EFFECT OF DIET SUPPLEMENTED WITH PUMPKIN (*CUCURBITA MOSCHATA*) AND BLACK SEED (*NIGELLA SATIVA*) OILS ON PERFORMANCE OF RABBITS: 3- PRODUCTIVE AND REPRODUCTIVE TRAITS, PUBERTY, SEXUAL ACTIVITY AND SEMEN CHARACTERISTICS OF NEW ZEALAND WHITE MALE RABBITS

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Abstract

Forty New Zealand White (NZW) rabbit males aged 12 weeks and weighing 1540.80±22.16 g were equally and randomly divided into four groups (10 in each). All groups were fed a commercial pelleted diet and the first group was served as a control, while diet of the second group was supplemented with 5 g pumpkin seed oil/kg diet (PSO), the third group received 5 g black seed oil/kg diet (BSO), however the fourth group received 5 g from both PSO and BSO/kg diet (PSO+BSO). Results showed that male rabbits in PSO+BSO group showed the heaviest (P<0.05) live body weight (LBW), total weight gain (TWG), average daily gain (ADG), feed conversion ratio and performance index, followed by those in PSO or BSO groups, while males in control group had the light values. Male rabbits received diet supplemented with PSO+BSO combination reached puberty significantly (P<0.05) faster compared with control group, while PSO or BSO groups were intermediate with insignificant differences. Moreover, buck rabbits in PSO+BSO group showed the highest (P<0.05) scrotal circumference, testicular index, weights of testes, epididymis and sexual accessory glands (included seminal vesicles, prostate and the paired bulbourethral (Cowper's gland), plasma testosterone concentration at puberty and the short reaction time and latency period, followed by those in either PSO or BSO groups, however bucks in control group had the lowest values. Semen volume and gel, pH value and semen color score were significantly (P<0.05) higher in PSO+BSO group than that in control group, PSO or BSO groups had intermediate values with insignificant differences. Semen color tended to be milky in PSO+BSO and BSO groups and heavy watery in PSO and control groups. All semen characteristics differ significantly (P<0.05) among the different collection weeks with a tendency to improve with advancing age. The PSO+BSO group showed highly (P<0.001) significant of percentages of sperm motility, livability, sperm cell concentration and the lowest sperm abnormality percentage, followed by PSO or BSO groups, however the control group had the lowest values. All sperm characteristics were significantly (P<0.05) differ among the different collection weeks and improved with age progress. Bucks in PSO+BSO group recorded the highly (P<0.001) significant of total count, total motile, total live and total normal per ejaculate, followed by those in PSO or BSO groups, however, the lowest in control group. Also, all counts of spermatozoa were significantly (P<0.05) differ among the different collection weeks and tended to increase with age.

Keywords: *NZW male rabbits, pumpkin oil, black seed oil, growth, puberty, semen characteristics.*

INTRODUCTION

Rabbits can play a significant role in solving the problem of meat shortage in many parts of the world, due to their high reproduction potential, rapid growth rate, short generation interval, ample nutritional spectrum, limited vital space and ease of rearing (Rashwan and Marai, 2000).

Nickavar *et al.* (2003) reported that eight fatty acids (99.5%) and thirty-two compounds (86.7%) have been identified in the fixed and volatile oils of *Nigella sativa* seeds, respectively. The main fatty acids of the fixed oil were linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%). The major compounds of the volatile oil were trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%) and carvone (4.0%). In pumpkin seed oil (PSO), the saturated fatty acid content was 27.73% and comprises of 16.41% palmitic acid and 11.14% stearic acid. The unsaturated fatty acid value was 73.03% and consisting mainly of 18.14% oleic acid and 52.69% linoleic acid (Alfawaz, 2004). Up to 60.8%, of the pumpkin seed oil (PSO) is from the fatty acids, oleic (up to 46.9%), linolenic (up to 40.5%) and palmitic and stearic (up to 17.4%), the ratio of monounsaturated to polyunsaturated acids from 0.60 to 0.75 g (Nakiae *et al.*, 2006).

Fluted pumpkin seed oil (FPSO) has been reported to posses some essential properties (vitamin A, linoleic acid, oleic acid and alkaloids), which suppress lipid peroxidation, hence, improving testicular function and subsequently improves semen parameters (Akang *et al.*, 2010).

The reproductive performance of rabbit males fed on diets supplemented with *Nigella sativa* (NS) was studied by El-Tohamy *et al.* (2010), who reported that the use of equal quantities of Raphanus sativus, Eruca sativa and NS meals in place of 50% soybean protein meal, improved the semen characteristics and reduced free radicals in the seminal plasma of the bucks. The use of NS also gave the best results regarding volume, motile sperm percentage, sperm concentration per ml, total sperm per ejaculation, reaction time, latency period, total motile sperm and total functional sperm fraction. Daader *et al.* (2004) found that rabbits fed on a diet supplemented with either 5% NS or Trigonella foenum-graecum had a significantly improved libido, semen quality, fertility rate and weight at first mating, and a significantly lower age of first mating compared to the animals fed with a standard diet.

The objective of this study was to investigate the effect of feed additive of pumpkin and/or black seeds oils on growth performance, puberty, reproductive traits and semen characteristics of NZW male rabbits.

MATERIALS AND METHODS

The current work was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture during the period from January to May 2015.

1. Experimental animals and treatments:

Forty New Zealand White (NZW) rabbit males aged 12 weeks and weighing 1540.80±22.16 g were equally and randomly divided into four groups (10 in each). All rabbits were fed a commercial pelleted diet according to NRC (1977) recommendations and the first group was kept untreated to serve as a control, while the other three groups (second, third and fourth) were supplemented with either 5 g pumpkin seed oil/kg diet (PSO) or 5 g black seed oil/kg diet (BSO) and combination of 2.5 g PSO plus 2.5 g BSO/kg diet (PSO+BSO), respectively. The formulation and chemical composition of commercial pelleted diet are shown in Table (1). Chemical composition of commercial pelleted diet was determined in duplicate according to AOAC (2012).

2. Housing:

Rabbits were housed separately in individual cages (35x35x60 cm) of conventional universal galvanized wire batteries. All cages were equipped with feeding hoppers, which were made of galvanized steel sheets, and nipples for automatic drinking. The batteries were located in a well ventilated building. Rabbits in all treatment groups were kept under similar managerial system and environmental conditions.

3. Experimental procedure:

Live body weight and daily feed intake were recorded weekly for each male rabbit during the experimental period. Then, daily weight gain, feed conversion ratio and economic efficiency were calculated. Also, performance index (PI) was calculated according to North (1981) as given below:

PI = [final body weight (kg)/feed conversion ratio] × 100

Males of bucks considered reached puberty when the semen ejaculate contain sperms. The weight and age of rabbit bucks from each group at puberty were recorded and three male rabbits from each experimental group were randomly slaughtered (by bleeding). Genitalia organs were taken immediately after slaughter and dissections were performed. Weights of testes, epididymis and sexual accessory glands (seminal vesicles, prostate and the paired bulbourethral (Cowper's gland) were recorded. Scrotal circumference was measured by the method described by Boiti *et al*. (2005). Testicular index (length x width x depth) was calculated in cubic centimeters as recorded by Castellini *et al*. (2006).

