

EFFECTS OF PARSLEY SUPPLEMENTATION ON THE SEMINAL QUALITY, BLOOD LIPID PROFILE AND OXIDANT STATUS OF YOUNG AND OLD MALE RABBITS

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Abstract: The high unsaturation levels of spermatozoal membrane make it very susceptible to oxidative damage and this problem increases with advancing age. In this study, the aim is to investigate whether parsley seed (PS) has a protective effect on semen quality, serum lipid profile and antioxidative status of old and young bucks. Male rabbits ($n=36$) (18 young 9-12 mo old and 18 old 36-42 mo old) were each assigned to 3 dietary treatments (a control and 2 levels of PS: 0.3 and 0.6 kg/100 kg diet) to evaluate the ability of parsley to enhance bucks' reproductive status. Most of the studied traits were adversely affected by age of rabbit bucks. On the other hand, the inclusion of PS significantly boosted ejaculate volume and improved mass motility concentration and total sperm output. Seminal plasma and blood serum total antioxidant capacity increased, while serum lipid peroxidase decreased with parsley treatments. In conclusion, dietary supplementation of parsley seed alleviates most semen quality parameters and counteracts oxidative stress, especially with the advance of age (seminal plasma and blood serum of total antioxidant capacity and malondialdehyde).

Key Words: rabbit, parsley, seminal quality, blood lipid, rabbit age.

INTRODUCTION

Sperm are particularly susceptible to oxidative stress (Aitken and Baker, 2002, 2004), as they are rich in mitochondria (Baccetti, 1985), the major source of the free radicals, the resultant reactive oxygen species (ROS) which are an integral component of cell metabolism regulating sperm movement, sperm capacitation, sperm-zona interaction, the acrosome reaction and sperm-oocyte fusion. Sperm are also rich in polyunsaturated fatty acids (Baker and Aitken, 2004), with low scavenging enzyme levels within the cytoplasm (Lewis *et al.*, 1997). Besides, the high rate of mitosis and various stages of meiosis in the seminiferous tubule expose the germinal cell chromosomes to the potentially damaging influence of free radicals in the local environment, thus creating a need for an effective antioxidant system (Oldereid *et al.*, 1998). Each cell is endowed with the antioxidant defence system, but an imbalance between reactive oxygen metabolites and antioxidant defence mechanisms of the cells, leading to excessive production of free radicals, creates a condition termed as oxidative stress. Moreover, the male reproductive function declines with age and aging is associated with decreased sperm quality, as the rate of ROS production per unit time increases with age (Droge, 2003), with progressive mitochondrial dysfunction (Bixi *et al.*, 2011) and a decline in immune function (Srinivasan *et al.*, 2005).

Parsley (*Petroselinum crispum*) is an important herb native in the Mediterranean area. It is considered to be a rich source of antioxidants that can help break up the chain reactions of free radical formation. It is widely distributed in Egypt and grown in gardens and fields. Parsley has been reported to have a number of possible medicinal attributes including antimicrobial (Wong and Kitts 2006; Farah *et al.*, 2015), anticoagulant, antihyperlipidemic, antihepatotoxic (Ozturk *et al.*, 1991) and antioxidant properties (Nielsen *et al.*, 1999; Hirano *et al.*, 2001). Parsley contains glycosides,

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apiole, terpenes, phthalides, coumarines, vitamins A, C and E, iron, calcium, phosphorus, manganese, quercetin and myristicin, which may help delay oxidant injury and cell death (Auger *et al.*, 1995). Our study was therefore conducted to investigate whether parsley seed (PS) has a preventive effect against oxidative stress on the physiological status and semen quality of young and old rabbit bucks.

MATERIALS AND METHODS

Animals and housing

The study was carried out at the Rabbit Research Laboratory of the Fish and Animal Production Department, Faculty of Agriculture, Saba Bash, Alexandria University. A total of 36 male V-line rabbits as defined by Ragab and Baselga, 2011, (18 young bucks at 9-12 mo of age with average initial live body weight 2.93 ± 0.05 kg and 18 old bucks at 36-42 mo of age with average initial live body weight 3.51 ± 0.06). Each age was divided between 3 homogeneous dietary treatments ($n=6$), a control and 2 levels of PS: 0.3 and 0.6 kg/100 kg diet. Animals were housed individually in flat-deck cages. The study protocol followed the Ethical Code for animal use by Alexandria University.

