ISSN 1819-1878

Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2020.44.53

Research Article
Effect of Oral Administration of Wheat (*Triticum aestivum* L.) Germ, *Eruca sativa* Mill. Seed Oils and their Mixture on Semen Evaluation of V-line Rabbit Bucks

[1]Sabrin A. Morshedy, [2]Mohamed Basyony, [1]Ahmed Abdelmodather, [1]Soliman A. Zahran and [1]Mohamed A. Hassan

[1]Department of Fish and Animal Production, Faculty of Agriculture Saba Basha, Alexandria University, Egypt
[2]Animal Production Research Institute, Agriculture Research Center, Dokki, Egypt

Abstract

Background and Objective: Different natural feed additives have been used to maintain semen quality characteristics of rabbit bucks at heat stress. This study was carried out to investigate the effect of administration of vegetable oils on the semen quality of male rabbits. **Materials and Methods:** Twenty adult V-Line rabbit bucks 6-7 months old, with average initial body weight (BW) 2792.40±66.39 kg were randomly distributed into four experimental groups (n = 5). The control group administered with 1.0 mL of distilled water, the second group received rocket seed oil (0.3 mL kg^{-1} b.wt., Rocket Seed Oil (RSO)), the third group received wheat germ oil (0.3 mL kg^{-1} b.wt., Wheat Germ Oil (WGO)) and the fourth group received 0.15 mL of RSO and 0.15 mL of WGO kg^{-1} b.wt., mixture. The rabbits were given oils orally once daily for 8 weeks under summer conditions (27.50°C and 76% relative humidity). **Results:** Feed intake of rabbits decreased ($p = 0.0001$) with orally received Rocket Seed Oil (RSO) and didn't differ with Wheat Germ Oil (WGO) or their mixture compared to the control group. Body weight change was tended to increase in rabbits received RSO compared to the control group. The treatments with oils significantly improved semen quality compared to the control group. **Conclusion:** The oral administration of RSO, WGO and their mixture improved semen quality characteristics under summer conditions induced heat stress.

Key words: Rocket seed oil, Wheat Germ Oil (WGO), semen evaluation, productive performance, rabbit bucks, motile sperm

Citation: Sabrin A. Morshedy, Mohamed Basyony, Ahmed Abdelmodather, Soliman A. Zahran and Mohamed A. Hassan, 2020. Effect of oral administration of wheat (*Triticum aestivum* L.) germ, *Eruca sativa* Mill. seed oils and their mixture on semen evaluation of V-line rabbit bucks. Asian J. Anim. Sci., 14: 44-53.

Corresponding Author: Mohamed Basyony, Animal Production Research Institute, Agriculture Research Center, Dokki, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In fact, the proper fertility in mammals depends on good semen quality[1] which affected by inappropriate environmental conditions[2]. Heat stress is one of the most environmental with adverse effects on the reproductive functions of male rabbits[3] throughout increasing levels of free radicals and disrupting the antioxidant-defence system[4,5]. Therefore, different natural feed additives have been used to maintain semen quality characteristics of rabbit bucks through heat stress period[6]. Vegetable oils such as; olive oil, rice bran oil, corn germ oil and wheat germ oil have been used as a source of plant antioxidants[7,8].

The rocket (*Eruca sativa*) seed contains up to 25-35% oil[9]. Rocket seed oil (RSO) constitutes of 17.84% total saturated fatty acids and 82.16% of total unsaturated fatty acids, which contain (alpha-linolenic acid 19.34%, oleic acid 15.53% and linoleic acid 13.67%), 49.09% mono unsaturated fatty acids and 33.01% polyunsaturated fatty acids[10]. Moreover, RSO composed of some important fatty acids, mainly erucic acid, which is present in large amounts in RSO[9,11]. The RSO is characterized by a high content of sulfur and nitrogen-containing compounds[12].

Moreover, *E. sativa* has been known as a rich source of health-promoting phytochemicals including vitamins (A, C and K), thiamin, riboflavin, niacin, vitamin B-6 (pyridoxine), pantothenic acid[13], carotenoids (16.2-275 μg g^{-1}) mainly lutein[14], glucosinolates and isothiocyanates, the hydrolytic product of glucosinolates[15], flavonoids (the main phenolic compounds, 4.68-31.39 g kg^{-1} dry weight) such as; flavonols, kaempferol, quercetin and isorhamnetin[16].