Table 1. Formulation, chemical composition and calculated composition of commercial rabbit diet

Formulation (% on DM basis):			
Berseem hay	30	limestone	0.7
Wheat bran	16	Common salt	0.5
Soybean meal	20	Premix*	0.5
Yellow corn	20	DL-lysine	0.15
Barley grain	10	DL-methionine	0.15
molasses	2	Total	100
Chemical composition (% on DM basis):			
DM	88.72	NFE	57.41
OM	93.17	Ash	7.21
CP	17.24	NDF	26.47
CF	12.26	ADF	15.35
EE	2.82	ADL	3.81
Calculated composition (% on DM basis):			
Hemicellulose	11.12	Phosphorus	0.45
Cellulose	11.54	Sodium	0.23
Starch	20.94	Chlorine	0.53
Sugar	4.55	Magnesium	0.24
Lysine	0.98	Potassium	1.40
Methionine	0.42	DCP	12.94
Threonine	0.80	DE	2520 kcal/kg
Tryptophan	0.24	ME	2356 kcal/kg
Calcium	0.78		

* Each one kg of premix (minerals and vitamins mixture) contains vit. A, 20000 IU; vit. D3, 15000 IU; vit. E, 8.33 g; vit. K, 0.33 g; vit. B1, 0.33; vit. B2, 1.0 g; vit. B6, 0.33 g; vit. B5, 8.33 g; vit. B12, 1.7 mg; pantothenic acid, 3.33 g; biotine, 33 mg; folic acid, 0.83 g; choline chloride, 200 mg.

4. Blood sampling:

About 3 ml of blood samples were collected at puberty from the slaughtered rabbits into heparinized tubes. Plasma was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept at -20 ^{0}C until hormonal assay. Blood serum testosterone (T) hormone concentration of the male rabbits was determined using RIA kits (Immunotech, A Coulter Co., Czech Republic) in accordance with the manufacturer's information.

5. Semen collection:

At reaching sexual maturity, bucks were trained to mount a teaser doe. Reaction time (the moment of subjecting a doe to the buck until the completion of ejaculation estimated in seconds using stopwatch. The latency period was assessed as the period from the dismount of the buck to the second mount with complete ejaculation (measured in seconds). Semen was collected twice a week for 9 consecutive weeks after puberty from all buck groups using artificial vagina of rabbits as described by Boiti *et al.* (2005). Semen was collected before feeding at 8 a.m. Gel was removed immediately after ejaculate collection and semen was keep at 35-37 ^{0}C in water bath, then the collected semen was taken immediately to the laboratory.

6. Semen evaluation:

Immediately after collection, the pH cooperative paper ranging from 0 to 14 with one grade was used to calibrate the pH value. Semen color was classified into a scale of 1 (watery), 2 (milky) and 3 (creamy) as described by Zemjanis (1970). Semen volume without gel fraction and gel volume (ml) were measured by graduated collection test tube.

Sperm motility percentage was assessed according to Amman and Hammerstedt (1980). Sperm livability percentage was determined using eosin and nigrosine mixture stain according to Hackett and Macpherson (1965). Live spermatozoa were counted in the field of a total of 200 spermatozoa. Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x) according to the classification adopted by Blom (1983). Sperm cell concentration (SCC) was evaluated by Neubauer hemocytometer.

Total sperm output (TSO) as well as count of motile sperm (MSO), live sperm (LSO) and normal sperm (NSO) per ejaculate were calculated as the following:

TSO/ejaculate = ejaculate volume (ml) x SCC (sperm/ml)

MSO/ejaculate = TSO/ejaculate x sperm motility (%)

LSO/ejaculate = TSO/ejaculate x live sperm (%)

NSO/ejaculate = TSO/ejaculate x sperm normality (%)

Where: Sperm normality = 100 – sperm abnormality (%)

7. Statistical analysis:

Data were statistically analyzed using general linear models (GLM) procedures adapted by IBM SPSS 22 (2014) for user's guide with one-way ANOVA. Duncan test within SPSS program was done to determine the degree of significance level among means (Duncan, 1955).

RESULTS AND DISCUSSION

1. Growth performance:

Results of growth performance are presented in Table (2). Rabbits fed diet supplemented with PSO+BSO combination showed significantly ($P<0.05$) the heaviest final LBW, the highest total and daily weight gain, which reflected in the best feed conversion ratio and the highest performance index as compared to rabbits fed diet supplemented with PSO or BSO and the control diet. While, feed intake was nearly similar to the different groups and insignificantly affected by PSO and/or BSO supplementation. Moreover, the live body weight (LBW) increased linearly, however, the average daily gain (ADG) decreased linearly with advancing age of male rabbits from 12 to 30 weeks. Males fed diet supplemented with PS and BS oils combination showed the best results (Figs. 1&2). These results agreed with

those obtained by Ragab *et al.* (2013a,b) who reported that PS and/or BS oils supplementation for growing and doe NZW rabbits improved body weight gain, feed conversion ratio and performance index.

Table 2. Live body weight, body weight gain, feed conversion ratio and performance index (mean ± SE) for the different groups.

Item	Treatments			
	Control	PSO	BSO	PSO+BSO
Initial live body weight (g)	1539±45	1541±46	1540±46	1545±47
Final live body weight (g)	3864±80^{c}	4065±81^{b}	4018±81^{bc}	4253±86^{a}
Total weight gain (g)	2325±51^{c}	2524±54^{b}	2478±53^{bc}	2708±60^{a}
Average daily weight gain (g)	18.5±0.41^{c}	20.0±0.43^{b}	19.7±0.42^{bc}	21.5±0.48^{a}
Total feed intake (kg)	16.4±0.36	16.530.32	16.5±0.34	16.6±0.36
Feed conversion ratio (kg/kg gain)	7.1±0.09^{a}	6.6±0.06^{ab}	6.7±0.07^{ab}	6.1±0.04^{b}
Performance index (%)	54.8±1.06^{c}	62.0±1.16^{b}	60.3±1.13^{b}	69.5±1.31^{a}

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

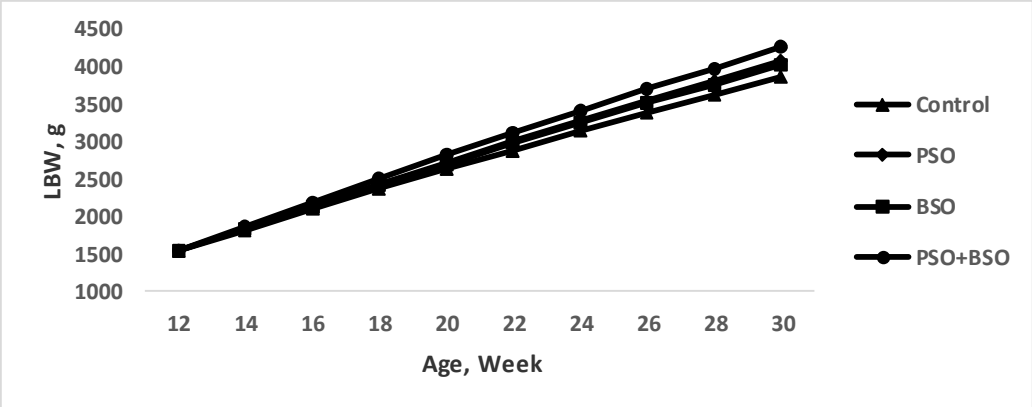

Fig. 1. Live body weight of the different groups of male rabbits during the different weeks of age.