Feeding

Parsley seed was obtained in a dried meal condition with moisture content of 9.8%. All experimental diets were formulated to ensure they were both isonitrogenous and isocaloric in accordance with De Blas and Mateos, (1998). Three experimental diets were prepared (a control and 2 levels of PS: 0.3 and 0.6 kg/100 kg diet) as shown in Table 1. Feed and water were offered *ad libitum* throughout the experimental period. Ingredients and chemical analysis of the PS and experimental diets were performed according to AOAC, standard methods (AOAC, 2000; method 934.01 for dry matter, method 942.05 for ash, method 954.01 for crude protein, method 962.09 for crude fibre and method 920.39 for ether extract) in Table 1.

Table 1: Ingredients and calculated chemical composition of the experimental diet.

Items	Parsley	Control	Parsley 0.3	Parsley 0.6
Ingredients (%)				
Yellow corn		11	11	11
Wheat bran		22	21.7	21.4
Barley		20	20	20
Clover Hay		22	22	22
Soybean meal (44% crude protein)		20.2	20.2	20.2
Molasses		3	3	3
Di-calcium phosphate		1	1	1
L-lysine		0.1	0.1	0.1
DL-Methionine		0.1	0.1	0.1
Premix ¹		0.3	0.3	0.3
NaCl		0.3	0.3	0.3
Parsley		-	0.3	0.6
Chemical composition				
Dry matter (DM)	90.20	91.74	91.34	91.65
Organic matter (OM)	82.78	84.52	84.22	84.55
Crude protein (CP)	17.68	17.45	17.44	17.93
Ether extract (EE)	12.45	3.32	3.73	3.93
Crude fibre (CF)	13.54	13.61	13.52	13.37
Nitrogen free extract (NFE)	39.11	50.14	49.53	49.32

¹Each kg of vitamin and mineral mixture contained: Vit A, 2000.000 IU; E, 10 mg; B₁, 400 mg; B₂, 1200 mg; B₆, 400 mg; B₁₂, 10 mg; D₃, 180000 IU; Colin chloride, 240 mg; Pantothenic acid, 400 mg; Niacin, 1000 mg; Folic acid, 1000 mg; Biotin, 40 mg; Manganese, 1700 mg; Zinc, 1400 mg; Iron, 15 mg; Copper, 600 mg; Selenium, 20 mg; Iodine, 40 mg and Magnesium, 8000 mg.

Semen sampling and traits

Semen was collected every 2 wk over the 8 wk, after a 4-wk adaption period to experimental conditions. During this period, rabbit bucks were trained for semen collection. Semen collection and handling were carried out and evaluated according to the international guidelines of (IRRG, 2005). Ejaculated volume was measured to the nearest 0.1 mL. Semen pH was determined just after collection using a pH cooperative paper ranging from 0 to 14 with 1 grade (Merck KgaA, 64271 Darmstadt, Germany). Semen mass motility was given an arbitrary score from 0 to 3 as described by Moule (1965). Individual sperm motility was estimated at 400 \times magnification (National Optical Model 162, Compound Microscope, [Kamar, 1960]). Percentage of dead sperm was determined using an eosin-nigrosin blue-staining mixture. Sperm-cell concentration ($N \times 10^6/\text{mL}$) and total-sperm output ($N \times 10^6/\text{ejaculate}$) were determined counting the cells for the evaluation of sperm concentration according to Smith and Mayer (1955), using the improved Neubauer haemocytometer slide. Evaluation of seminal initial fructose was carried out immediately after collection according to Mann (1948). Seminal plasma at the end of study (8th week of experiment) was separated by centrifugation at 1000 $\times g$ for 20 min and stored at -20°C in Eppendorf tubes for further analysis of total lipid, cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), aspartate transaminase (AST), total antioxidants capacity and lipid peroxide (malondialdehyde) using commercial kits obtained from BIO-DIAGNOSTICS, Egypt according to the procedure outlined by the manufacturer.

