{b}	11.83 ± 0.73^{ab}	12.50 ± 0.75^{ab}	13.53 ± 0.65^{a}	0.049
Hematocrit (%)	29.27 ± 1.37^{c}	32.03 ± 1.40^{bc}	35.50 ± 1.28^{ab}	36.70 ± 1.31^{a}	0.003
RBCs ($x10^{6}$/µL)	3.72±0.30	4.26±0.33	4.33±0.29	4.68±0.31	0.201
WBCs ($x10^{3}$/uL)	5.53 ± 0.36^{c}	6.23 ± 0.33^{bc}	7.03 ± 0.35^{ab}	7.47 ± 0.38^{a}	0.004

a, and b : Significant differences among means in the same row at P< 0.05.

Also, El-Sebai (2000) reported that Vit. E supplementation to broiler's diets caused a significant increase in WBCs count by a 4.65% comparing with the control. On the other hand, the insignificant effect of Vit. E on all hematological parameters in our study contrasted the results of many authors. Attia *et al.* (2020) found a significant increase in values of RBCs, Hb, PCV and WBCs by feeding Mandarah roosters on Vit. E (150 mg/kg) compared with control group. Abd El-Hack *et al.* (2019) found that 500 mg Vit. E/kg diet of Bovans Brown hens showed the highest value of packed cell volume (PCV) and Hb in comparing with other groups. Shahba *et al*. (2023) found that rabbits supplemented with 1.0 and 2.0 g PS powder/kg diet represented a significant increase in RBCs compared with the control. While, all supplemented doses of PS showed insignificant effect on WBCs, Hb, PCV compared to control group. In contrast to our results, Mathewos *et al*. (2019) found that 1% PS powder significantly decreased WBCs count compared to the control in the broilers. The confliction in the results may be attributed to marked variation in level of treatment, species, or sorce of treatment. Vitamin E elevate leucocytes in quails (Sahin *et al*., 2002a). On the other hand, El-Saadany *et al*. (2022) reported that addition of 5% PSO in the diet did not affect hematological traits in chicks.

Regarding the fractionation of WBCs in blood of cockers, only a combination treatment increased (P<0.05) eosinophil and monocyte percentages, and decreased (P<0.05) heterophil percentage as compared to control group. However, percentage of basophils and lymphocytes were not affected significantly by treatment (Fig. 2).

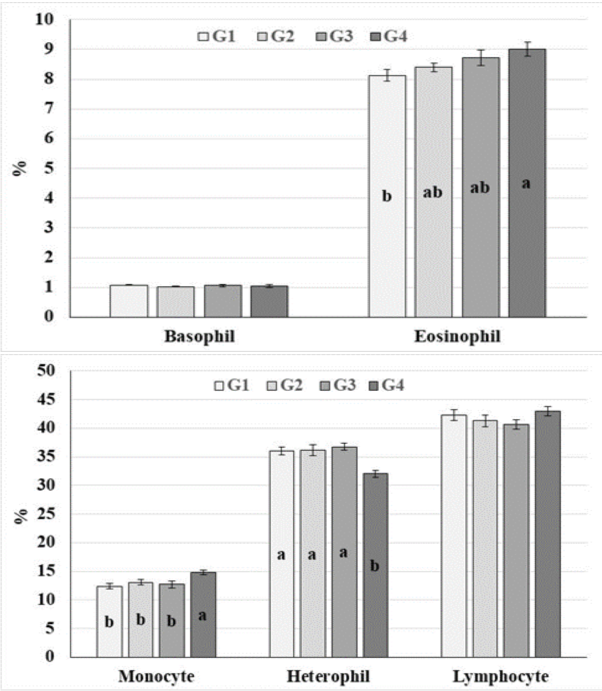

Fig. 2. Effect of Vit. E, pumpkin, or their combination on white cells differentiation in Sinai cockers blood.