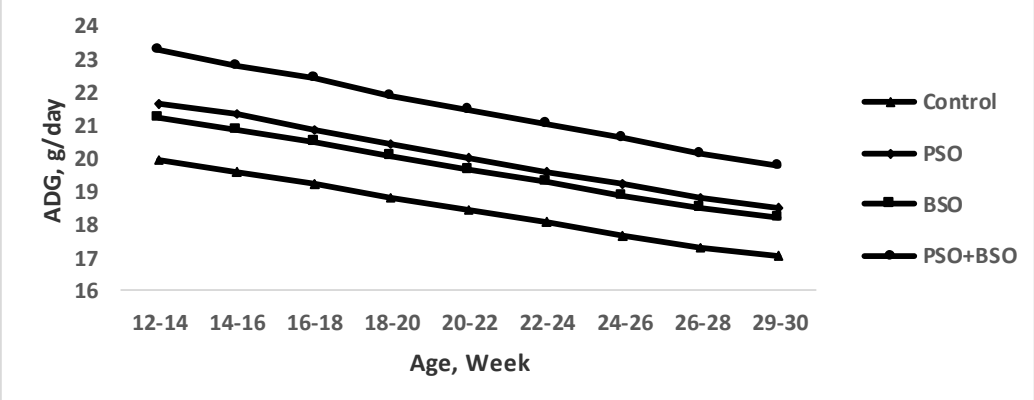

Fig. 2. Average daily weight gain of the different groups of male rabbits during the different weeks of age.

2. Reproductive performance:

Data of reproductive performance are shown in Table (3). Puberty in rabbits precedes the appearance of sperm in the ejaculate. Live body weight of bucks at puberty was mostly the same to the different groups with insignificant differences (P>0.05). However, male rabbits fed diet supplemented with PSO plus BSO combination was reached puberty (P<0.05) faster compared to the control group, while PSO or BSO groups were intermediate with insignificant differences. The shorter puberty age was recorded in PSO plus BSO bucks group being 142.70 days, however the oldest puberty age was 153.50 days in control group. These results could be related to the higher average daily gain obtained with PSO plus BSO group and also to the improving effect of PSO or BSO on testosterone concentration in buck blood serum that leads to fast maturity. Moreover, buck rabbits in PSO+BSO group showed significantly (P<0.05) the highest values of scrotal circumference and testicular index values at puberty, followed by those in PSO or BSO groups, however bucks in control group had the lowest values (Table 3). Rabbits are well known for their ability to reproduce quickly. Puberty in rabbits occurs between 4-6 months, and in smaller breeds it occurs earlier than in larger breeds (Harcourt-Brown, 2002). In rabbits, sexual maturity varies due to age (125-150 days), breed, lineage, food and environmental factors such as photoperiod, temperature and seasonality (Lebas *et al.*, 1997).

3. Genitalia organs weight:

The results given in Table (3) showed that the weights of testes, epididymis and sexual accessory glands included seminal vesicles, prostate and the paired bulbourethral (Cowper's gland) at puberty were significantly higher (P<0.05) in bucks received PSO plus BSO group, followed by those of PSO or BSO groups, however the lowest weights were noticed in control group. These results agreed with those obtained by Hashemi (2013) who found that combination of PSO and vit. E significantly increased testes weight in rats. Wahba (2011) reported that pretreatment with nigella sativa oil in rats induced significant increase in the weight of testis.

4. Plasma testosterone concentration:

The supplementation of pumpkin and/or black seed oils for male NZW rabbits increased significantly (P<0.05) plasma testosterone concentration as shown in Table (3). Group received PSO plus BSO recorded significantly (P<0.05) the highest testosterone concentration in blood plasma (5.23 ng/ml), followed by PSO group (4.88 ng/ml) and BSO group (4.74 ng/ml), however control group had the lowest concentration (4.44 ng/ml). These results agreed with those obtained by Hashemi (2013) who found that combination of PSO and vit. E significantly increased serum level of testosterone. Bashandy (2007)

reported that the testosterone level elevated significantly (P<0.01) in rats treated with *N. sativa*.

5. Sexual activity:

Sexual activity expressed as reaction time and latency period are shown in Table (3). Sexual activity improved significantly (P<0.05) in group received diet supplemented with PSO and/or BSO. Group received PSO+BSO diet recorded significantly (P<0.05) the shortest reaction time and latency period, followed by PSO or BSO groups, however control group had the longest values. The improved sexual activity of PSO+BSO group is parallel to the higher testosterone level of this group which reflected in higher libido and improved sexual performance. These results might be confirmed by the results of Cross and Roselli (1999) who suggested that testosterone acts synergistically with estradiol to stimulate male sexual behavior. Testosterone was found to acts in part through in situ conversion to estradiol by aromatase in the preoptic area stimulating mounting, ultimately improved the copulatory behavior. El-Tohamy *et al.* (2010) reported that the use of *Nigella sativa* gave the best results regarding reaction time and latency period. El-Nattat and El-Kady (2007) showed that the black cumin and the mixture diets gave the best results in case of reaction time and latency period in rabbits.

Table 3. Reproductive traits and internal genitalia organs weights (mean ± SE) of rabbit bucks at puberty.

Item	Treatments			
	Control	PSO	BSO	PSO+BSO
Weight at puberty (g)	2862±6.46	2867±2.61	2869±3.20	2859±5.50
Age at puberty (day)	153.5±3.62^{a}	148.2±3.40^{ab}	149.3±3.23^{ab}	142.7±2.98^{b}
Scrotal circumference (cm)	9.7±0.20^{c}	11.4±0.23^{b}	10.9±0.22^{b}	12.8±0.26^{a}
Testicular index (cm^3)	6.2±0.13^{c}	7.7±0.15^{b}	7.2±0.15^{b}	8.9±0.18^{a}
Testes (g)	11.2±0.23^{c}	12.6±0.25^{b}	12.1±0.24^{b}	13.6±0.28^{a}
Epididymis (g)	1.7±0.03^{c}	1.9±0.05^{b}	1.8±0.04^{b}	2.0±0.06^{a}
Sexual accessory glands (g)	6.2±0.12^{c}	6.7±0.13^{b}	6.6±0.13^{bc}	7.1±0.15^{a}
Testosterone (ng/ml)	4.4±0.01^{c}	4.9±0.02^{b}	4.7±0.03^{b}	5.2±0.04^{a}
Reaction time (sec.)	12.2±0.06^{a}	10.7±0.04^{b}	11.0±0.05^{b}	9.4±0.03^{c}
Latency period (sec.)	107.0±7.23^{a}	96.2±6.81^{ab}	98.6±6.46^{ab}	85.4±5.96^{b}

a, b, c: Values in the same row with different superscripts differ significantly (P<0.05).

