



DIETARY PARSLEY OIL MITIGATES THE NEGATIVE ALTERATIONS IN TESTICULAR HISTOMORPHOMETRIC AND SEMEN QUALITY IN JAPANESE QUAIL MALES DURING SUMMER

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Abstract

This study was conducted to investigate the effect of parsley oil (PO) on the testicular histomorphometric measurements and semen quality in Japanese quail males reared during summer in Iraq, the study lasting for 14 weeks from June to August, using 120 quail males, 6 weeks old which were randomly assigned to 4 dietary treatment groups in a completely randomized design. Each group (n=30) was subdivided into 3 replicates (10 males / replicate). The males in the control group (C) received a basal diet without any additives, while the other three test groups G1, G2, and G3 received a basal diet contained 0.3, 0.6 and 0.9 ml PO/ Kg diet respectively. At the 8 weeks of age, the semen was collected fortnightly throughout the experiment period to evaluate semen parameters which including the ejaculated volume, sperms motility, sperms concentration, and sperms morphology (dead and abnormal sperms). At the end of the experiment, nine males per group were randomly selected, weighed and slaughtered to assess the testicular weight and testicular tissue histomorphometric evaluation. The results revealed that the semen parameters were significantly improved in all treatment groups regardless of the dose in comparison to the control group (C). On the other hand, the results also showed that there were significant differences among the treatment groups, the most studied traits were significantly improved with increased PO dose in the diet, and the best results were achieved in G3 (0.9 ml PO/Kg diet) followed by G2 and G1 groups respectively. In conclusion, dietary supplementation of PO for Japanese quail males reared in summer conditions alleviates the negative effect of heat stress on the testis functions and semen quality.

Key words : Parsley oil, Quail, Testis, Histomorphometric, Semen, Summer.

Introduction

Summer in Iraq extends from June to August; it's thermally stressful to different domestic animals among them the poultry where the summer in Iraq is characterized by very high temperatures resulting in severe heat stress.

Heat stress causes severe economic losses on poultry sector as a result of suppressing growth, decrease in egg production, increased the cost of production, high rate of mortality due to depressed immunity, and impairment in the reproductive ability (Ayo *et al.*, 1996; Obidi *et al.*, 2008; Ayo *et al.*, 2010).

The reproductive performance in poultry extremely depressing during environmental stress and the male is affected by heat-induced infertility more than the female

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(McDaniel *et al.*, 1996). Heat stress raises the body temperature, leading to disruption in secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Li and Cui, 2013), resulting in deleterious changes in testis histological structure and testicular function, which is manifested through decreased seminiferous epithelial cell differentiation, inhibition of intracellular ion exchange and declined the semen traits (Obidi *et al.*, 2008). Recent studies proved that all negative effects of heat stress on the productive and reproductive aspects in the poultry are due to the fact that heat stress plays an important role in occurrence of oxidative stress (OS) in the different body cells (Lin *et al.*, 2006).

OS is defined as the overabundant production of free radicals (FR) compared with the antioxidant capacity in animal cells (Davies, 1995). Akbarian *et al.*, (2016) explained, during heat stress, mitochondrial substrate

oxidation, and electron transport chain are increased resulting in excessive FR production (Altan *et al.*, 2003), these compounds are highly reactive and modify cellular compounds, such as proteins, lipids, and nucleic acids (DNA and RNA) (Lin *et al.*, 2006). These phenomena participated in the metabolic dysfunctions and resulted in damage or death of the cells by OS (Halliwell and Whitman, 2004).

Previous studies have shown that the antioxidants nutrient supplementation such as vitamins and minerals are capable of reducing oxidative damage induced by heat stress in poultry (Surai and Dvorsky, 2002; Kapakin *et al.*, 2013). Furthermore, during the recent years, there has been an ever-increasing interest in the use of different natural phytochemicals that have potential antioxidant properties such as essential oils (EOs) that derived as a mixture of aromatic oily liquids obtained from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Oyen and Dung, 1999). EOs used in poultry feeding because they have many advantages regarding the health and production including antioxidant activity and also heat stress alleviator (Cervato *et al.*, 2000; Gopi *et al.*, 2013). The parsley (*Petroselinum crispum*) is one of the antioxidant plants that belong to Umbelliferae family. The origin of parsley is the Mediterranean region but now cultivated in different regions of the world. It is used for different medicinal purposes in traditional and folklore medicine in different countries (Farzaei *et al.*, 2013). New researches indicated that PO rich in flavonoids (apiin, luteolin, apigenin and glycosides) and essential oil (myristicin, apiole, α -pinene and β -penene) these compounds have proven antioxidant properties and increased cellular antioxidant enzymes activity in the tissues (Aziza and Abdel-Wahhab, 2006; Marin *et al.*, 2016; EL-Gindy *et al.*, 2017). Thus, we conducted this study to examine whether of PO has a protective role on heat stress-induced harmful effects in reproductive capacity of male Japanese quail by investigating the alterations in semen quality and Histomorphometric measurements of testicular tissue.