Blood data

The rabbits were prevented from eating for some hours before blood sampling. Blood samples were individually collected from marginal ear vein. Serum was separated by centrifugation for 15 min (1000 $\times g$) and stored in vials at -20°C for later analysis. Frozen serum was thawed and assayed calorimetrically for total lipids, cholesterol, HDL, LDL, triglycerides, total antioxidant capacity and malondialdehyde contents following the manufacturer's recommendations (Biodiagnostic, Egypt). Serum testosterone was determined by enzyme immunoassay using commercial kits purchased from Biosource.

Statistical analysis

Data were subjected to two-way analysis of variance with age, levels of parsley as the main effects and their interaction, using the GLM procedure of (SAS Institute, Cary, NC, USA). Significant means were compared according to Duncan's (1955). Semen quality was replicated 4 times through 8 wk. The mean values were simply averaged from replications and were presented along with standard error of the mean throughout the study (8 wk). Mean differences between treatments were compared using the Duncan's test (1955) ($P < 0.05$). Percentage values were transformed to Arc-Sin values to approximate normal distribution before statistical analysis.

RESULTS

Semen quality

The effect of age and parsley levels on the semen characteristics are shown in Table 2. Mass and individual motility, sperm concentration and sperm production output were significantly decreased in old rabbit bucks by 12.23, 12.29, 32.54 and 35.58%, respectively, while dead sperm was significantly increased by 66.16% compared to the rates in young males. Parsley levels (0.3 and 0.6%) significantly increased ejaculate volume (23.29, 16.44%), mass motility (17.71, 18.23%), concentration (15.03, 27.58%) and sperm production

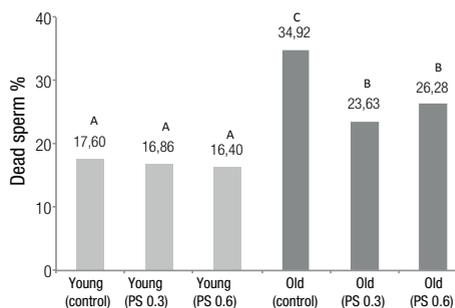


Figure 1: The interaction effect between age and parsley levels on percentage of dead sperm (standard error of mean=0.019). Bars not sharing letter are significantly different at $P < 0.05$.

Table 2: Effect of age, parsley levels and their interaction on some semen characteristics.

Semen characteristic	Age			Parsley level %				P-value		
	Youth	Old	SEM	Control	0.3	0.6	SEM	Age	Parsley level	Age×parsley level
Male (No)	18	18		12	12	12				
Volume (mL)	0.85	0.81	0.03	0.73 ^a	0.90 ^b	0.85 ^b	0.04	0.371	0.003	0.146
pH	7.60	7.33	0.12	7.44	7.45	7.51	0.15	0.12	0.937	0.563
Mass motility (1-3)	2.29	2.01	0.08	1.92 ^a	2.26 ^b	2.27 ^b	0.09	0.012	0.016	0.774
Individual motility (%)	77.71	68.16	3.70	68.16	76.43	74.46	4.4	0.008	0.142	0.993
Concentration (10 ⁶ /mm ³)	372.39	251.21	8.49	273.02 ^a	314.05 ^b	348.33 ^c	10.40	0.0001	0.0001	0.493
Dead sperm (%)	17.08	28.38	1.1	26.30 ^b	20.14 ^a	21.16 ^a	1.3	0.001	0.006	0.026
Sperm production (10 ⁶ /ejaculate)	316.12	203.65	10.55	199.55 ^a	282.27 ^b	297.84 ^b	12.92	0.0001	0.0001	0.231

^{abc}Means in a row and effect not sharing superscript are significantly different at $P<0.05$.

SEM: standard error of the mean.

output (41.45, 49.26%) compared to control bucks, respectively, while dead sperm decreased with 0.3 and 0.6% PS by 23.42 and 19.54%, respectively, compared to control bucks.

Interaction of age with parsley treatments showed no significant effect on all semen characteristics except on dead sperm (Figure 1) which showed PS supplementation (0.3 and 0.6%) significantly ($P<0.05$) reduced old rabbit bucks' dead sperm by 32.33 and 24.74% compared with un-supplemented old bucks.

