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 1946.35 g LWB) 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 analyzer and 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, , T3, T4, immunoglobulins, antioxidant capacity, and decreased AST and ALT activities. PSO alone or with Vit E increased, hematocrit, 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 lead 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 cockers (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).

Phytochemical 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 improve bird growth and production in association with improve immunity (Achilou et al., 2018; Mathewos et al., 2019). In PS extract, there are different 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; Zdúczyk 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 causes damage in cell membranes and it, as a natural antioxidant, has a guard role 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 libitum, 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 Kcalkg 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-Hedaida 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, T₃, T₄, 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’Industrie. B-1400 Nivelles, Belgium), and thyroid hormones (T₃ and T₄) 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 1954.6±27.24 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®ZEEDAM-HOLLAND) with capacity of 16,000 eggs, temperature (101 °F, 60% RH, 0.7-0.8% CO₂), while hatching conditions were 99 °F, 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: Yij = μ + Ti + ej, where: Yij = observed item, μ = the overall mean, Ti = treatment effect, and ej = 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>G1(Control)</th>
<th>G2(Vit. E)</th>
<th>G3(PSO)</th>
<th>G4(Vit. E+PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semen characteristics:</strong></td>
<td></td>
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<tr>
<td>Net semen volume (ml)</td>
<td>0.310±0.008</td>
<td>0.358±0.010</td>
<td>0.403±0.006</td>
<td>0.435±0.009</td>
<td>0.000</td>
</tr>
<tr>
<td>Sperm concentration (10⁵/ml)</td>
<td>2.143±0.028</td>
<td>2.483±0.082</td>
<td>2.536±0.043</td>
<td>2.608±0.056</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Sperm motility parameters:</strong></td>
<td></td>
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<tr>
<td>Progressive motility (%)</td>
<td>51.43±1.50</td>
<td>69.75±1.63</td>
<td>78.70±1.85</td>
<td>84.48±1.91</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-progressive motility (%)</td>
<td>22.86±0.21</td>
<td>8.40±0.26</td>
<td>5.40±0.32</td>
<td>5.17±0.29</td>
<td>0.000</td>
</tr>
<tr>
<td>Total motility rate</td>
<td>74.29±0.13</td>
<td>78.15±0.10</td>
<td>84.15±0.13</td>
<td>89.70±0.12</td>
<td>0.000</td>
</tr>
<tr>
<td>Rapid progressive motility (%)</td>
<td>28.60±0.17</td>
<td>50.45±0.15</td>
<td>63.60±0.19</td>
<td>66.38±0.12</td>
<td>0.001</td>
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<tr>
<td>Slow progressive motility (%)</td>
<td>22.86±0.22</td>
<td>19.33±0.19</td>
<td>15.10±0.20</td>
<td>18.10±0.18</td>
<td>0.004</td>
</tr>
<tr>
<td>Immotile sperm (%)</td>
<td>25.71±0.10</td>
<td>21.85±0.13</td>
<td>15.90±0.16</td>
<td>10.35±0.15</td>
<td>0.003</td>
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<tr>
<td><strong>Morphological sperm features:</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Normal forms</td>
<td>44.29±3.24</td>
<td>57.14±3.42</td>
<td>68.20±3.11</td>
<td>70.69±3.38</td>
<td>0.049</td>
</tr>
<tr>
<td>Abnormal head</td>
<td>30.14±1.87</td>
<td>22.51±1.61</td>
<td>18.27±1.70</td>
<td>17.94±1.54</td>
<td>0.041</td>
</tr>
<tr>
<td>Abnormal neck</td>
<td>15.39±1.11</td>
<td>11.28±1.03</td>
<td>08.23±1.09</td>
<td>07.34±1.05</td>
<td>0.037</td>
</tr>
<tr>
<td>Abnormal tail</td>
<td>10.18±1.12</td>
<td>09.07±1.09</td>
<td>05.30±1.06</td>
<td>04.03±1.24</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Sperm deformation:</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Mono deformation</td>
<td>24.72±0.42</td>
<td>20.06±0.51</td>
<td>15.42±0.49</td>
<td>14.17±0.44</td>
<td>0.039</td>
</tr>
<tr>
<td>Dual deformation</td>
<td>20.12±0.32</td>
<td>18.48±0.29</td>
<td>12.18±0.35</td>
<td>11.01±0.30</td>
<td>0.044</td>
</tr>
<tr>
<td>Tri deformation</td>
<td>5.60±0.19</td>
<td>4.32±0.14</td>
<td>4.23±0.16</td>
<td>4.13±0.11</td>
<td>0.652</td>
</tr>
<tr>
<td>Deformity index</td>
<td>0.83±0.15</td>
<td>0.77±0.17</td>
<td>0.40±0.12</td>
<td>0.29±0.11</td>
<td>0.047</td>
</tr>
</tbody>
</table>