Materials and Methods

Birds, Experimental Design and Management

The experiment was conducted from 1 June to 6 August (14 weeks) to examine the effect of different levels of PO on testicular function and semen quality in Japanese quail males during the summer season in Iraq. A total of 120 birds, 6 weeks old of Japanese quail males, approximately equal in body weight were used in this study. After 2 weeks of acclimation on experimental house conditions, the males were randomly distributed into 4

groups with 3 replicates (10 males/replicate) as the following: C (control) the quail males received control diet without parsley oil. The males in groups, G1, G2, and G3 were fed control diet contained 0.3, 0.6, and 0.9 ml PO/kg diet respectively. The males for all groups were housed within stainless wire cages (105×50×40 cm). The experimental diet containing 21% crude protein and 2888 (Kcal/Kg) metabolizable energy and drinking water were provided *ad libitum* until end of the experiment. The temperature was measured by thermometers placed in the center and sides of the experiment house, where it was 32.7 - 39.4° C. A lighting regime of 16h and continuous ventilation was provided; all birds were under symmetrical management conditions during the experiment.

Collection and analysis of semen characteristics

Semen was collected fortnightly from each male by a teaser female method as described by Chelmenska *et al.*, (2008). Freshly semen was used for measurement the ejaculated volume, sperms motility, sperms concentration, and sperms morphology (dead and abnormal sperms) according to AL-Draji (2007).

Testicular Histomorphometric Evaluation (THE)

At the end of the experiment, 3 males were randomly selected from each replicate, weighed individually and then slaughtered. The testis was completely removed from the abdominal cavity and weighed after removing excess tissue and washing with normal saline to measure the total testicular weight. For each male, one testis was fixed in 10% formalin for 24 hours, dehydrated through graded ethanol alcohols concentrations and xylene and then embedded in paraffin to obtain tissue blocks. Specimen blocks were then trimmed and sectioned, and 4- μ m sections were obtained with the use of an MRS 3500 Microtome (Pearse, 1964). Sections were then placed on glass slides and stained with hematoxylin and eosin (Luna, 1968). Due to the irregular shape of the seminiferous tubules, lumen of the seminiferous tubules and the germ cells layer, the histomorphometric analysis based on the measurement of the area and the diameter of the equivalent circle (D circle) for the mentioned criteria. Briefly, three seminiferous tubules were randomly examined for each section. The Area of the seminiferous tubules and area of lumen was measured in mm^2 by using the irregular shape tool in the Motic image plus (version 2.0 ml) image analysis system computer software after accurate calibration using a stage micrometer as shown in Fig.1. While the area of germ cells layer was estimated by subtracting lumen area from the seminiferous tubule area, and then the diameter of the equivalent circle for these parameters was measured by using the following statistical equation:

Diameter of the equivalent circle (D circle) mm= $(\text{Area} / 3.14)^{1/2} \times 2$. According to Mobark *et al.*, (2002) and Jalil *et al.*, (2017).

Statistical Analysis

Data were presented as means \pm standard error mean (SEM). The data were analyzed by the Statistical Analysis System (SAS) (2012) used to study the effect of different coefficients in the studied parameters in full randomized design (CRD). The differences between the averages were compared by Duncan (1955) test.