In addition, RSO has a hypocholesterolemia effect, this may be due to the high percentage of unsaturated fatty acids[17,18]. Furthermore, RSO promotes the regeneration of hepatic tissue, decrease hepatic lipid and possess a potent free radical scavenging[19], inhibit of melanoma tumour growth in mice[20]. In addition, the supplementation of RSO significantly improved semen quality, including ejaculate volume, percentages of motility, live sperm, sperm concentration, total sperm output and sperm concentration in rabbit bucks[21], in male goat[22], in ram lambs[23].

The wheat (*Triticum aestivum*) germ contains about 8-14% oil[24]. Wheat Germ Oil (WGO) is rich in triglycerides (57% of total lipids), which, consists of total unsaturated and polyunsaturated fatty acids (PUFA) about 64-81%[25,26]. The WGO includes the following fatty acids 56% of linoleic acid (omega-6), 16% of palmitic acid, 14% of oleic acid and 7-9% of alpha-linolenic acid (omega-3) as reported by Megahad and El Kinawy[27] and Niu *et al.*[28]. Moreover, WGO has a high content of bioactive compounds, including α-tocopherol (vitamin E, 1300-2700 mg kg^{-1}), carotenoid (56 mg kg^{-1}), policosanols and phytosterols[29-31].

The WGO has several nutritional and health benefits such as reducing plasma and liver cholesterol levels, improving body fitness, lowering diabetic, reducing oxidative stress, delaying the effects of ageing and enhancing fertility[32,33]. Also, the dietary supplementation of WGO increased body weight of male broilers[34]. Moreover, the supplementation of wheat germ 4 g/rabbit significantly increased ejaculate volume, pH, mass motility, individual motility and live sperm in rabbits[35].

Therefore, the objective of the present study was to investigate the effects of RSO, WGO and their mixture on productive and reproductive performance of buck rabbits under Egyptian summer condition.

MATERIALS AND METHODS

Animals and experimental design: The experimental work was carried out at the Rabbit Research Laboratory belonging to The Animal and Fish Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt. This study was carried out during the Egyptian summer and early autumn seasons (from 15 July-1 October, 2018). The daily mean temperature during the experimental period was 27.50°C and average relative humidity was 76%. According to the data obtained from the records of a nearby Meteorological Station, the experimental rabbits exhibited to heat stress.

The study investigated the effects of wheat (*Triticum aestivum* L.) germ, rocket (*Eruca sativa* Mill.) seed oils and their mixture on semen evaluation of V-line rabbit bucks. Twenty adults, fertile V-Line rabbit bucks 6-7 months old, with average initial body weight 2792.40±66.39 kg were randomly distributed into 4 experimental groups (n = 5) as follows:

- First group served as negative control administered 1.0 mL distilled water orally once daily (T1)
- Second group received rocket seed oil at level 0.3 mL kg^{-1} b.wt., (T2)
- Third group received wheat germ oil at level 0.3 mL kg^{-1} b.wt., (T3)
- Forth group received a mixture of 0.15 mL of rocket oil and 0.15 mL of wheat germ oil per kilogram BW (T4)

Two local genotypes of wheat germ and rocket seed oils were obtained from El Medina Factory for natural seeds extract at Borg El Arab belong to the governorate of Alexandria, Egypt. Rabbits were given oils once daily via gavage (oral administration). The experimental treatments lasted for 10 consecutive weeks, including 2 weeks for adaptation and the later 8 weeks for semen evaluation and data collection. The basal ration was formulated and pelleted to meet the nutrient requirements of rabbits according to National Research Council[36]. The rations were offered to rabbits *ad libitum*. Rabbits were offered free access to fresh water. All bucks were kept under similar managerial and environmental condition. Bucks were individually housed in galvanized wire cage batteries (50×50×40 cm) provided with feeders and automatic drinkers in a naturally ventilated and lighted Rabbitry.

Feed intake and body weight: The feed intake was calculated bi-weekly by subtracting the unconsumed feed from the total amount offered during this period and then daily feed consumption was calculated by dividing the bi-weekly feed intake by 14 days. Buck rabbits were weighed bi-weekly in the morning before offering a feed.

Semen collection and evaluation: Semen samples were weekly (weeks 3-10) collected during the experimental trial artificial vagina used to collect semen with exposing four mature females as a teaser handling and collection of semen carried out according to Boiti *et al.*[37].

