

EFFECTS OF PARSLEY SEEDS AT DIFFERENT LEVELS AS NATURAL ADDITIVES ON SEMEN QUALITY IN RABBIT MALES

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Abstract

This study is designed to evaluate the effect of using parsley seeds (PAS) at different levels as a natural on semen quality and reduce the effect of lipid peroxidation of bucks. Forty New Zealand rabbit bucks 8 month old were divided into four groups (each 10 rabbit bucks). The groups were divided as follows: Control diet (free from PAS), T1: Control diet + 5g/kg PAS, T2: Control diet + 10g/kg PAS; T3: Control diet + 15g/kg PAS. Semen sample were collected twice a week; from each rabbit buck by artificial vagina. The first semen collection was used to evaluate semen quality parameters. However, the second semen collection was used for analysis semen plasma. Results revealed that feeding diets containing different levels of PAS significantly ($P<0.05$) increased semen volume, mass activity, individual motility, PSV, sperm concentration, total sperm and seminal plasma activity of ALP enzyme and significant ($P<0.05$) decrease in dead sperm and seminal plasma concentrations of total lipids, triglycerides, cholesterol and MDA and seminal plasma activities of AST and ALT enzymes and TAC as compared with the control group. In conclusion, dietary supplementation with different levels of PAS, especially at the level of 15 g/kg (T3) resulted in significant improvement in semen quality, antioxidant status and reproductive traits of rabbit bucks.

Key words: Rabbits, Parsley seeds, Semen Quality, Antioxidant status.

Introduction

The medicinal plants and herbs have been used in the treatment of various diseases in both of animals and human. Nowadays, utilization of these medicinal plants is increasing. These are used in animal feed as the growth promoters and for enhancement of productive performance (Chairs, 2000). Parsley is powerhouse and nutrients containing high levels of beta, carotene and vitamin C. Parsley enhances and stimulates the energy of organs, improving their ability to assimilate and utilize nutrients. Parsley is a source of alpha-linolenic acid, an important essential fatty acid for growth and reproduction (Bardley, 1992). Richmond and Mackley (2000) reported that parsley is rich in minerals such as calcium, potassium, iron and vitamins such like A, C, thiamin, riboflavin and niacin. Parsley seed is also used as a

diuretic and the hypoglycemic activity of parsley has been shown by Ozsoy et al., (2006). The constituents of parsley which include carotenoids, flavo-noids, coumarins, apiole, various terpenoid compounds, phenyl propanoids, phthalides, furanocoumarins, and tocopherol, have been chemically investigated (Tunali *et al.*, 1999). Components of fresh parsley leaf scavenge superoxide anion in vitro (Campanella *et al.*, 2003), and the methanol extracts of parsley scavenge hydroxyl radical in addition to protecting against ascorbic acid induced membrane oxidation (Fejeset *et al.*, 2000). Supplementation of rats with a fresh parsley leaf in diets can increase antioxidant capacity of the blood plasma. Embark (2016) found that the supplementation of male rabbit's diets with 6g/kg parsley seeds improved reproductive performance. So, the objective of the present experiment was to investigate the impact of dietary supplementation with different levels of parsley seeds on semen quality of rabbit bucks.

Materials and Methods:

Forty New Zealand rabbit bucks 8 months old of proven fertility, with an average initial live body weight of 2.90 ± 0.02 kg were used in this study. The rabbits involved in this study were housed in 4 separated floor pens under an artificial lighting program of 16 h light / 8 h dark photo period from the beginning of February to the end of April 2017. Pools of water were made available to rabbits. For 3 months of experiment all rabbits were fed 150 to 200 g/day, a commercial ration for rabbits breeding which contains 12metabolisable energy (MJ) and 17 % crude protein. Parsley was offered in the form of seeds. The rabbits separated into 4 treatment groups containing 10 rabbits each. Treatment groups were as follows:

Control diet (free from parsley; Control)