Similarly, the effect of thyme treatment on lymphocyte percentage in cockers was not significant (El-Hadad *et al*., 2024). Also, different levels of PS did not represent any statistical changes compared to the control with respect to basophil, eosinophil, heterophil percentages

(Shahba *et al*., 2023). In Mandarah roosters fed diet supplemented with Vit. E (150 mg/kg diet) had no effects in basophil and eosinophil percentages (Attia *et al*., 2020). Insignificant effect of PSO on WBCs differentiation of growing rabbits was reported by Ragab *et al*. (2013).

In comparable with our results, rabbit bucks treated with PS power (1.0 and 2.0 g/kg diet) increased lymphocyte, while decreased monocyte percentage compared with those in control (Shahba *et al*., 2023). In Mandarah roosters fed diet supplemented with Vit. E at a level of 150 mg/kg diet, Attia *et al*. (2020) found that lymphocytes and monocytes increased with lower values were noted in heterophils compared to control values. Dietary Vit. E at a level of 250 or 500 mg/kg significantly impacted monocyte and basophil counts in Bovans Brown hens (Abd El-Hack *et al*., 2019), and increased number of lymphocytes in quails (Sahin *et al*., 2002a).

Protein metabolism:

Treatment affected significantly on concentration of total protein and their fraction in blood plasma of cockers (Fig. 3). All treatments increased (P<0.05) plasma total protein concentration. The observed increase in total protein level was due to increased globulin by Vit E treatment (P<0.05), increased albumin by PSO (P>0.05), and Vit. E+PSO (P<0.05).

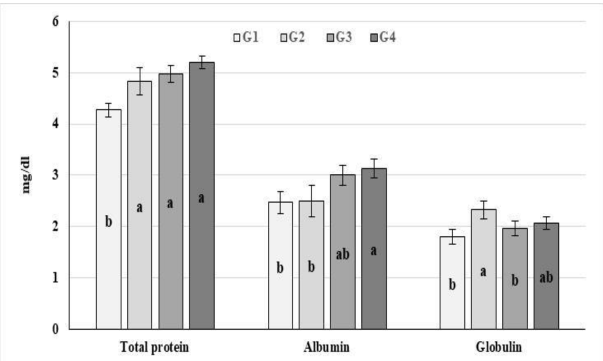

Fig. 3. Effect of Vit. E, pumpkin, or their combination on total protein and its fractions in Sinai cockers blood.

In agreement with the obtained results, Vit. E (150 mg/kg diet) increased total protein of 32-week-old Mandarah roosters under heat stress (Attia *et al*., 2020). An improvement in plasma concentration of total protein was recorded by feeding broilers on diets supplemented with Vit. E compared with the control (El-Sebai, 2000). Also, Sahin *et al*. (2002b) reported that the treatment with Vit. E elevated serum total protein concentration of broilers under heat stress. However, Abd El-Hack *et al*. (2015) assured that Vit. E (250 mg/kg diet) did not have any significant effects on serum albumin and globulin of laying hens. In another study, Vit. E supplementation in the diet had no significant effects on albumin and globulin in Bovans Brown hens under summer ambient temperature Abd El-Hack *et al*. (2019).

Regard to the effect of PSO, Abdelnour *et al*. (2023) observed that dietary PSO supplementation (0.5, 1 and 2 ml/kg) increased plasma total protein concentration compared to control rabbits. However, Shahba *et al*. (2023) showed that rabbits supplemented with 0.5, 1.0, and 2.0 g PSO/kg diet had no significant effect on albumin and globulin. Also, addition of 5% PSO showed no significant effect on blood albumin levels in chickens (El-Saadany *et al*., 2022). Dietary supplementation with different levels of PSO had no significant effect on serum albumin and globulin in Japanese quail (Abbas *et al*., 2016). Conversely, plasma albumin and globulin levels were increased by PSO supplementation compared to control rabbits (Abdelnour *et al*., 2023). However, Vit. E increased serum concentrations of albumin in broilers (Sahin *et al*., 2002b) and in Mandarah roster (32 week-old) (Attia *et al*., 2020) under heat stress conditions.

Plasma glucose, creatinine, and lipid profile:

Concentration of plasma of glucose and creatinine in cockers was significantly affected by treatment (Table 4). Concentration of glucose increased (P<0.05), while creatinine concentration was decreased (P<0.05) by PSO or Vit. E+PSO as compared to Vit. E and control groups.