Results

Semen Characteristics

As shown in table 1, 2, 3, 4 and 5, the ejaculate volume, sperms motility, sperms concentration, dead and abnormal sperms were significantly improved ($p < 0.05$) by dietary supplementation with different doses (0.3, 0.6 and 0.9 ml/kg) of PO in comparison to the control group (0 ml/kg). However, the results of this study revealed significant differences among PO groups, the group G3 (0.9ml/kg) recorded the best results ($p < 0.05$) regarding all semen parameters compared with G1 group, only concerning sperm motility and concentration compared with group G2 (table 2 and 3). Also, we noted that there was a numerical increase in the ejaculated volume and a decrease in dead and abnormal sperms in G3 group compared with G2 group (Table 1, 4 and 5) as well the percentage of sperm motility in G2 group compared with

G1 group, but these variables were not statistically significant.

Discussion

Heat stress has detrimental effects on each organ of the avian body including the reproductive organs (Deeb and Cahaner, 2002). High environmental temperatures considered to be one of the significant risk factors causing infertility in males (Durairajanayagam *et al.*, 2014), Heat stress causes reduction in testes weight, degeneration in testicular tissue, disrupt spermatogenesis, decreased semen volume, sperms concentration, sperm motility, increase abnormal and dead sperms (McDaniel *et al.*, 2004; Ebeid, 2012; Turk *et al.*, 2015). These findings of the previous studies are consistent with our results in the present study regarding testes weight, testicular histomorphometric values, and semen traits of quail males in the C group which showed a significant decline in all studied parameters compared with quail males treated with PO. The responsible mechanism for this deterioration that the heat stress increase body temperature resulting in increased intra-testicular temperature and greater production of reactive oxygen species (ROS) that leading to lipid peroxidation due to the increased metabolic activity under stress conditions (Ebeid, 2012). It has been reported that excessive production of free radicles and ROS induce negative changes in the different types of testicular spermatogenic cells through disrupting there proliferation, differentiation, trigger apoptosis, and cell death (Lysiak

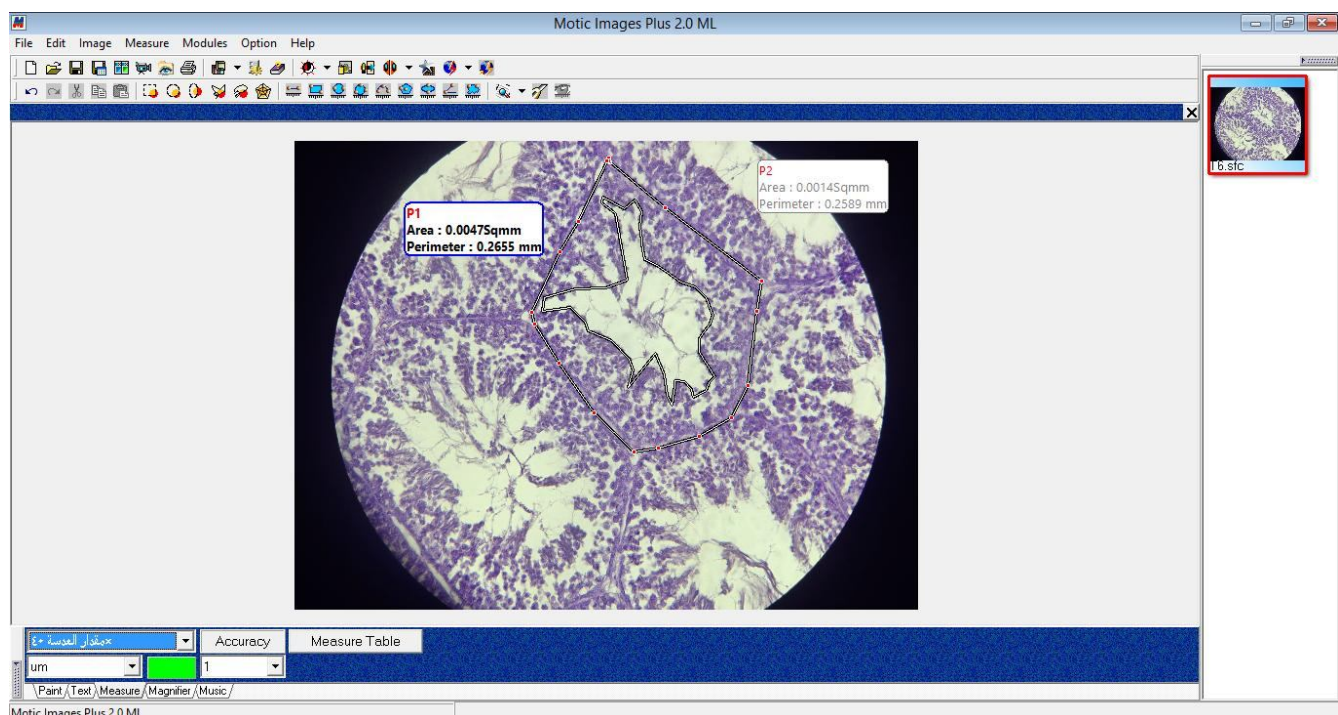


Fig. 1: Area measurement by irregular tool in the Motic Images Plus Software.