T1: Control diet + 5 g/kg parsley seeds

T2: Control diet + 10 g/kg parsley seeds

T3: Control diet + 15 g/kg parsley seeds

Semen samples were collected weekly over 8 weeks using an artificial vagina and the samples of each week were subjected to chemical analysis. Semen collection and handling were carried out and evaluated according to the international guidelines of (IRRG, 2005). Ejaculated volume was measured to the nearest 0.01 ml. The volume of each ejaculate was recorded after removal of the gel mass. Immediately after collection, semen was maintained at 37°C in a water bath for evaluation. Semen mass motility was given an arbitrary score from 0 to 3 based on the following assessment and the following variables were estimated: 0= No current, (0.5) =Very few slow current, 1 = Few slow current, 1.5= Many moderate waves, 2=Many sweeping waves, 2.5=Numerous vigorous waves, 3= Numerous rapid and vigorous waves, as described by Moule (1965). A weak eosin-formalin (10% formalin) solution was

used at a rate of 1: 99 before counting the cells for evaluation of sperm concentration by the improved Neubauerhemocytometer slide method as described by Smith and Mayer (1955). Individual sperm motility was estimated at 400× magnification (Kamar, 1960). Assessment of live and abnormal spermatozoa was performed using an eosin–nigrosin blue-staining mixture (Blom, 1950). 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). Serum testosterone was determined by enzyme immunoassay using commercial kits purchased from Biosource. Packed sperm volume (PSV) was recorded using Micro-AID® microhematocrit tubes and microhematocrit-centrifuge which were centrifuged for 5 min at 4000 rpm. Evaluation of seminal initial fructose was carried out immediately after collection according to Mann (1948). However, the second semen collection was used after pooled the semen from 5 rabbits in each treatment group to determine seminal plasma concentrations of cholesterol, high densitylipoprotein (HDL) and low density lipoprotein (LDL), total lipids, triglycerides, fructose, total antioxidant capacity (TAC), Malondialdehyde (MDA) and activities of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes by using the procedures indicated by Al-Daraji (2007b). The data were assessed by analysis of variance using the SPSS (2004). Test of significance for the difference between different treatments was done by Duncan's multiple range test (Duncan, 1955).

Results and discussions:

The result of this experiment indicated that the supplementation of the rabbit bucks ration with parsley seeds (T1,T2 and T3) resulted in significant ($P<0.05$) increase in ejaculate volume, mass activity, individual motility, PSV, sperm concentration and total sperm count and significant ($P<0.05$) decrease in dead sperm as compared with control group (C), while there was no significant differences among treatment groups with relation to the pH of semen (Table 1). Furthermore, there were no significant differences between T2 and T3 concerning mass activity, individual motility, and PSV (Table 1). As shown in Table 2 supplementing ration of buck rabbits with parsley seeds (T1,T2 and T3) caused significantly ($P<0.05$) decrease in seminal plasma concentrations of cholesterol, LDL, total lipids, triglycerides, MDA and activities of AST and ALT enzymes and significant ($P<0.05$) increase in seminal plasma activity of fructose, HDL,TAC and ALP (alkaline phosphatase) enzyme in comparison with the control group. Moreover, it is also clear from (Table 2) that there was no significant difference between T1 and T2 with respect to seminal plasma concentrations of total lipid and triglycerides and activities of AST, ALT enzymes So no significant difference between T2 and T3 with respect to seminal plasma concentrations of HDL, ALP, fructose, TAC and MDA. The effects of parsley seed on ejaculate volume and sperm concentration were mirrored on the overall total sperm output where it increased significantly ($P \leq 0.01$) in comparison with the

control groups. Sperm mass activity also significantly ($P \leq 0.01$) improved with parsley seed treatment compared to control groups which was reflected on sperm individual motility. Motility is critical in enabling the sperm to ascend the female reproductive tract to the site of fertilization, which is necessary if fertilization is to be achieved (Aitken, 1990). The increase in sperm, motility of treatment containing parsley seeds compared with control could be due to the protective effect of parsley. Also, these protective effects were reflected in the decrease of malondialdehyde (MDA) level and increase of total antioxidant capacity (Table 2) the present findings are in agreement with the recent results of Al-Janabi, (2014) who found a significant negative correlation between sperm motility and seminal plasma concentration of MDA. Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin present in corn, as well as food mixture for farm animals (Molto *et al.*, 1997; Placinta *et al.*, 1999). ZEN and its metabolites were associated with hyperestrogenism and several physiological alterations of the reproductive tract in several laboratory animals (mice, rat and rabbits) (Creppy, 2002). Hassan and Abdel-Wahhab, (2006) reported that ZEN-induced genotoxic effects of germ cell's chromosomes, sperm abnormality as well as testosterone level in male mice, however, the treatment with parsley caused a significant decrease in total chromosomal aberrations, sperm abnormalities and increase in testosterone level and sperm counts and motility. The antioxidant activity of parsley has been reported previously.