Ejaculate volume was recorded using a graduated collection tube connected to the end of the artificial vagina after removal of the gel mass. After that, the ejaculate volume (mL) was measured. The pH value was determined by using pH paper (Merck KgaA, 64271 Darmstadt, Germany). The percentage of progressive motile sperm (percentage of forward motility) was immediately performed after semen collection in several microscopic fields for individual semen samples by visual examination under 100 magnifications using a light microscope with heated stage and subjectively assessed from 0-100%. Sperm concentration (10^6 mL^{-1}) was determined after semen dilution (1:100) using the improved Neubauer hemocytometer slide (GmbH and Co, Brandstwiete 4, 2000 Hamburg 11, Germany).

Semen mass motility was given a score (0-3) according to Moule[38]. A dried smear of a drop of each semen ejaculates stained with an eosin-nigrosin blue staining mixture was prepared to assess the percentage of sperm viability (live or dead) and sperm abnormality by counting 200 sperm cells. The sperm cells were classified according to the staining pattern into complete or partial purple-stained sperm cells and non-stained sperm cells representing dead and live sperm cell, respectively. Packed sperm volume was measured by using Micro-AID® microhematocrit tubes and microhematocrit-centrifuge which centrifuged for 5 min at 4000 rpm.

All visual semen parameters i.e., sperm progressive motility, sperm viability and sperm morphology were determined by the same trained technician according to Salisbury and Van Demark[39]. The previous measurements were used to calculate the Total Sperm Output (TSO, 10^6/ejaculate) as a product of semen ejaculate volume (mL) by sperm concentration (10^6 mL^{-1}); total motile sperm (TMS, 10^6/ejaculate) as a product of TSO by percentage of progressive motility and Total Functional Sperm Fraction (TFSF, 10^6/ejaculate) as a product of TMS by percentage of normal sperm morphology.

Statistical analysis: Data were statistically analyzed using SPSS 11.0 statistical software[40]. Data were analyzed by using the two-way repeated measures Analysis of Variance (ANOVA). The statistical model included the fixed effect of treatment (control, rocket seeds oil, wheat germ oil and their mixture), time of sampling/data collection and the interactions, also the random effect of an individual buck was considered. Differences among means were determined using Duncan[41] test. Statistical significance was accepted at $p<0.05$.

RESULTS

Feed intake and body weight of V-line rabbit bucks: The present results showed that the oral incorporation of 0.3 mL of RSO per kilogram BW caused ($p = 0.0001$) decreasing feed intake of rabbit bucks compared with other groups (Table 1). However, the oral administration of 0.03 mL of WGO per kilogram BW or their mixture (0.15 mL of RSO plus 0.15 mL WGO per kilogram BW) didn't differ feed intake of rabbit bucks compared to the control group. Body weight of rabbit bucks treated with RSO and WGO decreased ($p = 0.03$) in

Table 1: Live body weight (g) and daily feed intake (g) of rabbit bucks administrated orally with Rocket Seed Oil (RSO) and Wheat Germ Oil (WGO) or their mixture (Means±SE)

	Treatments				p-value		
Items (g)	Control	Rocket seed oil	Wheat germ oil	Mixture	T	W	T*W
Daily intake	138.22±1.61[a]	131.58±1.24[b]	141.09±2.12[a]	142.37±1.85[a]	0.00	0.03	0.58
Body weight	3270.84±66.29[a]	3068.72±71.05[b]	3070.40±69.12[b]	3149.96±63.64[ab]	0.03	0.00	1.00
Body change	146.45±13.50[ab]	170.10±17.00[a]	162.00±15.53[ab]	128.55±21.90[b]	0.05	0.00	0.02

[a-b]Means with a different superscript in the same row are significantly different (p<0.05), T: RSO, W: WGO, T*W: Mixture

Table 2: Semen characteristics of rabbit bucks given orally Rocket Seed Oil (RSO) and Wheat Germ Oil (WGO) and their mixture (Means±SE)