6. Semen characteristics:

Data in Table (4) showed the effect of PSO and/or BSO supplementation was differ significantly (P<0.05) in all semen characteristics. Volume of semen and gel fraction was significantly higher (P<0.05) in group received PSO plus BSO than that in control group, while it was intermediate in PSO or BSO groups with insignificant differences. Moreover, semen pH value was significantly higher (P<0.05) in PSO plus BSO group than those in BSO and control groups, while it was intermediate in PSO group with insignificant difference. While, semen color score was significantly milky (P<0.05) in PSO plus BSO and BSO group compared to that heavy watery in PSO and control groups. Generally, values of semen volume, semen gel, pH values and semen color were the highest for bucks received diet supplemented with PSO plus BSO as compared to other treatments and control.

Concerning the effect of collection week, there were significant differences (P<0.05) in all semen characteristics among the different collection weeks with a tendency to improvements with advancing age. The highest semen volume was detected in week 5, the highest gel volume in weeks 3 and 4, the highest pH value in week 3 and the highest color score was in week 6 (Table 4).

Analysis of variance revealed that the effect of interaction between treatments and collection weeks was highly significant (P<0.05) on the studied semen characteristics (Table 4) and Figs. (3-6).

According to this study, the supplementation of PSO plus BSO combination had beneficial effects on semen volume and regulation of semen pH value toward neutrality by increasing gel volume in semen of buck rabbits and to some extent milky color.

The reproductive performance of rabbit males fed on diets supplemented with NS was studied by El-Tohamy *et al.* (2010), who reported that the use of equal quantities of Raphanus sativus, Eruca sativa and NS meals in place of 50% soybean protein meal, improved the semen characteristics. According to Daader *et al.* (2004), rabbits fed on a diet supplemented with either 5% NS or Trigonella foenum-graecum had significantly improved semen quality compared to the animals fed with a standard diet. Akang *et al.* (2010) found that fluted pumpkin seed oil (FPSO) supplemented at dose of 400 mg/kg live body weight of rates improves semen parameters. El-Nattat and El-Kady (2007) showed that semen characteristics revealed that the black cumin and the mixture diets gave the best results in case of volume.

Table 4. Semen characteristics (mean ± SE) of New Zealand White rabbit bucks.

Item	Semen volume (ml)	Semen gel volume (ml)	Semen pH value	Semen color
Treatments:				
Control	0.7±0.04^{b}	0.2±0.03^{b}	7.3±0.08^{b}	1.3±0.08^{b}
PSO	0.8±0.08^{ab}	0.3±0.03^{a}	7.6±0.13^{ab}	1.5±0.08^{b}
BSO	0.7±0.04^{b}	0.2±0.03^{b}	7.5±0.07^{ab}	1.8±0.11^{a}
PSO+BSO	0.9±0.05^{a}	0.3±0.03^{a}	7.8±0.09^{a}	1.9±0.13^{a}
Collection weeks:				
1	0.7±0.07^{bc}	0.3±0.04^{ab}	7.5±0.13^{bc}	1.5±0.16^{b}
2	0.7±0.08^{bc}	0.2±0.04^{bc}	7.5±0.14^{bc}	1.4±0.13^{b}
3	0.9±0.12^{ab}	0.4±0.04^{a}	7.9±0.21^{a}	1.6±0.13^{ab}
4	0.8±0.10^{abc}	0.3±0.04^{a}	7.8±0.15^{ab}	1.4±0.16^{b}
5	1.0±0.08^{a}	0.2±0.04^{bc}	7.8±0.13^{ab}	1.8±0.19^{ab}
6	0.8±0.07^{abc}	0.1±0.05^{c}	7.4±0.12^{bc}	2.0±0.18^{a}
7	0.6±0.05^{c}	0.3±0.05^{abc}	7.3±0.10^{c}	1.9±0.15^{ab}
8	0.6±0.05^{c}	0.3±0.04^{ab}	7.3±0.12^{c}	1.6±0.18^{ab}
9	0.7±0.06^{bc}	0.3±0.04^{ab}	7.5±0.12^{bc}	1.4±0.18^{b}
P-value:				
Treatments	0.049	0.043	0.015	0.005
Weeks	0.005	0.011	0.008	0.048
Interaction	0.028	0.049	0.038	0.034

a, b, c: Values in the same column for each item with different superscripts differ significantly (P<0.05).

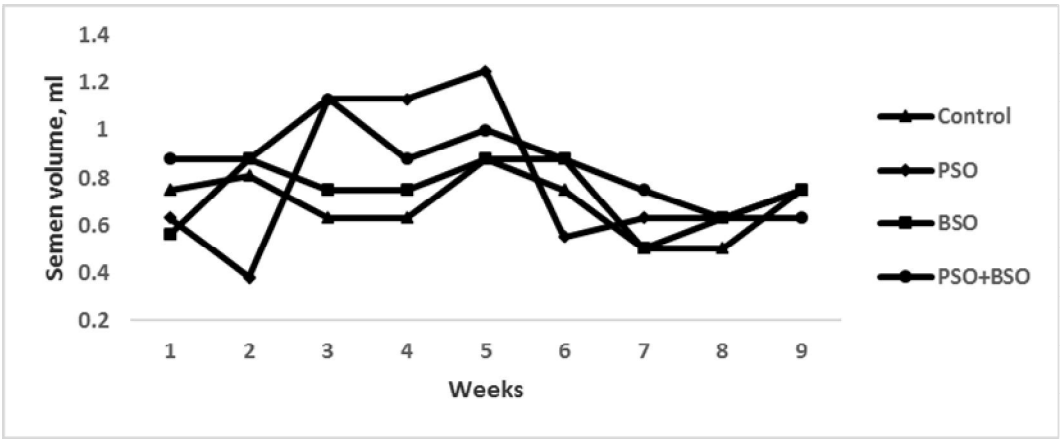

Fig. 3. Ejaculate semen volume of the different groups of male rabbits during the different collection weeks.

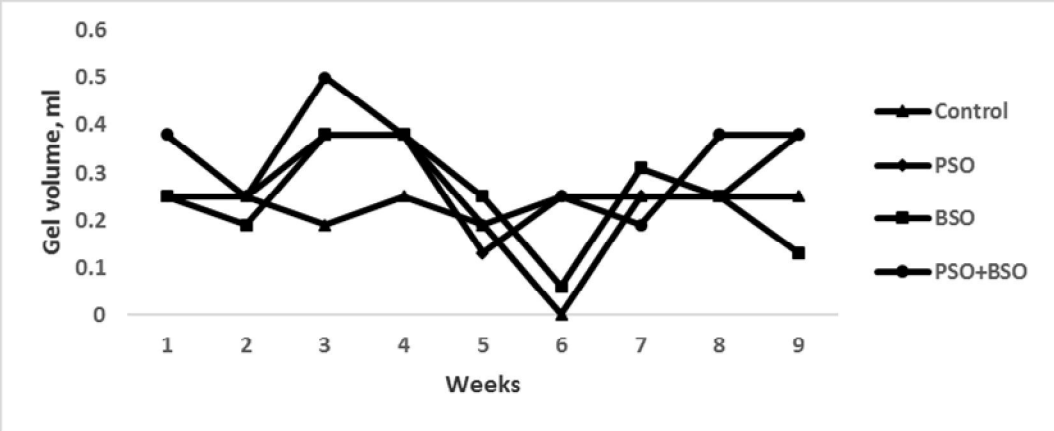

Fig. 4. Ejaculate gel volume of the different groups of male rabbits during the different collection weeks.