Seminal plasma analysis

Data on seminal plasma are presented in Table 3. The effect of age was recognised, where old rabbit bucks showed significant increase in LDL and malondialdehyde (MAD) (12.78 and 26.99%) and decrease in HDL, HDL/LDL ratio and total antioxidant capacity (TAC) (6.28, 17.36 and 27.52%), respectively, while initial fructose was significantly decreased by 11.12% compared to the young rabbit bucks. The highest level of PS supplementation (0.6%) significantly increased seminal plasma total lipid and HDL by 4.32 and 7.91%, respectively, compared to the non-treated bucks. Moreover, PS at both doses led to a significant increase ($P<0.05$) in seminal plasma fructose (18.25, 14.29%) cholesterol (9.68, 16.53%), HDL/LDL (14.58, 30.21%) and TAC (18.75, 35%) compared to the control group. The opposite trend was shown in seminal plasma LDL and MAD, which showed a significant decrease with parsley supplementation (0.3 and 0.6%) by 8.38, 16.91 and 19.81, 22.14%, respectively, compared to the

Table 3: Effect of age, parsley levels and their interaction on seminal plasma characteristics.

Seminal plasma	Age			Parsley level %				P-value		
	Youth	Old	SEM	Control	0.3	0.6	SEM	Age	Parsley level	Age×parsley level
Male (No)	18	18		12	12	12				
Fructose (mg/100 mL)	239.70	213.04	7.57	204.22 ^a	241.50 ^b	233.40 ^b	9.28	0.019	0.020	0.137
Total lipid (mg/dL)	154.47	157.44	1.7	153.38 ^a	154.50 ^a	160.00 ^b	1.44	0.084	0.006	0.064
Cholesterol (mg/dL)	60.14	52.22	1.24	51.67 ^a	56.67 ^b	60.21 ^b	1.53	0.081	0.002	0.705
LDL (mg/dL)	16.75	18.89	0.37	19.46 ^c	17.83 ^b	16.17 ^a	0.46	0.0001	0.0001	0.817
HDL (mg/dL)	19.92	18.67	0.30	18.46 ^a	19.50 ^a	19.92 ^b	0.37	0.006	0.026	0.848
HDL/LDL	1.21	1.00	0.03	0.96 ^a	1.10 ^b	1.25 ^c	0.03	0.0001	0.0001	0.705
AST (U/L)	23.93	24.75	0.58	25.10	24.27	23.66	0.71	0.327	0.365	0.516
TAC mM/L	1.09	0.79	0.03	0.80 ^a	0.95 ^b	1.08 ^c	0.04	0.0001	0.0001	0.049
MAD (nmol/mL)	4.89	6.21	0.18	6.46 ^b	5.18 ^a	5.03 ^a	0.21	0.001	0.0001	0.518

^{abc}Means in a row and effect not sharing superscript are significantly different at $P<0.05$.

LDL: Low density lipoprotein; HDL: High density lipoprotein; AST: Aspartate transaminase; TAC: Total antioxidant capacity; MDA: Malondialdehyde. SEM: standard error of the mean.

Table 4: Effect of age, parsley levels and their interaction on blood compounds.

	Age			Parsley level (%)				P-value		
	Youth	Old	SEM	Control	0.3	0.6	SEM	Age	Parsley level	Age×parsley level
Blood serum										
Male (No)	18	18		12	12	12				
Total lipid (mg/dL)	144.36	235.33	2.72	206.54 ^b	179.17 ^a	183.83 ^a	3.37	0.0001	0.0001	0.466
Triglycerides (mg/dL)	161.57	217.93	6.83	198.02	189.86	181.38	8.3	0.0001	0.382	0.949
Cholesterol (mg/dL)	55.25	73.97	1.53	67.37	63.19	63.27	1.95	0.0001	0.249	0.165
LDL (mg/dL)	15.97	24.00	0.30	19.96 ^b	19.00 ^b	17.83 ^a	0.36	0.0001	0.0001	0.014
HDL (mg/dL)	20.53	24.00	0.30	19.85 ^a	23.67 ^b	23.17 ^b	0.38	0.0001	0.0001	0.004
HDL/LDL	1.31	1.03	0.03	0.95 ^a	1.23 ^b	1.30 ^b	0.03	0.0001	0.0001	0.271
Testosterone (ng/mL)	3.10	2.58	0.08	2.62 ^a	2.95 ^b	2.91 ^b	0.09	0.0001	0.034	0.508
TAC (mM/L)	1.31	0.84	0.04	0.88 ^a	1.23 ^b	1.11 ^b	0.04	0.0001	0.0001	0.025
MAD (nmol/mL)	19.26	19.93	0.31	21.50 ^b	18.26 ^a	19.03 ^a	0.38	0.154	0.0001	0.008

^{abc}Means in a row and effect not sharing superscript are significantly different at $P<0.05$.