Table 1: Effect of different levels of Parsley oil on the ejaculated volume (ml) (Mean \pm SE) in Japanese quail males.

Groups				Period (14 days)
G3	G2	G1	C	
0.027 \pm 0.007a	0.025 \pm 0.006a	0.022 \pm 0.006b	0.019 \pm 0.007c	1
0.028 \pm 0.008a	0.027 \pm 0.008a	0.023 \pm 0.006b	0.018 \pm 0.007c	2
0.028 \pm 0.007a	0.027 \pm 0.006a	0.022 \pm 0.008b	0.020 \pm 0.005c	3
0.029 \pm 0.006a	0.029 \pm 0.006a	0.024 \pm 0.007b	0.019 \pm 0.008c	4
0.031 \pm 0.008a	0.030 \pm 0.007a	0.024 \pm 0.007b	0.021 \pm 0.005c	5
0.033 \pm 0.009a	0.033 \pm 0.004a	0.022 \pm 0.005b	0.020 \pm 0.007c	6
0.029 \pm 0.008a	0.028 \pm 0.007a	0.023 \pm 0.007b	0.019 \pm 0.005c	Mean

C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet. The different letters within the rows mean a significant difference ($P < 0.05$) between the experimental groups.

Table 2: Effect of different levels of Parsley oil on sperms motility (100%) (Mean \pm SE) in quail males.

Groups				Period (14 days)
G3	G2	G1	C	
79.6 \pm 3.8a	74.6 \pm 4.6ab	69.1 \pm 6.1b	61.5 \pm 8.6c	1
79.3 \pm 2.6a	73.3 \pm 4.1ab	70.6 \pm 3.6b	61.3 \pm 3.7c	2
81.4 \pm 3.3a	76.7 \pm 5.2ab	70.2 \pm 3.5b	60.8 \pm 4.8c	3
81.7 \pm 6.8a	77.2 \pm 6.1ab	71.1 \pm 6.0b	63.3 \pm 5.1c	4
84.2 \pm 5.2a	75.6 \pm 4.8ab	70.5 \pm 2.5b	62.7 \pm 6.8c	5
84.9 \pm 4.5a	75.8 \pm 3.7ab	71.4 \pm 3.2b	61.6 \pm 4.7c	6
81.8 \pm 5.3a	75.8 \pm 5.0ab	70.4 \pm 6.0b	61.8 \pm 5.3c	Mean

C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet. The different letters within the rows mean a significant difference ($P < 0.05$) between the experimental groups.

Table 3: Effect of different levels of Parsley oil on sperms concentration ($\times 10^6$ ml) (Mean \pm SE) in Japanese quail males.

Groups				Period (14 days)
G3	G2	G1	C	
612.5 \pm 88.5a	582.6 \pm 75.3b	451.2 \pm 55.9c	341.2 \pm 64.2d	1
614.3 \pm 97.8a	588.8 \pm 61.2b	438.5 \pm 74.6c	354.4 \pm 53.8d	2
682.3 \pm 89.8a	587.7 \pm 82.9b	471.2 \pm 81.2c	376.1 \pm 67.1d	3
679.1 \pm 93.2a	514.6 \pm 91.8b	474.9 \pm 66.8c	387.7 \pm 43.8d	4
704.2 \pm 99.3a	523.8 \pm 92.9b	518.3 \pm 71.7c	412.3 \pm 56.7d	5
721.6 \pm 95.9a	579.4 \pm 89.8b	527.1 \pm 70.1c	403.1 \pm 62.5d	6
669.0 \pm 96.7a	562.8 \pm 87.7b	480.2 \pm 77.5c	379.1 \pm 47.3d	Mean

C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet. The different letters within the rows mean a significant difference ($P < 0.05$) between the experimental groups.

et al., 2007; Paul *et al.*, 2009). Turk *et al.*, (2015; 2016) reported, heat stress has a significant role to enhance OS in testicular tissue of Japanese quail males exposed to a high-temperature manifested by increasing malondialdehyde concentration as an indicator of lipid peroxidation and OS level in the testis cells with a decrease anti-apoptosis value in germ cells and limited amounts of androgen receptors in Sertoli and Leydig cells.