Table 4. Effect of pumpkin seed oil, Vit. E, and their combination on glucose and creatinine concentration in plasma of Sinai cockers.

Item	G1 (Control)	G2 (Vit. E)	G3 (PSO)	G4 (Vit. E +PSO)	P-value
Glucose (mg/dl)	186.00±2.39^{b}	188.32±2.76^{b}	197.68±2.64^{a}	198.35±2.20^{a}	0.003
Creatinine (mg/dl)	1.03±0.02^{a}	0.96±0.02^{a}	0.87±0.03^{b}	0.81±0.02^{b}	0.000

^{a}, and b: Significant differences among means in the same row at P< 0.05.

In agreement with increasing plasma glucose level in cockers fed diet supplemented with PSO or their combination, Abdelnour *et al*. (2023) found increased plasma glucose level in growing rabbits fed diet supplemented with PSO at a level of 2 ml/kg. However, El-Saadany *et al*. (2022) showed a significant decrease in glucose compared with control group by addition of 5% PSO to chicken diet because it increases insulin secretion in the blood (Mukherjee *et al*., 2022). Although, Vit. E had no significant effect on plasma glucose level of cockers in our study, Attia *et al*. (2020) found that dietary addition of Vit E (150 mg/kg) significantly increased glucose level in Mandarah roosters (32-week old) compared with control under heat stress. This finding may indicate that the effect of Vit. E+PSO combination on glucose level was mainly related to a positive effect of PSO in this combination.

The reduction in creatinine concentration in plasma of cockers in our study by PSO or Vit. E+PSO combination was indicated in growing rabbits by Abdelnour *et al*. (2023), who showed that, rabbits fed diet supplemented with PSO at a level of 2 ml/kg significantly reduced creatinine compared to the control group under hot condition. On contrast, Shahba *et al*. (2023) reported that PSO supplementation with PSO (0.5, 1.0 and 2.0 g/kg diet) represented no significant effect on creatinine level in rabbits. Also, dietary addition of Vit. E (150 mg/kg) lowered creatinine level Mandarah roosters under heat stress condition (Attia *et al*., 2020).

Regarding the effect of treatment on lipid profile in blood plasma of cockers, total lipids concentration was increased (P<0.05) and total cholesterol concentrations was decreased (P<0.05) by PSO or Vit. PSO combination compared to Vit. E and control group (Fig. 4).

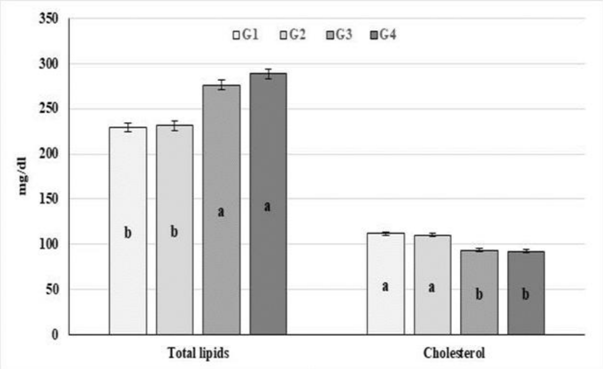

Fig. 4. Effect of Vit. E, pumpkin, or their combination on total lipids and cholesterol in Sinai cockers blood.

As proved in the current study, feeding diets supplemented with PSO strongly affects blood levels of total cholesterol, LDL, and HDL in poultry (Murata *et al*., 2003; Martınez *et al*., 2010 a, b; Martınez *et al*., 2012). In this respect, El-Saadany *et al*. (2022) found a decrease in cholesterol and LDL concentrations compared with control by feeding diet supplemented with 5% PSO to chicken. Shahba *et al*. (2023) found that PS powder (0.5, 1.0 and 2.0 g/kg diet) decreased serum cholesterol and total lipids compared with the control rabbits. Regard to the effect of Vit. E, the present results are in agreement with the results of Abd El-Hack *et al*. (2015), who assured that 250 mg Vit. E/kg diet did not have any significant impact on serum total cholesterol. On the other hand, addition of Vit. E (150 mg/kg) in the diet of Mandarah roosters decreased blood cholesterol level under heat stress condition (Attia *et al*., 2020). Also, Abd El-Hack *et al*. (2019) found positive impacts on total lipids in Bovans Brown hens by Vit. E at levels of 250 and 500 mg/kg diet under heat stress condition. Moreover, El-Sebai (2000) found an increase in plasma total lipids as affected by dietary supplementation with Vit. E as compared to the controls.