LDL: Low density lipoprotein; HDL: High density lipoprotein; TAC: Total antioxidant capacity; MDA: Malondialdehyde. SEM: standard error of the means.

control bucks. Interaction of age with parsley treatments showed significant effect ($P<0.05$) on seminal plasma TAC (Figure 2), which indicated that PS had a positive effect on seminal plasma TAC of old rabbit bucks (both levels of PS) and young rabbit bucks (level 0.6% PS only).

Blood biochemical study

Serum total lipid, triglycerides, cholesterol, LDL and HDL (Table 4) increased ($P<0.001$) in old rabbit bucks by 63.02, 34.88, 33.88, 50.28 and 16.90% compared to the young group. In contrast, HDL/LDL, testosterone and TAC decreased significantly in old rabbit bucks by 21.37, 16.77 and 35.88% compared with young rabbits. Parsley treatments (0.3 and 0.6%) caused a reduction ($P<0.001$) in serum total lipid to reach 86.75, 89.00 and MAD to reach 84.93, and 88.51% of control. However, parsley treatments (2 levels 0.3 and 0.6%) significantly increased ($P<0.05$) serum HDL (19.24; 16.73%), HDL/LDL (29.47; 36.84%), testosterone (12.60; 11.07) and TAC (39.77; 26.14%) compared with control bucks, without significant differences between the different PS doses. Meanwhile, a high level of parsley (0.6%) decreased serum LDL by 10.67% compared with un-supplemented bucks. Interaction between age and parsley treatments showed no significant effect ($P<0.05$) on studied blood components except LDL, HDL, TAC and MAD. Parsley seed supplementation (Figure 3) significantly decreased LDL with increased HDL in both old and young bucks. Similar effects of PS were found in an increase ($P<0.05$) in the TAC concentration and a decrease in the MAD concentration of old bucks, while significant increase was determined in the TAC concentration of young rabbit bucks (0.6% PS) compared to control.

DISCUSSION

Semen quality

Our study findings showed that with increased male age most semen quality parameters decreased, except the dead sperm %, which increased. These results agree with (Kidd *et al.*, 2001; Narendra *et al.*, 2003; Henkel *et al.*, 2005; Stone *et al.*, 2013). Previous results from Wang *et al.* (1978) reported a significantly reduced total sperm production among older rats. Several mechanisms

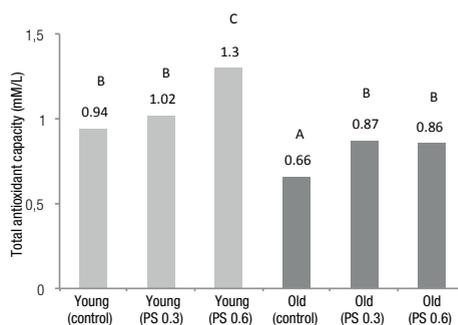


Figure 2: The interaction effect between age and parsley levels on seminal plasma total antioxidant capacity (standard error of mean= 0.056). Bars not sharing letter are significantly different at $P<0.05$.