Table1: Effect of dietary supplementation with different levels of parsley seeds on semen traits (Mean \pm SE) of buck rabbits

Traits	Treatments				P.Value
	Control	T1	T2	T3	
Ejaculate volume(ml)	0.63 \pm 0.005 ^d	0.71 \pm 0.003 ^c	0.74 \pm 0.003 ^b	0.82 \pm 0.004 ^a	0.0001
Mass activity (1-3)	2.20 \pm 0.006 ^c	2.43 \pm 0.08 ^b	2.54 \pm 0.003 ^a	2.55 \pm 0.004 ^a	0.0001
Individual motility %	72.82 \pm 0.24 ^c	75.85 \pm 0.18 ^b	81.60 \pm 0.23 ^a	81.83 \pm 0.28 ^a	0.0001
Dead sperm %	22.81 \pm 0.29 ^a	16.41 \pm 0.21 ^b	13.56 \pm 0.20 ^c	11.91 \pm 0.20 ^d	0.0001
Ph	7.96 \pm 0.008	7.96 \pm 0.005	7.97 \pm 0.003	7.96 \pm 0.004	0.976
*PSV %	11.09 \pm 0.16 ^c	12.61 \pm 0.05 ^b	13.54 \pm 0.07 ^a	13.53 \pm 0.04 ^a	0.0001
Sperm concentration (10 ⁶ mm ³)	256.41 \pm 1.84 ^d	271.25 \pm 1.27 ^c	283.66 \pm 1.56 ^b	303.08 \pm 1.80 ^a	0.0001
Total sperm (10 ⁶ / ejaculate)	163.66 \pm 1.22 ^d	194.62 \pm 1.05 ^c	212.26 \pm 1.32 ^b	250.56 \pm 2.14 ^a	0.0001

Each value represented the mean of 6 semen evaluations; C: Control group; T1, T2 and T3: Diet supplemented with 5, 10 and 15 g/kg of parsley seeds; a-f Values within rows followed by different letters differ significantly ($P < 0.05$).

*PSV = Packed sperm volume.

Table2: Effect of dietary supplementation with different levels of parsley seeds on seminal plasma traits (Mean \pm SE) of buck rabbits.

Traits	Treatments				P.Value
	Control	T1	T2	T3	
Cholesterol (mg/dl)	52.05 \pm 0.29 ^a	45.10 \pm 0.19 ^b	42.20 \pm 0.24 ^c	42.15 \pm 0.36 ^c	0.0001
LDL (mg/dl)	17.70 \pm 0.30 ^a	15.71 \pm 0.22 ^b	13.30 \pm 0.18 ^c	12.12 \pm 0.22 ^d	0.0001
HDL mg/dl)	19.30 \pm 0.23 ^c	20.87 \pm 0.21 ^b	24.45 \pm 0.16 ^a	24.50 \pm 0.14 ^a	0.0001
Total lipids (mg/dl)	145.50 \pm 0.64 ^a	141.75 \pm 0.25 ^b	141.25 \pm 0.40 ^b	130.25 \pm 0.40 ^c	0.0001
Triglyceride (mg/dl)	130.50 \pm 0.64 ^a	124.75 \pm 0.47 ^b	123.75 \pm 0.47 ^b	121.25 \pm 0.40 ^c	0.0001
*ALP (IU/L)	35.35 \pm 0.30 ^c	37.75 \pm 0.35 ^b	44.65 \pm 0.25 ^a	44.15 \pm 0.23 ^a	0.0001
**AST (U/L)	24.47 \pm 0.17 ^a	23.45 \pm 0.20 ^b	23.50 \pm 0.24 ^b	22.35 \pm 0.21 ^c	0.001
***ALT (U/L)	12.12 \pm 0.12 ^a	11.43 \pm 0.02 ^b	11.21 \pm 0.06 ^b	10.76 \pm 0.08 ^c	0.001
Fructose (mg/100ml)	199.85 \pm 1.62 ^c	224.05 \pm 1.67 ^b	242.70 \pm 1.66 ^a	243.50 \pm 1.14 ^a	0.0001
****TAC mmol/l	0.98 \pm 0.02 ^c	1.16 \pm 0.01 ^b	1.28 \pm 0.01 ^a	1.29 \pm 0.04 ^a	0.0001
*****MDA nmol/ml	5.52 \pm 0.11 ^a	4.63 \pm 0.04 ^b	4.34 \pm 0.04 ^c	4.33 \pm 0.02 ^c	0.0001

Each value represented the mean of 6 semen evaluations; C: Control group; T1, T2 and T3: Diet supplemented with 5, 10 and 15g/kg of parsley seeds; a-f Values within rows followed by different letters differ significantly ($P < 0.05$).