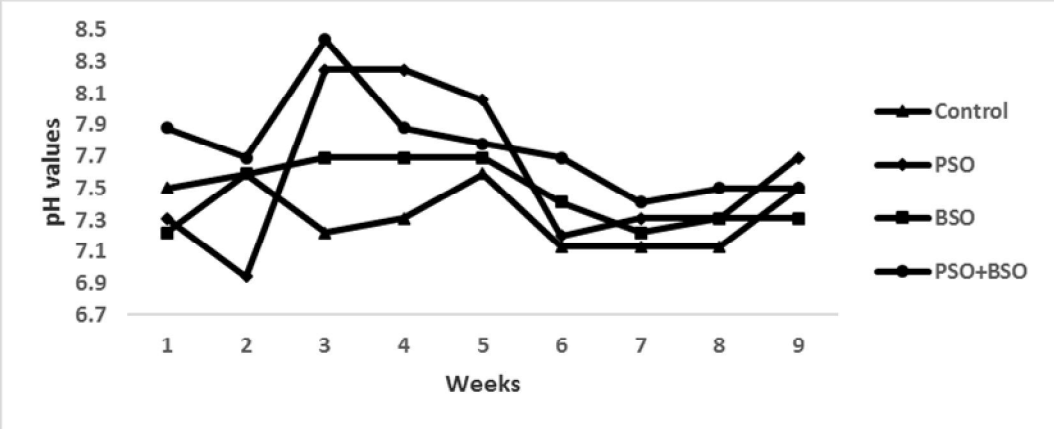

Fig. 5. Semen pH values of the different groups of male rabbits during the different collection weeks.

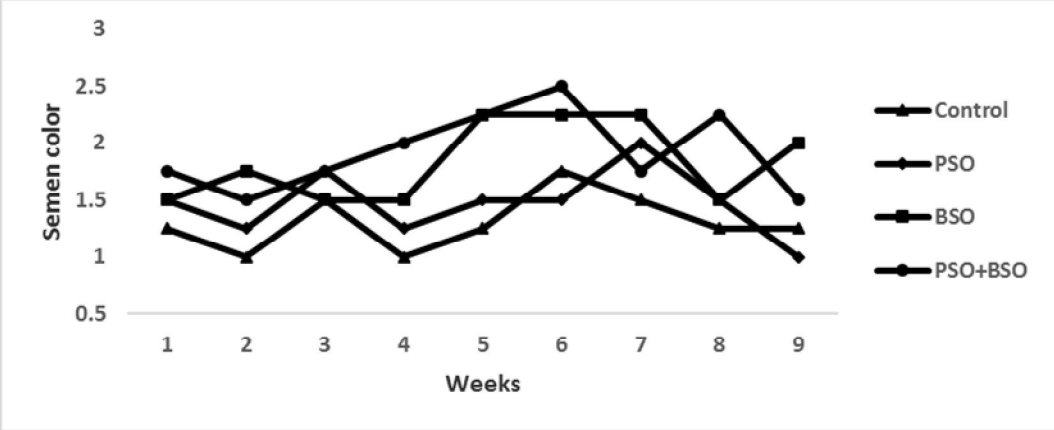

Fig. 6. Semen color of the different groups of male rabbits during the different collection weeks.

7. Sperm characteristics:

Results of sperm characteristics in Table (5) show that PSO and/or BSO supplementation effect significantly (P<0.001) the percentages of sperm motility, livability and abnormality as well as sperm cell concentration. The PSO plus BSO group showed significantly (P<0.001) the highest percentages of sperm motility and livability and sperm cell concentration and the lowest sperm abnormality percentage, followed by PSO or BSO groups, compared with control.

All sperm characteristics were significantly (P<0.05) differ among the different collection weeks and improved with age progress. The highest sperm motility was detected in weeks 6 and 8 and the highest sperm livability and sperm cell concentration was found in week 8. While, the highest sperm abnormality was recorded in weeks 1 and 2, however the lowest value was in week 6 (Table 5).

The interaction between treatments and collection weeks was highly significant (P<0.001) on all sperm characteristics (Table 5). The significant effect of this interaction reflected in the highest sperm motility and livability as well as sperm cell concentration and the lowest sperm abnormality in semen of bucks fed diet supplemented with PSO plus BSO group at all collection weeks (Figs. 7-10).

These results indicated that PSO and/or BSO supplementation to buck diet had beneficial effects on sperm characteristics and this improvement was observed by advancing age.

Table 5. Sperm characteristics (mean ± SE) of New Zealand White rabbit bucks.

Item	Motility (%)	Livability (%)	Abnormality (%)	Concentration (x 10^6/ml)
Treatments:				
Control	65.8±1.70^{c}	77.7±0.19^{c}	15.0±0.41^{a}	310.6±14.33^{c}
PSO	74.7±1.21^{b}	79.9±0.59^{b}	11.1±0.24^{b}	356.1±10.98^{b}
BSO	72.4±1.57^{b}	80.1±0.80^{b}	12.1±0.31^{b}	338.5±13.14^{bc}
PSO+BSO	81.3±1.47^{a}	84.8±1.00^{a}	7.3±0.39^{c}	426.5±14.79^{a}
Collection weeks:				
1	67.5±3.65^{b}	80.8±1.39^{ab}	12.3±1.14^{a}	312.3±25.76^{c}
2	71.6±2.53^{ab}	80.3±1.32^{ab}	11.9±1.03^{a}	329.5±18.90^{bc}
3	73.8±2.26^{ab}	79.6±0.64^{b}	11.6±0.70^{abc}	369.5±21.40^{abc}
4	72.2±2.09^{ab}	79.0±0.61^{b}	11.7±0.66^{abc}	328.7±18.39^{bc}
5	74.4±2.81^{ab}	80.5±1.30^{ab}	11.3±0.83^{abc}	372.2±24.76^{abc}
6	77.2±2.33^{a}	81.5±1.20^{ab}	10.2±0.91^{c}	382.7±24.06^{ab}
7	74.4±2.28^{ab}	79.8±0.94^{b}	11.3±0.79^{abc}	371.5±18.95^{abc}
8	77.5±2.70^{a}	83.7±1.68^{a}	10.3±0.99^{bc}	402.9±23.22^{a}
9	73.4±2.36^{ab}	80.3±1.56^{ab}	11.8±0.61^{ab}	350.7±22.38^{abc}
P-value:				
Treatments	0.001	0.001	0.001	0.001
Weeks	0.049	0.047	0.044	0.033
Interaction	0.002	0.003	0.001	0.001

a, b, c: Values in the same column for each item with different superscripts differ significantly (P<0.05).

The reproductive performance of male rabbits fed on diets supplemented with NS was studied by El-Tohamy *et al.* (2010), who reported that the use of NS gave the best results regarding motile sperm percentage and sperm concentration. Daader *et al.* (2004) found that rabbits fed on a diet supplemented with either 5% NS or Trigonella foenum-graecum had a significantly improved semen quality compared to the animals fed with a standard diet. El-Nattat and El-Kady (2007) showed that the semen characteristics revealed that the black cumin and the mixture diets gave the best results in case of motile sperm percentage and sperm concentration. Fluted pumpkin seed oil (FPSO) supplemented at a dose of 400 mg/kg live body weight of rates increased sperm count (Akang *et al.*, 2010). Hashemi (2013) showed that combination of PSO and Vit E significantly increased sperm motility, count and vitality and decreased sperm cell abnormalities in rats.