Liver function:

In our study, all treatments significantly affected the activity of AST and ALT in plasma of cockers (Fig. 5). All treatments decreased ($P<0.05$) AST and ALT activities as compared to control group, but the reduction in AST and ALT activities was higher ($P<.05$) by PSO or a combination treatment than in Vit. E.

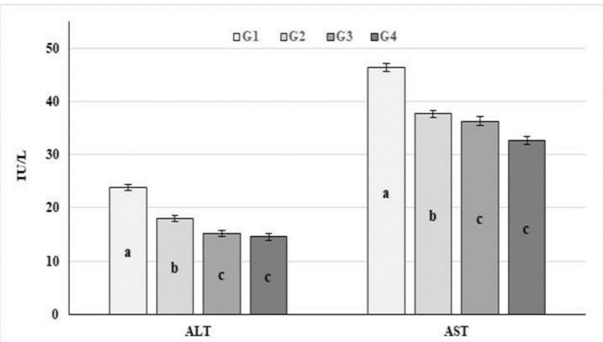

Fig. 5. Effect of Vit E, pumpkin, or their combination on ALT and AST in Sinai cockers blood.

Similar to the reduction in AST and ALT activities in plasma of cockers in our study, El-Saadany *et al*. (2022) found that dietary supplementation of 5% PSO decreased ALT and AST activities in chicken as compared to controls. Also, Abdelnour *et al*. (2023) indicated that PSO treatment (2 ml/kg diet) significantly decreased activity of AST and ALT compared with the controls under hot condition. Additionally, Attia *et al*. (2020) reported a decrease in the activity of AST and ALT in blood of Mandarah roosters by feeding Vit. E (150 mg/kg) under heat stress. Conversely, no effects of PS powder were observed on AST and ALT activities in blood by presence of PS powder in the diet of rabbits (Shahba *et al*., 2023) and laying hens (Abdel-Fattah and Abdel-Azeem, 2007).

Thyroid function:

A significant effect was found for treatment on plasma concentration of T_3 (triiodothyronine) and T_4 (thyroxin). All treatments increased ($P<0.05$) both T_3 and T_4 concentrations in plasma of cockers as compared to control (Fig. 6). The differences in T_3 level among treatment groups were not significant, however, T_4 level was the highest in a combination, followed by PSO, and Vit. E groups, respectively ($P<0.05$).

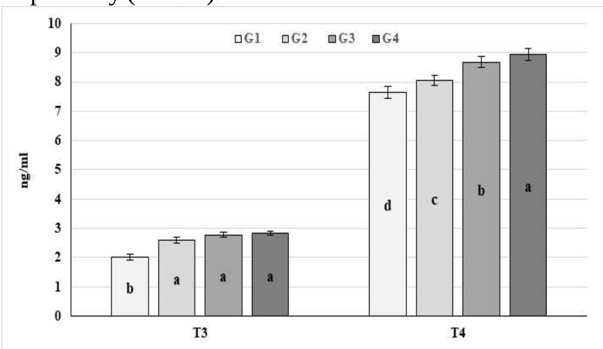

Fig. 6. Effect of Vit E, pumpkin, or their combination on thyroid hormones (T_3 and T_4) in Sinai cockers blood.