*ALP= alkaline phosphatase.

**AST= aspartate aminotransferase.

***ALT= alanine aminotransferase.

****TAC= total antioxidant capacity.

*****MDA= malondialdehyde.

Zheng *et al.*, (1992) reported that parsley oil is rich in myristicin which Showed a high activity as an inducer of the detoxifying enzyme glutathione S-transferase (GST) in the liver and small intestinal mucosa of female mice. Reduction of myristicin yielded dihydromyristicin that retained the GST-inducing activity. Fejes *et al.*, (1998) indicated that parsley oil contain flavonoids (apiin, luteolin-, apigenin-glycosides), essential oil (apiol, miriszticin), cumarines, (bergapten, imperatorin) and vitamin C. The protective role of parsley oil may be attributed to its higher content of these flavonoids which either scavenge free radicals or increase the production of GST. Ozsoy-Sacan *et al.*, (2006) concluded that, parsley extract probably, due to its antioxidant property, has a protective effect against hepatotoxicity caused by diabetes and have free radical scavenging and membrane protective effects (Fejes *et al.*, 2000). In the same regards, Nielsen *et al.*, (1999) indicated that treatment with parsley oil resulted in increased levels of glutathione reductase and SOD activity.

The protective effects of flavonoids may occur through inhibitory effect on CYP1A1/2 among CYP enzymes involved in ZEN metabolism by rat microsomes as well as the decreased DNA damage and activating the phase II enzymes glutathione S-transferase (GST) and GSH peroxidase (GSH-Px). These results suggest that parsley oil is capable of counteracting ZEN toxicity by suppressing cytochromes P-450 mediated bioactivation of the mycotoxin (Abdel-Wahhab and Aly, 2003, 2005). The increased level of testosterone reported in the current study accompanied with the increase of sperm counts is supported by the previous reports of (McDonald and Capen, 1989) who stated that the testosterone hormone promotes the growth development and secretory activity of the accessory sex organs of the male. Al-Janabi, (2014) reported that a various concentrations of parsley oil had inhibitory effect on the growth of *P. mirabilis*, *E. coli*, *S. Areas* and *p. Klebsiella sp*, 7×10^{-8} g/ml concentration was The more effective concentration on the various types of bacteria. The effect of all parsley oil concentrations

(0.07×10^{-2} , 0.07×10^{-4} , 0.07×10^{-5} , 7×10^{-6} g/ml) motile spermatozoa in seminal fluid on the number of infertile men was a significant ($P \leq 0.05$) increase when compared with the control. Where as the concentration 0.07×10^{-3} g/ml was a significant ($P \leq 0.01$) increase in the number of motile spermatozoa when compared with control. Tavalani *et al.*, (2014) studied the correlation between serum lipid concentrations and variations in seminal lipid parameters in infertile men. They found no relationship between the concentration of cholesterol, phospholipids and triacylglycerols in serum, spermatozoa or seminal plasma of the infertile men, which is consistent with the findings of several other authors (Grizard *et al.*, 1995). Grizard *et al.*, (1995) compared the effect of hypercholesterolemia and normocholesterolemia on the spermatozoa and seminal content of cholesterol and phospholipids. They suggested that hyper cholesterolemia has no effect on cholesterol and phospholipid levels in spermatozoa and seminal plasma (Grizard *et al.*, 1995). Since cholesterol has a major role in the sperm membrane, which is essential for sperm cell function, it can be assumed that an increase of cholesterol level in the blood will also increase the cholesterol content of semen. This hypothesis was not confirmed in the study of Tavalani *et al.*, (2014). Seminal plasma alkaline phosphatase (ALP) and AST showed significant ($P \leq 0.01$) reduction due to inclusion of different herbal seeds used in the present study and the decrease ALP and AST in comparison with the control group. These results get along with the finding of (Roussaland Stalleup, 1965) who found negative correlation between AST activity of seminal plasma and with each of ejaculation volume, sperm motility, sperm concentration and percent live cells. Also Chauban *et al.*, (1993) found a positive correlation between enzyme release and sperm acrosomal damage. The results presented in Table (2) showed significant increase ($P \leq 0.0001$) in seminal plasma fructose level and this increase due to parsley as compared with control group. In seminal vesicle, the seminal plasma is synthesized which is a medium for sperms. It consists of proteins, fructose,

mucus, vitamin c, flavins, phosphoryl choline and prostaglandins. The high fructose concentration provides nutrient energy for the spermatozoa (Wilke *et al.*, 2009), which reflects testosterone action and better quality of semen (Taha, 2008).