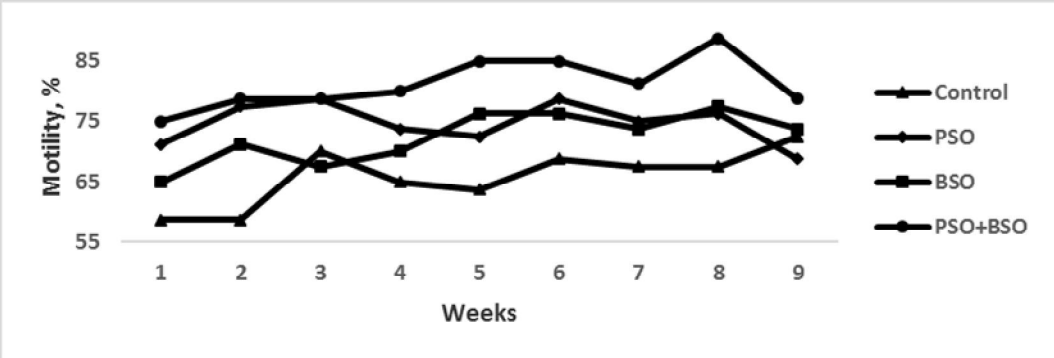

Fig. 7. Sperm motility of the different groups of male rabbits during the different collection weeks.

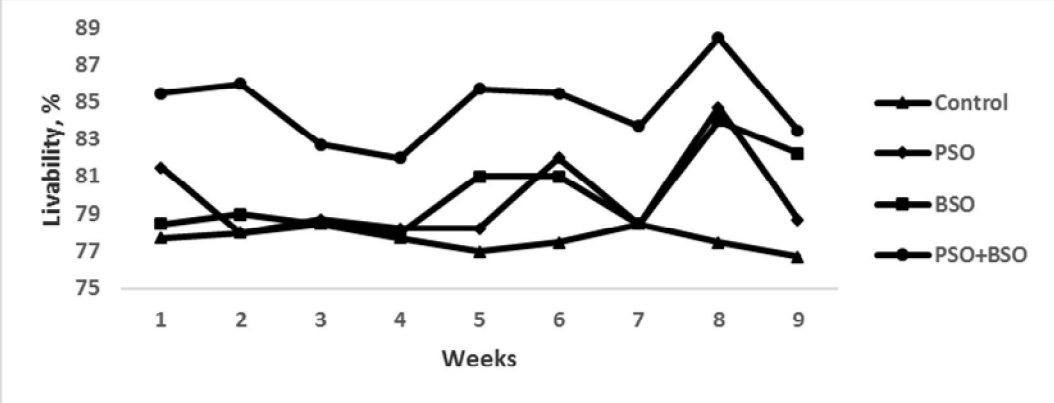

Fig. 8. Sperm livability for the different groups of male rabbits during the different collection weeks.

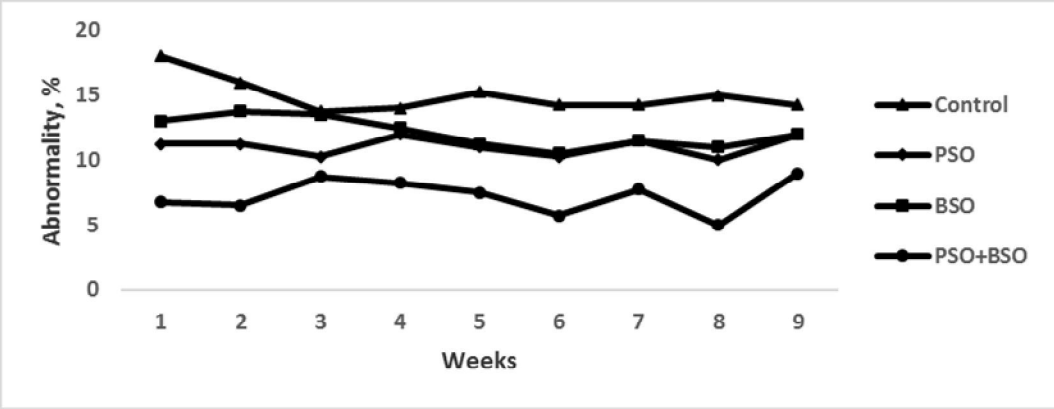

Fig. 9. Sperm abnormality of the different groups of male rabbits during the different collection weeks.

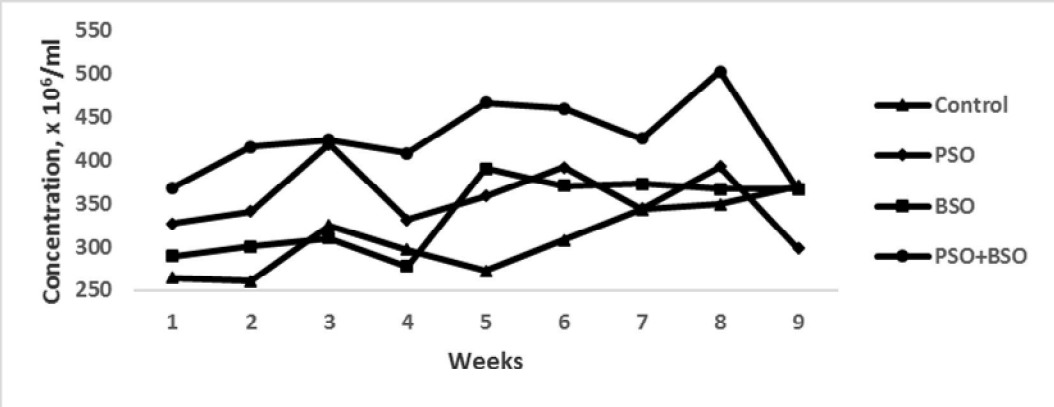

Fig. 10. Sperm cell concentration of the different groups of male rabbits during the different collection weeks.

8. Total sperm output:

Results in Table (6) show that sperm output in terms of total count, total motile, total live and total normal per ejaculate was affected significantly ($P<0.001$) by PSO and/or BSO supplementation. Which, bucks of PSO plus BSO group recorded ($P<0.001$) the highest total count, total motile, total live and total normal sperm per ejaculate, followed by those in PSO or BSO groups, however, the lowest values in control group.

Also, data in Table (6) show that all traits of spermatozoa were significantly ($P<0.05$) differ among the different collection weeks and have a trend to increase with advancement age. The total motile, total live and total normal sperm per ejaculate increased gradually with the advancing collection week, which was the highest in week 8 (Table 5).

Table 6. Total sperm output (mean ± SE) of New Zealand White rabbit bucks.