These phenomena caused a significant reduction in seminiferous tubules size, numbers of all spermatogenic cells, germinal cell layer thickness and testis weight. These results are in agreement with findings of Chen *et al.*, (2015), he observed that the seminiferous tubules diameter were lower, an absence of spermatogenic cells, and the histological structure of testis was destroyed in heat-stressed birds. The impairment in testicular histological structure, spermatogenesis and spermatozoa functions due to the increased heat stress and lipid peroxidation negatively affects semen quality and fertility (Surai *et al.*, 2001).

Hassan *et al.*, (2003a) showed a positive and significant correlation coefficient between ejaculated volume and testicular weight, diameter of seminiferous tubules and the thickness of germinal cell layer, while the correlation was positive and highly significant between the sperm concentration and both testicular weight, thickness of germinal cell layer. On the other hand, the correlation coefficient was negative and significant between testicular weight and the percentage of dead and abnormal sperms. Also, Hassan *et al.*, (2003b) reported that cocks which selected for high sperms concentration and low abnormal sperms have high testes weight, seminiferous tubule diameter and germinal cell layer compared to those elected in the opposite trend. These findings of previous studies illustrate the deterioration reasons of the testis histological traits and semen quality in the C group males due to constant heat stress and don't treat with PO compared with G1, G2, and G3 groups respectively.

Recently, use of natural antioxidant products derived from medicinal plants has increased in poultry diets to alleviate the deterioration in productive and reproductive performance relating to heat stress-induced oxidative damage in the poultry sector (Raskovic *et al.*, 2014). The results of the present study indicated that the adding PO regardless of the doses led to mitigate negative effects of heat stress-induced oxidative damage in testis tissue and semen quality. PO contains active anti-oxidative substances such as flavonoids (apiin, luteolin, apigenin and glycosides) and essential oil (myristicin, apiole, α -pinene and β -penene), that are responsible for blocking and detoxifying of the harmful action of free radicals (Hassan and Abdel – Wahhab, 2006; Marin *et al.*, 2016; EL – Gindy *et al.*, 2017). Previous reports indicated that

Table 4: Effect of different levels of Parsley oil on dead sperms (100%) (Mean ± SE) in Japanese quail males.

Groups				Period (14 days)
G3	G2	G1	C	
5.7±0.6c	6.3±1.1c	11.0±2.5b	16.2±2.6a	1
5.7±0.8c	9.3±1.6bc	13.1±2.7b	20.0±2.9a	2
6.1±0.8c	8.2±1.8c	17.4±2.7b	21.3±2.9a	3
5.0±0.9d	9.9±1.3c	13.9±2.0bc	17.5±2.9a	4
5.6±0.7d	10.3±1.1c	13.6±2.7b	17.8±2.8a	5
4.9±0.9d	9.1±1.7c	12.4±2.3b	17.3±2.3a	6
5.5±0.9d	8.9±1.4c	13.6±2.6b	18.4±2.9a	Mean

C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet. The different letters within the rows mean a significant difference (P<0.05) between the experimental groups.

Table 5: Effect of different levels of Parsley oil on abnormal sperms (100%) (Mean±SE) in Japanese quail males.

Groups				Period (14 days)
G3	G2	G1	C	
6.4±0.8c	6.6±0.8c	10.7±0.9b	16.3±2.9a	1
6.5±0.3c	8.4±0.3c	12.2±3.4b	17.2±3.0a	2
6.3±0.6c	8.1±1.3c	14.3±1.7b	17.8±2.7a	3
6.1±1.3c	8.6±1.8c	14.2±2.9b	16.7±3.0a	4
5.6±0.9c	7.7±1.3c	11.6±2.4b	16.8±1.6a	5
5.1±0.9c	7.1±1.0c	11.3±1.9b	15.2±1.7a	6
6.0±1.2c	7.8±1.5c	12.3±2.1b	16.6±1.8a	Mean