In harmony with the present results, Abdel-Fattah and Abdel-Azeem (2007) showed that Vit. E (375 to 500 mg/kg diet) increased serum levels of T_3 and T_4 in blood of laying hens with increasing Vit. E at a level of 250 ppm. However, Abd El-Hack *et al*. (2015) showed that Vit. E (250 mg/kg diet) did not have any significant effect on serum T_3 and T_4 levels in laying hens. The rise in the levels of T_3 and T_4 in plasma of cockers may be attributed to the antioxidative properties of PSO alone or in a combination with Vit. E. In this line, PSO, as omega-3 fatty acid source supplementation, has shown anti-peroxidative activities (Elfiky *et al*., 2012). The same trend was observed in T_3 and T_4 levels by thyme treatment (100 mg/kg) in 96 day-old boiler chicks. Antioxidants ameliorate level of T_3 and T_4 via flavonoid contents which have an ability to enhance iodide uptake and sodium-iodide symporter expression and thyroperoxidase (the key enzyme in thyroid hormones biosynthesis) (Gonçalves *et al*., 2017).

Immunity and antioxidant status:

Regarding the immune response, all treatments improved ($P<0.05$) plasma concentration of immunoglobulin (IgG and IgM) in plasma of cockers as compared to controls (Table 5). In birds, many investigators (Stevenson *et al*., 2007; Elfiky *et al*., 2012; Hashemi 2013; Deshmukh *et al*., 2017; Al-Sayed *et al*., 2019) reported that PSO contains large amounts of Vit. E, Zn, L-tryptophan, omega 3- and 6-fatty acids, and these compounds have the ability to increase immunity and e antioxidant capacity (Alagawany *et al*., 2021). Also, all treatments improved ($P<0.05$) the antioxidant status of cockers, in terms of increasing TAC level and decreasing MDA level in plasma of treated cockers as compared to controls. The highest impacts of treatments were recorded for cockers fed diet supplemented with a combination of Vit. E+PSO.

Table 5. Effect of pumpkin seed oil, E vit and their combination on concentration of immunoglobulins (IgG and IgM) in plasma of Sinai cockers.

Item	G1(control)	G2(Vit. E)	G3(PSO)	G4(Vit. E+PSO)	P-value
		Immunoglobulin concentration:			
IgG (ng/ml)	46.40±0.71[c]	51.28±0.75[b]	52.17±0.70[a]	55.14±0.73[a]	0.001
IgM (ng/ml)	8.60±0.09[c]	9.72±0.11[b]	10.80±0.12[a]	11.10±0.08[a]	0.001
		Antioxidant status markers:			
TAC (mmol/L)	0.68±0.01[c]	0.76±0.02[b]	0.87±0.02[a]	0.91±0.03[a]	0.000
MDA (mmol/L)	2.72±0.11[a]	2.02±0.08[b]	1.77±0.06[b]	1.52±0.10[c]	0.000

a,b,c Mean in the same row was different significantly at P< 0.05.

Similarly, diet supplemented with 150 mg Vit. E/kg diet increased TAC contents and decreased MDA level in blood plasma of Mandarah roosters under heat stress (Attia *et al*., 2020). This may be due to the crucial role of Vit. E, as antioxidant, in the defense system of the cells in birds (Attia *et al*., 2017; Kutlu *et al*., 2019). Dietary Vit. E (100 and 200 mg/kg) supplementation enhanced the antioxidant status of chicken cockers as reported by Biswas *et al*. (2009) and Ebeid (2012), respectively.

In addition, role of PSO, as antioxidant, was proved by different investigators. In this context, Bakeer *et al*. (2021) found that 0.5% PSO increased level of TAC. Rouag *et al*. (2020) found a decrease in MDA level by 0.5% PSO compared with control group. El-Saadany *et al*. (2022) observed that MDA decreased in hens treated with 5% PSO compared with control group. Lotfi *et al*. (2021) showed that a combination of 2% PSO+200 mg Vit. E/kg diet) decreased MDA levels in Ross roosters, on days 40 and 60 of age, compared with control group.

In control group, higher ROS level than the natural antioxidant defense mechanisms cause lipid peroxidation, then MDA increased more than TAC. The levels of TAC are valuable indication of reducing blood ROS, so TAC level in blood is important to neutralization of ROS, generated via different pathways of oxidation. In harmony with our results, PSO as antioxidant like thyme could be effective in reducing level of MDA to increase antioxidant enzyme activities and to eliminate lipid peroxidation and ROS generation. Also, PSO may has a great defensive impact on protection against the peroxidation of lipids within the cells through decreasing MDA levels. In this concern, Vit. E has a role in protecting the cells from oxidative stress (Deivendran and Yeong, 2015) and Vit. E increased antioxidant enzymes, like SOD and GPx, which act as ROS scavengers (Wang *et al*., 2007). In rabbits, Ragab *et al*. (2016) found that PSO plus black seed oil mixture improved antioxidant capacity.