Item	Sperm output (x 10^6)/ejaculate			
	Total count	Total motile	Total live	Total normal
Treatments:				
Control	210.1±9.36^{c}	143.5±9.39^{c}	163.5±7.48^{c}	179.7±8.58^{c}
PSO	281.9±10.92^{b}	214.0±11.00^{b}	226.1±9.65^{b}	251.4±10.26^{b}
BSO	255.3±12.11^{b}	190.5±13.77^{b}	207.3±12.43^{b}	225.4±11.47^{b}
PSO+BSO	359.0±17.69^{a}	299.3±18.85^{a}	309.5±18.25^{a}	334.9±17.58^{a}
Collection weeks:				
1	242.4±25.26^{b}	176.8±27.71^{b}	200.6±25.21^{b}	216.1±25.34^{b}
2	255.2±21.19^{ab}	189.0±20.78^{ab}	208.0±21.14^{ab}	227.8±21.67^{ab}
3	285.4±23.13^{ab}	216.4±21.62^{ab}	228.9±20.00^{ab}	254.5±22.23^{ab}
4	254.2±19.71^{ab}	189.0±19.18^{ab}	202.2±17.17^{ab}	226.1±19.01^{ab}
5	289.8±26.58^{ab}	225.4±28.19^{ab}	238.0±26.13^{ab}	260.1±25.95^{ab}
6	298.5±28.49^{ab}	239.3±30.13^{ab}	248.0±27.69^{ab}	271.1±28.36^{ab}
7	285.9±19.79^{ab}	218.4±20.73^{ab}	230.1±18.61^{ab}	255.9±20.00^{ab}
8	310.1±24.34^{a}	249.0±27.70^{a}	264.1±25.35^{a}	281.3±24.90^{a}
9	267.8±21.55^{ab}	203.3±25.02^{ab}	219.7±23.79^{ab}	237.6±20.97^{ab}
P-value:				
Treatments	0.001	0.001	0.001	0.001
Weeks	0.049	0.036	0.041	0.038
Interaction	0.001	0.001	0.002	0.001

a, b, c, d: Values in the same column for each item with different superscripts differ significantly (P<0.05).

The interaction between treatments and collection weeks was highly significant (P<0.001) on total output characteristics (Table 5). The significant effect of this interaction reflected in the highest total count, total motile, total live and total normal sperm per ejaculate of bucks in PSO plus BSO group increased with advancing collection period up to week 8 (Figs. 11-14).

These results indicated that PSO and/or BSO supplementation for diet of buck rabbits had beneficial effects on total sperm output and this improvement was observed by advancing age.

The reproductive performance of rabbit males fed on diets supplemented with NS was studied by El-Tohamy *et al.* (2010), who reported that the use of NS gave the best results regarding total sperm per ejaculation, total motile sperm and total functional sperm fraction. El-Nattat and El-Kady (2007) showed that the semen characteristics revealed that the black cumin and the mixture diets gave the best results in case of total sperm per ejaculate, total motile sperm and total function sperm fraction.

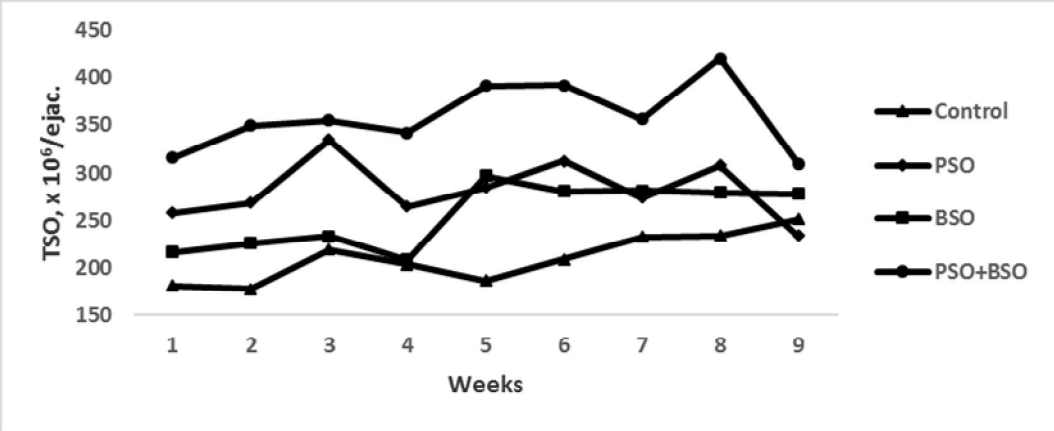

Fig. 11. Total sperm output for the different groups of male rabbits during the different collection weeks.

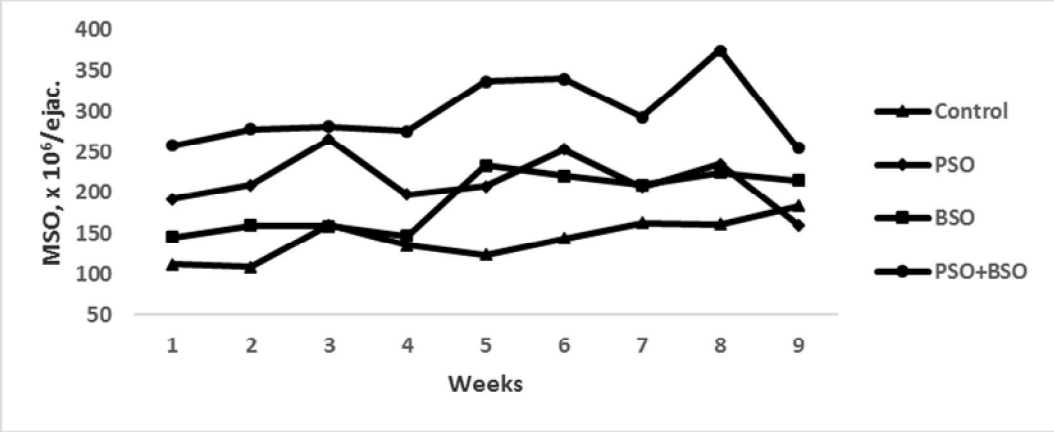

Fig. 12. Total motile sperm output for the different groups of male rabbits during the different collection weeks.

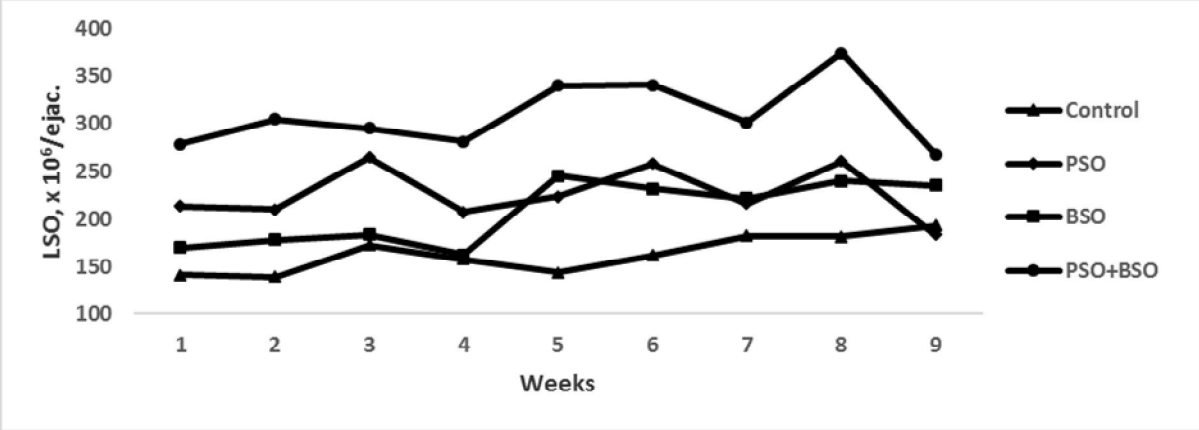

Fig. 13. Total live sperm output for the different groups of male rabbits during the different collection weeks.