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.
Straightness

VSL (µm/s)

Table 2. Effect of pumpkin seed oil, Vit. E, and their combination on sperm motion parameters and kinetic indexes of cell abnormalities in rats. (2021) found that Vit. E+PSO treatment showed the greatest synergetic effect with 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 viability as compared to control (Eid et al., 2006).

Sperm kinetics:

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>G1(Control)</th>
<th>G2(Vit. E)</th>
<th>G3(PSO)</th>
<th>G4(Vit.E+PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCL(µm/s)</td>
<td>30.69±4.16</td>
<td>32.62±4.78</td>
<td>34.40±4.62</td>
<td>36.94±4.92</td>
<td>0.030</td>
</tr>
<tr>
<td>VSL(µm/s)</td>
<td>12.91±1.25</td>
<td>15.14±3.14</td>
<td>17.28±1.21</td>
<td>20.18±1.28</td>
<td>0.041</td>
</tr>
<tr>
<td>AVP(µm/s)</td>
<td>25.54±2.40</td>
<td>27.29±2.37</td>
<td>30.03±2.25</td>
<td>32.94±2.10</td>
<td>0.027</td>
</tr>
<tr>
<td>Linearity(%)</td>
<td>42.07±2.51</td>
<td>46.41±2.29</td>
<td>50.23±2.46</td>
<td>54.63±2.34</td>
<td>0.039</td>
</tr>
<tr>
<td>Straightness(%)</td>
<td>50.55±2.63</td>
<td>55.47±2.91</td>
<td>57.54±2.77</td>
<td>61.26±2.52</td>
<td>0.037</td>
</tr>
<tr>
<td>Wobble(%)</td>
<td>83.21±10.29</td>
<td>83.66±10.64</td>
<td>87.29±11.02</td>
<td>89.17±11.19</td>
<td>0.040</td>
</tr>
</tbody>
</table>

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-mI thymine 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 note 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 Nigella sativa oil increased the level of...
testosterone as compared to control. Moreover, intramuscular Vit. E injection with ChnRH analogue improved the level of testosterone in blood serum of chicken broiler breeders (Hezarjari 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.

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.

Regarding the fractionation of WBCs in blood of cockers, only a combination treatment increased (P<0.05) heterophil percentage 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).

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. Shaliba 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 source 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.

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

<table>
<thead>
<tr>
<th>Item</th>
<th>G1(control)</th>
<th>G2(Vit. E)</th>
<th>G3(PSO)</th>
<th>G4(Vit. E + PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>10.77±0.60a</td>
<td>11.83±0.73b</td>
<td>12.50±0.75bc</td>
<td>13.53±0.65c</td>
<td>0.049</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>29.27±1.37a</td>
<td>32.03±1.40ab</td>
<td>35.50±1.28bc</td>
<td>36.70±1.31c</td>
<td>0.003</td>
</tr>
<tr>
<td>RBCs (x10⁴/µL)</td>
<td>3.72±0.30a</td>
<td>4.26±0.33b</td>
<td>4.33±0.29a</td>
<td>4.68±0.31a</td>
<td>0.201</td>
</tr>
<tr>
<td>WBCs (x10⁴/µL)</td>
<td>5.53±0.36c</td>
<td>6.23±0.33bc</td>
<td>7.03±0.35ab</td>
<td>7.47±0.38a</td>
<td>0.004</td>
</tr>
</tbody>
</table>

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

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.