C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet. The different letters within the rows mean a significant difference (P<0.05) between the experimental groups.

the addition of parsley leaf in poultry diets enhance egg yield and hatchability in quail (Tahan and Bayram, 2011). Improve hematological characteristics in geese (Al-Daraji *et al.*, 2012), raise the body weight and serum biochemical traits in broiler chicken (Jaafer, 2013; Ali *et al.*, 2016). To our knowledge, there has been no previous study regarding the protective effect of PO on heat stress-induced degradation in testicular tissue and semen quality in Japanese quail. Therefore, we can explain our results based on the findings of other studies carried out in different

Table 6: Effect of different levels of parsley oil on testes weight (g), diameter of seminiferous tubule, lumen of seminiferous tubule and germ cells layer (mm) (Mean±SE) in Japanese quail males.

Groups				Parameters
G3	G2	G1	C	
7.10 ± 0.15 a*	6.55 ± 0.17 b	5.75 ± 0.21 c	5.05 ± 0.12 d	Testes weight(g)
0.107 ± 0.001 a**	0.104 ± 0.003 ab	0.096 ± 0.004 b	0.082 ± 0.001 c	S.T.D (mm)
0.048 ± 0.001 a	0.057 ± 0.005 a	0.059 ± 0.004 a	0.054 ± 0.001 a	L.S.T.D (mm)
0.096 ± 0.001 a**	0.087 ± 0.001 b	0.082 ± 0.001 c	0.062 ± 0.003 d	G.C.L.D (mm)

C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet. S.T. D (mm): Seminiferous Tubule Diameter, L.S.T. D (mm): Lumen of Seminiferous Tubule Diameter, G.C.L. D (mm): Germ Cells Layer Diameter. The different letters within the rows mean a significant difference (*P<0.05), (** P< 0, 01) between the experimental groups.

models.

Generally, many studies pointed out that the protective role of PO against oxidative damage caused by different stressors attributed to its compounds (flavonoids and volatile substances) which acting either scavenging free radicals or inducing antioxidant enzymes production in tissues cells such as superoxide dismutase (SOD), Glutathione transferase and glutathione peroxidase (GSH-Px) (Zheng *et al.*, 1992; Nilsen *et al.*, 1999; Fejes *et al.*, 2000; Ozsoy-Sacan *et al.*, 2006). Aziza and Abdel- wahhab (2006) reported that PO showed a protective effect against sever OS induced by Zearalenone (ZEN) (A mycotoxin present in corn, as well as food mixture for farm animals) in the reproductive performance of mice males through decreasing deviations in spermatocytes, improve sperm motility and count, reducing the dead and abnormal sperms.

Liu *et al.*, (2016) reported that the administration of Apigenin (a Flavons subclass of flavonoids, commonly found in PO) to scrotal heat-induced damage in the mice testis resulted in increased testosterone, antioxidant enzymes such as SOD, GSH-Px and decreased Malondialdehyde (MDA). On the other hand, the histological examination revealed that Apigenin restrained testicular injury, preserves diameter of seminiferous tubules, and normal spermatogenic series and spermatids formation. Furthermore, the thickness of the basement membrane, Sertoli cells, and interstitial Leydig cells seemed intact and normal. Therefore, we can be supposed that the improvement in the testicular structure by increasing the diameters of seminiferous tubules, germ cells layer and

semen quality in the males of G1, G2 and G3 groups compared to the control birds (C), may be attributed to that bioactive substances in PO that inhibit OS due to chronic exposure to high temperatures during summer either by eliminating free radicals directly or indirectly via increasing the production of antioxidant enzymes which are the main defense systems of the body against the harmful effects of OS in animals. Thus, reduce the apoptosis in spermatogenic cells,