In the present study, seminal plasma total antioxidant capacity increased significantly ($P \leq 0.01$) as a result of feeding rabbits on diet containing parsley seed and this increase surpassed the control one by. Also, significant increase in total antioxidant capacity due to parsley seeds as compared with the control group. It could be observed that, parsley seed was more effective in increasing seminal plasma total antioxidant capacity. In support of this finding, lipid peroxidation (malondialdehyd) levels of rabbits fed parsley seed in their diet resulted in significant decrease as compared with the control group. In the present study, administration of parsley seeds through the experiment significantly improved semen quality, beside the obtained results showed that level of TAC was significantly increased and level of MDA was decreased ($P \leq 0.01$). The increase in sperm quality of parsley group in comparison to control group could be due to the protective effect of parsley seeds administration. This result suggests that parsley seeds may be promising in enhancing sperm healthy and consequently improve the reproductive performance of the male rabbits.

The improvement in semen quality traits due to treatment the rabbit bucks with parsley in traits of semen and seminal plasma may be explained by the supplementation of parsley seeds which is rich source of vitamins, minerals, essential fatty acids, and volatile oil components (Duke, 1997). Wichtl (1994) reported that parsley stimulates sexual activity in both men and women, stimulates the sensory nerves, and increases sexual wish. However, parsley may increase sexual activity through several mechanisms such as increase testosterone and other sexual hormones production and increase energy supply for reproductive organs. Al-Daraji (2002) found significant positive correlation between spermatozoa motility, spermatozoa concentration and spermatocrit. Moreover, Al-Daraji (2001a) noticed significant positive correlation between percentages of dead and abnormal spermatozoa. Al-Daraji (2001b) concluded that the highly significant negative correlation between numbers of spermatozoa and fructose concentration in seminal plasma suggests the utilization of glucose by spermatozoa. Al-Daraji (2002) indicated that spermatozoa utilize the fructose in their metabolism. (Al-Daraji *et al.*, 2001). The lowest seminal plasma activities of AST and ALT enzymes were obtained in semen of T1, T2, T3 and T4 groups. When sperm cell membrane damaged, AST and ALT enzymes are released into the extracellular medium (Al-Daraji *et al.*, 2002a). Al-Daraji *et al.* (2002b) reported significant correlation between seminal plasma activities of AST and ALT enzymes following cellular disruption. Brown *et al.* (1971) examined several enzymes and selected AST and ALT release as the best indicator of cellular damage. Buckland (1971) suggested that the observed increase in AST and ALT

activities of seminal plasma and semen during storage may be due to structural instability of the sperm. A positive correlation between activities of AST and ALT in seminal plasma and percentages of dead and abnormal spermatozoa (Al-Daraji *et al.* (2000), between ALP activity and spermatozoa concentration and liveability Al-Daraji (2002b), and between the amounts of ALP in the seminal plasma and the number of spermatozoa per ejaculate Al-Daraji *et al.* (2002a). Differences in seminal plasma ALP activity for different treatments included in this study closely resemble differences in spermatozoa liveability and concentration. Al-Daraji *et al.* (2001) reported that both of alkaline and acid phosphatase enzymes are involved in the metabolism of spermatozoa *via* the hydrolysis of carbohydrates. Cholesterol concentration in seminal plasma exhibited differences between treatment groups. Cholesterol concentration was highest in the semen samples of control group, whereas the lowest cholesterol concentrations were recorded for semen samples of T1, T2, T3 and T4 groups (Table 2). Gruenz and Deuticke (1974) suggested that higher cholesterol to phospholipids ratio of cells such as spermatozoa promotes higher degree of membrane cohesion and impermeability. Davis (1976) suggested that cholesterol in seminal plasma of rabbits may inhibit fertilization by inhibiting membrane fusion during the acrosome reaction as a result of its incorporation into the lipid bilayers. Ansah and Buckland (1982) reported that the phenotypic correlation of seminal plasma cholesterol with the fertility of frozen-thawed semen were negatively correlated as were the phenotypic correlation of seminal plasma cholesterol with fertility of fresh semen. In conclusion, the results of this study showed that supplementing parsley seeds in the diet of buck rabbits resulted in significant improvement in semen and seminal plasma characteristics. Therefore, parsley seeds can be used as a beneficial tool for improving reproductive performance of buck rabbits by inclusion this plant in their feeding program.

Conclusion

The results of this study showed that supplementing parsley seeds in the diet of buck rabbits resulted in significant improvement in semen and seminal plasma characteristics.

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