The responsible compounds for high antioxidant capacity of PSO are total phenolics or phytochemicals (Al-Sayed *et al*., 2019; Vlaicu and Panaite 2022); these phenolics stimulate activity of catalase, which detoxifies H2O2 and converts lipid hydroperoxides to non-toxic substances (Fki *et al*., 2005). PSO prevents the alterations in plasma lipids and has anti-peroxidative abilities (Elfiky *et al*., 2012; Vlaicu and Panaite, 2022).

Fertility and hatchability of eggs:

Fertility rate of eggs was increased (P<0.05) when hens were copulated with cockers fed only diet supplemented with Vit. E+PSO combination. However, hatchability rates (scientific and commercial) were improved (P<0.05) by feeding Vit. E+PSO-diet (Table 6).

Table 6. Effect of thyme treatment on fertility, and hatchability of Sinai hens.

Item	G1(control)	G2(Vit. E)	G3(PSO)	G4(Vit. E+PSO)	P-value
Fertility rate (%)	83.31±1.13[b]	84.34±1.28[ab]	86.16±1.21[ab]	87.48±1.18[a]	0.049
Hatchability rate[1] (%)	84.54±1.04[b]	84.58±1.13[b]	86.45±1.17[a]	88.32±1.11[a]	0.035
Hatchability rate[2] (%)	70.18±1.17[b]	71.39±1.12[b]	74.34±1.08[a]	77.31±1.10[a]	0.002

1 Hatchability rate from fertile eggs, 2 Hatchability rate from all set eggs a,b Mean in the same row was different significantly at P< 0.05.

It is of interest to observe that Vit. E or PSO alone had no significant effects on fertility, but a significant increase of fertility was more pronounced by their combination, indicating a synergetic relationship between Vit. and PSO on egg fertility. Regard to hatchability rate, PSO was found to be more effective than Vit. E as an enhancer of hatchability. In this concern, Vit. E has a protective ability against ROS on sperm cells leading to a reduction in lipid peroxidation and maintenance of the fertilizing capacity in quails, but had no significant effect on hatchability compared to the controls as reported by Biswas *et al*. (2007). Dietary addition of 150 mg alpha-tocopherol acetate per kg, as a source of Vit. E, increased fertility rate of Mandarah roosters compared with control under heat stress (Attia *et al*., 2020). Also, chicks fed diet containing 200 mg Vit. E /kg boosted good quality of semen (Khan *et al*., 2012). Vit. E, as a natural antioxidant, can enhance the quality of semen, then improving fertilizing ability (Cerolini *et al*., 2006). Vit. E at a level of 100 mg/kg was found to be better to maintain the fertility in male chickens (Keskes-Ammar *et al*., 2003). Administration of roosters with Vit. E (200 and 400 IU), increased the egg fertility compared with the control group. The egg hatchability was significantly higher in by both Vit. E levels as compared to control, and higher by 400 than 200 IU Vit. E (Asrol and Abdul Rashid, 2017). Supplementing turkey hens with (50 g PS powder/kg feed) can improves the fertility and hatchability of the eggs (Machebe *et al*., 2013).

Change in live body weight:

Final live body weight and total gain of Sinai males were increased (P<0.05) only by feeding diet supplemented with Vit. E+PSO combination as compared to those fed the control unsupplemented diet (Table 7).

These results indicated a beneficial impact of Vit. E+PSO combination on LBW of cockers. However, a tendency of increasing LBW of cockers fed Vit. E or PSO diets indicated the save use of each on growth performance of cockers

Table 7. Effect of pumpkin seed oil, Vit. E and their combination on live body weight of Sinai cockers.