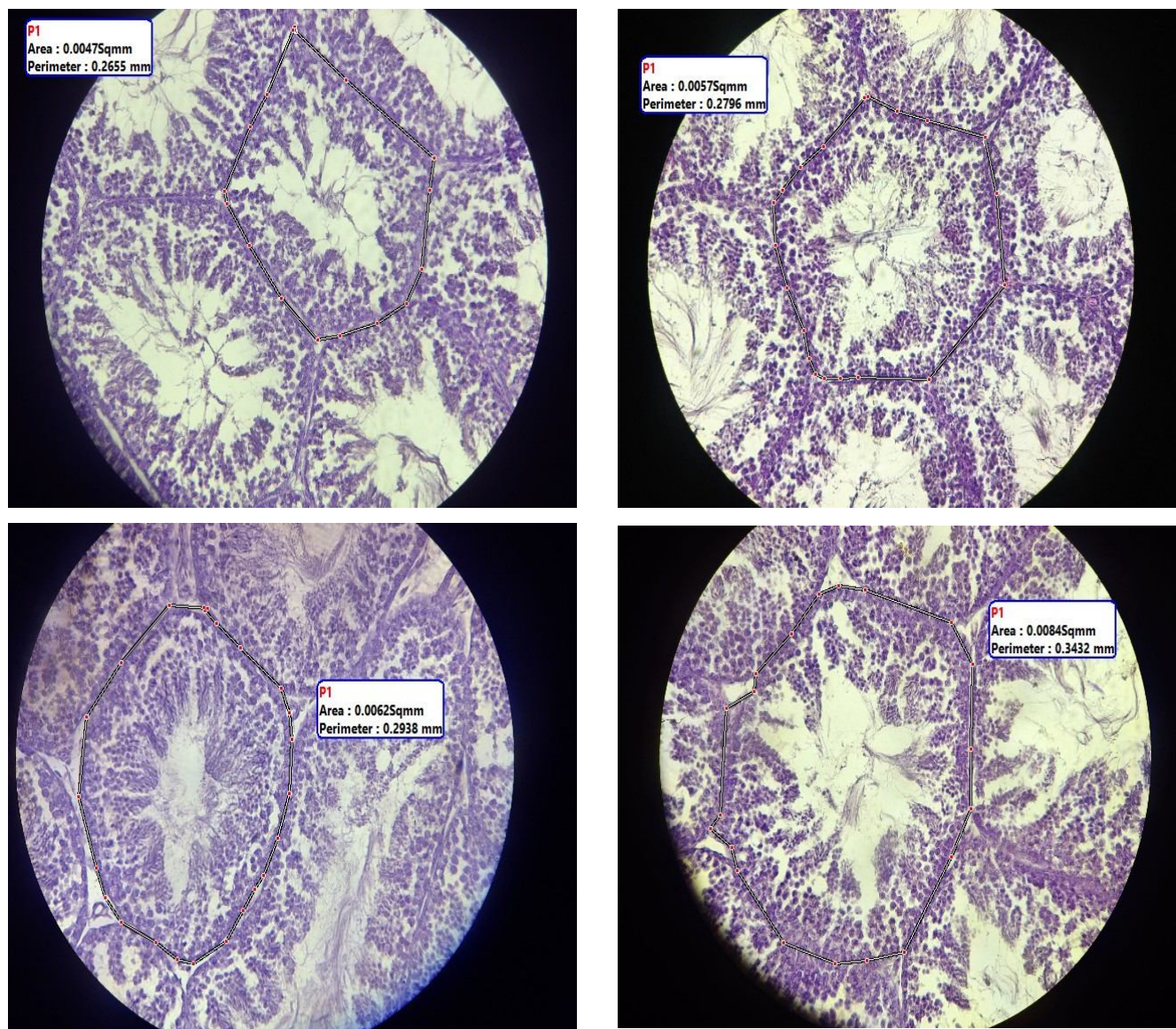


Fig. 2: Effect of different levels of parsley oil on the diameter of seminiferous tubule in the testis of Japanese quail male. C: parsley oil 0 ml/ Kg diet, G1: parsley oil 0.3 ml/Kg diet, G2: parsley oil 0.6 ml/Kg diet, G3: parsley oil 0.9 ml/Kg diet.

maintain their proliferation and differentiation normally, which leads to an increase in the thickness of the germ cell layer, seminiferous tubules size and consequently increase testicular weight because 98% of the testis mass consists of seminiferous tubules and germinal cells layer (Ferrouk *et al.*, 2015). The results of the above-mentioned studies and our results presented in tables 1 to 6 and Fig. 2 confirm our hypothesis that PO has a protective role in maintaining the reproductive efficiency in quail male during the high heat conditions.

Conclusion

The present study revealed that PO addition to diets of male quail housed under heat stress conditions reduced the negative alterations in the seminiferous tubules, spermatogenic cells, and semen traits. This positive effect

of OP may be attributed to its antioxidant activity.

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