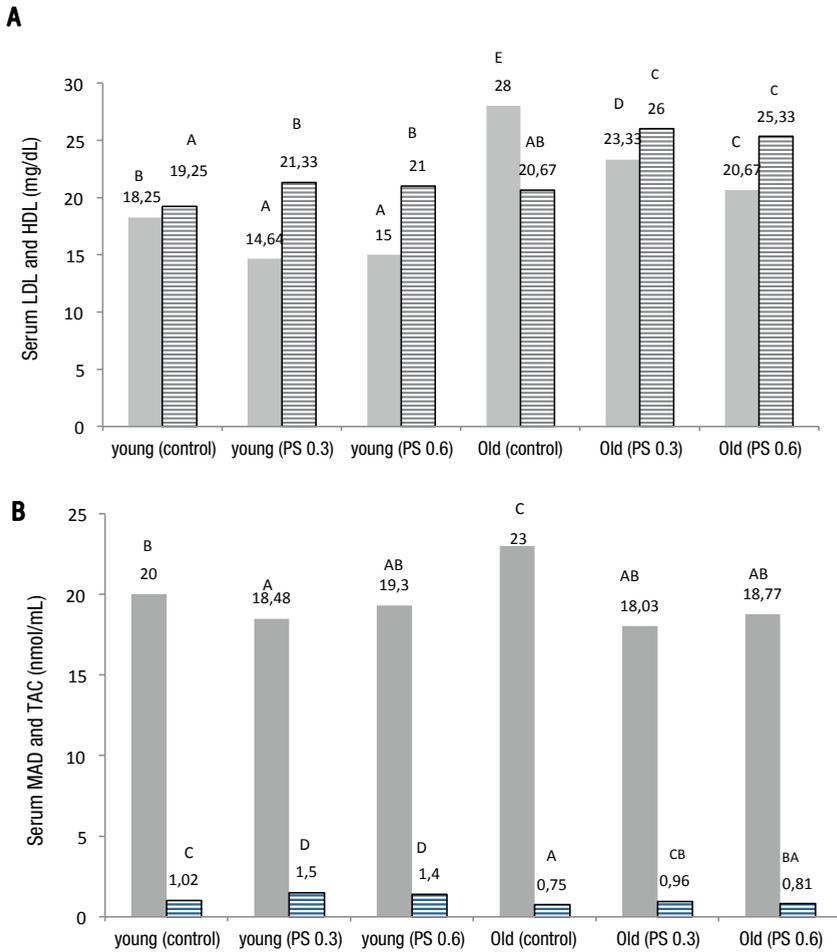


Figure 3: A) The interaction effect between age and parsley levels on blood serum low density lipoprotein (LDL) and high density lipoprotein (HDL) (■ LDL [mg/dL]; ▨ HDL[mg/dL]. LDL standard error of mean [SEM]: 0.49; HDL SEM: 0.51) B) blood serum malondialdehyde (MAD) and total antioxidant capacity (TAC) (■ MAD [nmol/mL]; ▨ TAC [nmol/mL]. MAD SEM: 0.54; TAC SEM: 0.05). Bars for each trait not sharing letter are significantly different at $P < 0.05$.

have been proposed to explain how aging in males may cause changes in semen parameters, as these changes can be related to seminal vesicle inadequacy which changes in prostate, in terms of prostate atrophy, such as reduction in water and protein content which might affect sperm motility (Kidd *et al.*, 2001) with decrease in fructose values (Molina *et al.*, 2010), or perhaps to a decline in the sperm concentration in testis (Wang *et al.*, 1993), with a reduction in the percentage of motile spermatozoa and an increase in the proportion of spermatozoa with cytoplasmic droplet in cauda epididymis (Syntin and Robaire, 2001). Likewise, age has been negatively correlated with sperm motility and plasma testosterone levels (Henkel *et al.*, 2005). At the cellular level, oxidative and other accumulated damage in sperm successively lead to degeneration of the cell membrane, increased cell permeability, leakage of vital constituents, decrease in ATP content and disturbance of ionic exchange (Salisbury and Hert, 1970; Vishwanath and Shannon, 1997; Tarin *et al.*, 2000).

Evidently, parsley supplementation improved the quality of rabbit semen. The ejaculate volume and sperm concentration were mirrored in overall sperm production output, where it increased significantly with parsley seed treatment in comparison with the control. Increased ejaculate volume in PS treatments may be due to increased secretion seminal fluid from the sex accessory gland or increased testosterone level compared to the control, as shown in Table 4. The dead sperm percentage of old rabbit bucks was significantly ($P=0.026$) decreased with PS supplementation. These results concur with those of El-Damrawy *et al.* (2008), who found that antioxidant intake was associated with greater sperm numbers and motility in old rabbit bucks. The increase in sperm mass motility of PS treatments compared with control could be due to increased seminal plasma fructose (Khatoun *et al.*, 2014), or the protective effect of parsley, reflected in the increased seminal plasma total antioxidants capacity (Koca *et al.*, 2003) and decreased MDA level (Keskes-Ammar *et al.*, 2003) (as shown in Table 2 and 3) or to preserving the morphology and the motility of sperm by binding antioxidants to end-peroxides (Marin-Guzman *et al.*, 2000). Our findings are in agreement with the results of Baghdadi (2014), who found a significant negative correlation between sperm motility and seminal plasma concentration of MDA.