![Fig. 1. Effect of pumpkin seed oil, Vit. E, and their combination on plasma testosterone concentration and semen characteristics of Sinai cockers.](image1)

![Fig. 2. Effect of Vit. E, pumpkin, or their combination on white cells differentiation in Sinai cockers blood.](image2)
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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).

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).

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

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 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>G1 (Control)</th>
<th>G2 (Vit. E)</th>
<th>G3 (PSO)</th>
<th>G4 (Vit. E+PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>186.00±2.39</td>
<td>188.32±2.76</td>
<td>197.68±2.64</td>
<td>198.35±2.30</td>
<td>0.003</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.03±0.02</td>
<td>0.96±0.02</td>
<td>0.87±0.03</td>
<td>0.81±0.02</td>
<td>0.000</td>
</tr>
</tbody>
</table>

a, 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. E+PSO combination compared to Vit. E and control group (Fig. 4).
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; Martinez et al., 2010 a, b; Martinez 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. Shabha 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. Regarding 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<0.05) by PSO or a combination treatment than in Vit. E.

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 T3 and T4 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 T3 and T4 levels in laying hens. The rise in the levels of T3 and T4 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 T3 and T4 levels by thyme treatment (100 mg/kg) in 96 day-old boiler chicks. Antioxidants ameliorate level of T3 and T4 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

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

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

Fig. 6. Effect of Vit. E, pumpkin, or their combination on thyroid hormones (T3 and T4) in Sinai cockers blood.
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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.

<table>
<thead>
<tr>
<th>Item</th>
<th>G1(control)</th>
<th>G2(Vit. E)</th>
<th>G3(PSO)</th>
<th>G4(Vit. E+PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (ng/ml)</td>
<td>46.40±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.28±0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.17±0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.14±0.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>IgM (ng/ml)</td>
<td>8.60±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.72±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.80±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.10±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antioxidant status markers:</th>
<th>TAC (mmol/L)</th>
<th>MDA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.68±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.72±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.76±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.02±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.87±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.91±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> 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 have 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; Vlaiuc 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-oxidative abilities (Effinky et al., 2012; Vlaiuc 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>G1(control)</th>
<th>G2(Vit. E)</th>
<th>G3(PSO)</th>
<th>G4(Vit. E+PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertility rate (%)</td>
<td>83.31±1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.34±1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.16±1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.48±1.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.049</td>
</tr>
<tr>
<td>Hatchability rate&lt;sup&gt;1&lt;/sup&gt; (%)</td>
<td>84.54±1.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.58±1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.45±1.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.32±1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.035</td>
</tr>
<tr>
<td>Hatchability rate&lt;sup&gt;2&lt;/sup&gt; (%)</td>
<td>70.18±1.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.39±1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.34±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.31±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<sup>1</sup>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 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 improve 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>G1(control)</th>
<th>G2(Vit. E)</th>
<th>G3(PSO)</th>
<th>G4(Vit. E+PSO)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial live body weight (g)</td>
<td>1946.0±6.86</td>
<td>1963.5±6.01</td>
<td>1948.0±8.67</td>
<td>1961.0±7.45</td>
<td>0.244</td>
</tr>
<tr>
<td>Final live body weight (g)</td>
<td>2037.5±9.26b</td>
<td>2059.0±7.88b</td>
<td>2052.0±8.27b</td>
<td>2070.5±6.30b</td>
<td>0.044</td>
</tr>
<tr>
<td>Chang in live body weight (g)</td>
<td>91.5±4.35b</td>
<td>95.5±4.74b</td>
<td>104.0±4.88b</td>
<td>109.5±3.53b</td>
<td>0.028</td>
</tr>
</tbody>
</table>

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.

REFERENCES


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الملخص


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