Item	G1(control)	G2(Vit. E)	G3(PSO)	G4(Vit. E+PSO)	P-value
Initial live body weight (g)	1946.00±6.86	1963.50±6.01	1948.00±8.67	1961.00±7.45	0.244
Final live body weight (g)	2037.50±9.26^{b}	2059.00±7.88^{ab}	2052.00±8.27^{ab}	2070.50±6.30^{a}	0.044
Chang in live body weight (g)	91.50±4.35^{b}	95.50±4.74^{b}	104.00±4.88^{ab}	109.50±3.53^{a}	0.028

a,b Means in the same row with different superscripts are different significantly at P< 0.05.

CONCLUSION

According to our findings, it can conclude that the dietary supplementation with a combination of 100 mg Vit. E+1% PSO had beneficial effects on semen quality, sperm function, blood testosterone, liver, kidney, and thyroid functions, immunoglobulins immunity, egg fertility and hatchability rates of cockers of Sinai local strain chicken.

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جودة السائل المنوي وخصائص الحيوانات المنوية وصورة الدم والمناعة والقدرة المضادة للأكسدة لديوك سينا المغذاة على عليقة مضاف لها فيتامين E و/أو زيت بذرة اليقطين

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الملخص

تهدف هذه الدراسة إلى تقييم القدرة المضادة للأكسدة لزيت بذرة اليقطين وفيتامين هـ أو خليطهما على جودة السائل المنوي، وصفات الحيوانات المنوية ، والحالة الصحية ، ومناعة دجاج سلالة سينا المحلية. تم تقسيم 40 طائر (عمر 40 أسبوع ووزن 1946- 1963.5 جم) إلى أربع مجاميع (10 في كل منها). في المجموعة الأولى تم تغذية الطيور على العليقة الأساسية بدون أي إضافات (الضابطة). في المجموعة الثانية والثالثة والرابعة تم إضافة 100 ملجم فيتامين هـ/ كجم من العليقة الضابطة ، 1٪ زيت بذور اليقطين، و100 ملجم فيتامين هـ/ كجم + 1٪ زيت بذرة اليقطين، على التوالي. في نهاية فترة المعاملة والتي بلغت 8 أسابيع، تم تجميع السائل المنوي مرتين أسبوعيا لمدة أربعة أسابيع من 10 ديوك في كل مجموعة. تم تقييم جميع القذفات بواسطة برنامج CASA. في نهاية فترة جمع السائل المنوي، تم سحب عينات الدم من وريد الجناح للديوك في كل مجموعة. أظهرت النتائج أن:1- أظهرت جميع المعاملات تحسن معنوى (P<0.05) فى حجم وتركيز السائل المنوي، والنسبه المئويه للخصائص المختلفة للحركة، الشواذ، وسرعة الحيوانات المنوية مقارنة بالضابطة. 2- أظهرت جميع المعاملات زيادة (P<0.05) فى مستوى كل من هرمون التستوستيرون ، البروتين الكلي، هرموني الثيروكسين والتراي أيودو ثيريونين، والجلوبيولينات المناعية، قدرة مضادات الأكسدة، مع خفض معنوى فى نشط انزيمات الترانس اميناز (ALT & AST) فى اللازما الدمو التأكسديه في بلازما الدم. 3- حسنت أضافة زيت بذور اليقطين بمفردها أو مع فيتامينهـ بزياده معنويه فى معظم الخصائص الهيماتولوجيه، الجلوكوز، الدهون الكليه، معدل الفقس، ونقص معنوى فى مستوى الكوليسترول والكرياتينين فى بلازما الدم. 4- زادت خصوبة البيض معنويا نتيجة التلقيح بالذكور المعامله فقط بخليط زيت بذرة اليقطين مع فيتامين هـ.الأستنتاج:المعاملة بخليط من 100 ملجم فيتامين هـ + 1٪ زيت بذرة اليقطين كان لها تأثيرات مفيدة على جودة السائل المنوي، وظائف الحيوانات المنوية، هرمون التستوستيرون في الدم، وظائف الكبد والكلى والغدة الدرقية، قدرة مضادات الأكسدة، المناعة، معدلات خصوبة وفقس البيض في دجاج سلالة سينا المحلية.