Seminal plasma

Seminal plasma components are very important for sperm metabolism, as well as sperm function, survival and transport in the female reproductive tract (Juyena and Stelletta, 2012). Old rabbit bucks showed significant increase in LDL and MAD and a decrease in HDL, HDL/LDL ratio and TAC compared to the young bucks. Parsley seed supplementation (0.6%) significantly increased total lipid, TAC and HDL/LDL, while LDL was decreased compared with un-supplemented male ones. Likewise, seminal plasma cholesterol of parsley treatments (0.3 and 0.6%) significantly increased compared with control bucks. This is a good indicator for improved semen quality of parsley bucks, as cholesterol plays a major role in the sperm membrane, which is essential for sperm cell function.

The high fructose concentration provides nutrient energy for the spermatozoa (Wilke *et al.*, 2009), which is reflected in the testosterone concentration (Table 4) and quality of semen. Our result suggests that parsley seeds may be promising for healthy sperm and consequently improve the reproductive performance of male rabbits.

Blood compounds

Testosterone is required for maturation of male germ cells and sperm production and quality (Walker, 2009). Our data showed that male rabbits supplemented with PS significantly improved blood plasma testosterone concentration compared to the control group.

Sustaining the normal levels of circulating testosterone is clearly important for the well-being of male reproductive function. The increased level of testosterone reported in the current study, accompanied by the increase in sperm counts (Table 2) is supported by the previous reports of (McDonald and Capen, 1989) who stated that the testosterone hormone promotes growth development and secretory activity of the accessory sex organs of the male. Al-Janabi (2014) reported that parsley oil significantly increased the number of motile spermatozoa in seminal fluid of infertile men as compared with control.

The increase in serum HDL and HDL/LDL of parsley treatments (2 levels: 0.3 and 0.6%) could be mainly attributed to the presence of flavonoids, which play a role in decreasing the activity of both hepatic enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and Acyl Coenzyme A: Cholesterol O-acyltransferase (ACAT), or may be due to the presence of some HMG-CoA reductase inhibitors (Marzouk *et al.*, 2013), or could be referred to the stabilising effect of polyphenols on plasma lipoproteins or due to the systemic effect of flavonoids to modulate various enzyme activities that can have an effect on lipoprotein, leading to an augmentation of high density lipoprotein. The positive effect of parsley extract on high density lipoprotein seemed to be in agreement with the results of Abd El-Baky (2011) and Baghdadi (2014). Our results indicated that inclusion of parsley in the diet caused an increase in the total antioxidant capacity, and these outcomes were in line with the results reported by (Nielsen *et al.*, 1999; Rajeshwari *et al.*, 2011a,b; Dixit *et al.*, 2005). Reactive oxygen species concentrations increase with age, concomitantly with decreases in antioxidant levels (Weir and Robaire 2007; Vazquez-Memije *et al.*, 2008). Malondialdehyde is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid (Vaca *et al.*, 1988). Therefore, the content of MDA is used as an indicator of tissue damage (Ohkawa *et al.*, 1979). In the present study, we observed a significant

decreased in the MDA concentration of PS treatment, indicating decreased lipid peroxidation, which could be attributed to decreased formation or adequate clearance of free radicals by cellular antioxidants. A high level of parsley (0.6%) decreased serum LDL compared with un-supplemented bucks. These results support that parsley exerts an antioxidant activity (Ahsan *et al.*, 1990; Kery *et al.*, 2001; Jimenez-Alvarez *et al.*, 2008).

CONCLUSION

The study results clearly suggest that addition of parsley seed to diets of young and old rabbit bucks played an important role in male reproduction, especially in old bucks, but the exact effect and mechanism need to be further investigated.

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