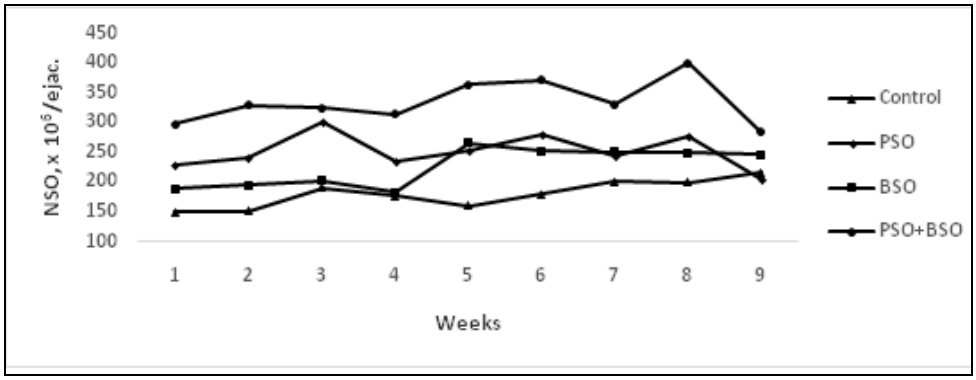

Fig. 14. Total normal sperm output for the different groups of male rabbits during the different collection weeks.

CONCLUSION

From the previous results, it could be concluded that male rabbits fed diets supplemented with a combination of pumpkin and black seeds oils (2.5 g PSO plus 2.5 g BSO/kg diet) improve growth performance, feed conversion ratio, reproductive traits, puberty, sexual activity, semen and sperm characteristics and total sperm output.

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تأثير اضافة زيوت بذور اليقطين وحبة البركة فى العليقة على أداء الأرانب ٣- الصفات الانتاجية والتناسلية، البلوغ، النشاط الجنسى وخصائص السائل المنوى لذكور الأرانب النيوزيلندى البيضاء

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أجريت هذه الدراسة على ٤٠ من ذكور الأرانب النيوزيلندى البيضاء عمر ١٢ أسبوع ومتوسط وزنها ١٥٤٠.٨٠±٢٢.١٦ جم وزعت عشوائيا الى أربعة مجاميع متساوية (١٠ فى كل مجموعة)٠ غذيت جميع الأرانب على عليقة الأرانب المكعبات التجارية بدون اضافة فى المجموعة الأولى (مجموعة المقارنة) أو مع اضافة ٥ جم زيت بذور اليقطين/كجم عليقة فى المجموعة الثانية (زيت بذور اليقطين)، ٥ جم زيت حبة البركة/كجم عليقة فى المجموعة الثالثة (زيت حبة البركة)، ٢.٥ جم زيت بذور اليقطين + ٢.٥ جم زيت حبة البركة فى المجموعة الرابعة (زيت بذور اليقطين + زيت حبة البركة)٠

أظهرت ذكور مجموعة خليط زيوت بذور اليقطين وحبة البركة معنويا (عند مستوى ٠.٠٥) أعلى قيم لكل من وزن الجسم، الزيادة الكلية واليومية فى الوزن، معدل التحويل الغذائى و دليل الأداء، تلاها مجموعة زيت بذور اليقطين ومجموعة زيت حبة البركة، بينما كانت أقل القيم مع ذكور مجموعة المقارنة٠

انخفض عمر البلوغ معنويا (عند مستوى ٠.٠٥) فى ذكور مجموعة خليط زيوت بذور اليقطين وحبة البركة بالمقارنة مع ذكور مجموعة المقارنة، بينما كانت مجموعة زيت بذور اليقطين ومجموعة زيت حبة البركة وسط بينهما بدون أى اختلافات معنوية٠ علاوة على ذلك حققت ذكور مجموعة خليط زيوت بذور اليقطين وحبة البركة معنويا (عند مستوى ٠.٠٥) أعلى قيم لمحيط كيس الصفن، دليل الخصية، ووزن كل من الخصيتين، البربخ، الغدد الجنسية الملحقة (الحويصلات المنوية، البروستاتا وغدة كوبر) وتركيز التستيسترون فى البلازما عند البلوغ وقصر المدة من دخول الأنثى حتى القذف والفترة بين وثبتين، تلاها مجموعة زيت بذور اليقطين ومجموعة زيت حبة البركة، بينما أظهرت مجموعة المقارنة قيما مضادة٠

ارتفاع حجم السائل المنوى والجل وقيمة درجة الحموضة ودرجة اللون معنويا (عند مستوى ٠.٠٥) فى مجموعة خليط زيوت بذور اليقطين وحبة البركة عنه فى مجموعة المقارنة وكانت وسط فى مجموعة زيت بذور اليقطين ومجموعة زيت حبة البركة بدون اختلافات معنوية٠ كما لوحظ اختلافات معنوية فى كل صفات السائل المنوى بين أسابيع الجمع المختلفة مع الميل الى التحسن مع تقدم العمر٠

سجلت ذكور مجموعة خليط زيوت بذور اليقطين وحبة البركة معنويا (عند مستوى ٠.٠٥) أعلى نسبة مئوية للحيوانات المنوية المتحركة والحية وتركيز الحيوانات المنوية وأقل نسبة مئوية للحيوانات المنوية الشاذة، تلاها مجموعة زيت بذور اليقطين ومجموعة زيت حبة البركة، بينما أظهرت مجموعة المقارنة قيما مضادة٠ كذلك وجد اختلافات معنوية فى صفات الحيوانات المنوية بين أسابيع الجمع المختلفة وتحسنها مع تقدم العمر٠

وجدت زيادة معنوية (عند مستوى ٠.٠٥) فى اجمالى عدد الحيوانات المنوية الكلية والمتحركة والحية والطبيعية فى القذفة فى ذكور مجموعة خليط زيوت بذور اليقطين وحبة البركة، تلاها مجموعة زيت بذور اليقطين ومجموعة زيت حبة البركة، بينما كانت أقل القيم فى مجموعة المقارنة٠ كذلك وجد اختلافات معنوية فى تعداد الحيوانات المنوية بين أسابيع الجمع المختلفة وميلها الى الزيادة مع تقدم العمر٠

نستخلص من هذه الدراسة أن اضافة خليط زيوت بذور اليقطين وحبة البركة (٢.٥ جم + ٢.٥ جم/ كجم عليقة) فى عليقة ذكور الأرانب النيوزيلندى البيضاء حسنت معدل النمو، معدل التحويل الغذائى، صفات التناسل، البلوغ، النشاط الجنسى، خصائص السائل المنوى والحيوانات المنوية واجمالى الحيوانات المنوية الكلية فى القذفة٠