	Treatments				p-value		
Items	Control	Rocket seed oil	Wheat germ oil	Mixture	T	W	T*W
Ejaculate volume (mL)	0.44±0.01[c]	0.60±0.02[a]	0.48±0.01[c]	0.54±0.02[b]	0.00	0.42	0.88
pH	7.73±0.07	7.88±0.05	7.73±0.07	7.78±0.07	0.38	0.21	1.00
Mass motility (%)	2.60±0.05[c]	2.90±0.03[a]	2.78±0.04[b]	2.85±0.04[ab]	0.00	0.00	0.61
Progressive motility (%)	73.83±0.57[c]	82.38±0.74[a]	77.13±0.53[b]	77.73±0.63[b]	0.00	0.00	0.99
Normal sperm morphology (%)	89.85±0.32[b]	91.00±0.26[a]	90.33±0.29[a]	90.60±0.23[a]	0.03	0.03	0.91
Abnormal sperm morphology (%)	10.15±0.32[a]	9.00±0.26[b]	9.68±0.29[b]	9.40±0.23[b]	0.03	0.03	0.91
Live sperms (%)	74.05±0.73[c]	80.18±0.72[a]	75.75±0.66[c]	77.88±0.59[b]	0.00	0.00	0.68
Dead sperms (%)	25.95±0.73[a]	19.83±0.72[d]	24.25±0.66[b]	22.13±0.59[c]	0.00	0.00	0.68

[a-c]Means with a different superscript in the same row are significantly different (p<0.05), T: RSO, W: WGO, T*W: Mixture

Table 3: Semen parameters of rabbit bucks given orally Rocket Seed Oil (RSO) and Wheat Germ Oil (WGO) and their mixture (Means±SE)

	Treatments				p-value		
Items	Control	Rocket seed oil	Wheat germ oil	Mixture	T	W	T*W
Concentration, 10^6 mL^{-1}	339.75±6.58[c]	396.50±9.96[a]	346.75±5.87[bc]	357.00±7.96[b]	0.00	0.00	0.00
Packed sperm volume (%)	43.92±13.89[b]	47.44±15.00[a]	41.72±13.19[b]	43.68±13.81[b]	0.01	0.00	0.75
[#]Total sperm output (10^6/ejaculate)	148.84±5.78[c]	237.35±10.00[a]	164.79±5.21[c]	191.89±6.62[b]	0.00	0.00	0.44
[∞]Total motile sperm (10^6/ejaculate)	110.63±4.73[d]	197.21±9.36[a]	127.13±4.09[c]	149.73±5.62[b]	0.00	0.00	0.17
[&]Total functional sperm fraction (10^6/ejaculate)	99.56±4.36[d]	179.72±8.70[a]	115.83±3.85[c]	135.49±4.99[b]	0.00	0.00	0.14

[a-d]Means with a different superscript in the same row are significantly different (p<0.05), [#]Total sperm output (TSO, 10^6/ejaculate): Semen ejaculate volume (mL)×sperm concentration (10^6 mL^{-1}), [∞]Total motile sperm (TMS, 10^6/ejaculate): Total sperm output (10^6/ejaculate)×progressive motility (%)/100 , [&]Total functional sperm fraction (TFSF, 10^6/ejaculate): Total motile sperm (10^6/ejaculate)×Normal sperm morphology (%)/100

comparison with the control group. While there weren't any difference observed in body weight in the group of rabbits received the mixture of RSO and WGO compared to other groups. Body weight change was (p = 0.05) higher in rabbits received RSO than the rabbits orally administrated with the mixture of RSO and WGO, while it didn't differ compared with WGO and control groups.

Semen characteristics of V-line rabbit bucks: Table 2 and 3 summarized the impact of oral administration of RSO, WGO or their mixture on some semen characteristics under summer condition. The current results showed that semen ejaculate volume and percentages of mass motility, progressive motility, normal sperm morphology, total motile sperm and total functional sperm fraction were significantly higher in rabbits received RSO, WGO and their mixture compared to the control group. Moreover, live sperms percentage, sperm concentration and total sperm output increased significantly in rabbits received RSO and their mixture compared to WGO and control groups. Also, the percentage of packed sperm volume was significantly higher in rabbits received RSO compared to other groups.

While, percentages of abnormal sperm morphology and dead sperms decreased significantly in rabbits received RSO, WGO and their mixture compared to the control group. However, semen pH showed a non-significant effect between treatments, however, the results showed numerically increase in pH values with RSO and their mixture treatments compared to WGO and control groups. Generally, the best values of semen evaluation were recorded in the group of bucks received 0.3 mL of RSO as compared to the control or the other experimental groups. The mean effect of experimental weeks was significant in feed intake, body weight, body weight changes and most semen evaluation parameters (Table 1). However, the interaction between treatments and weeks were significant with body weight change and insignificant with feed intake, body weight of V-line rabbits (Table 1).

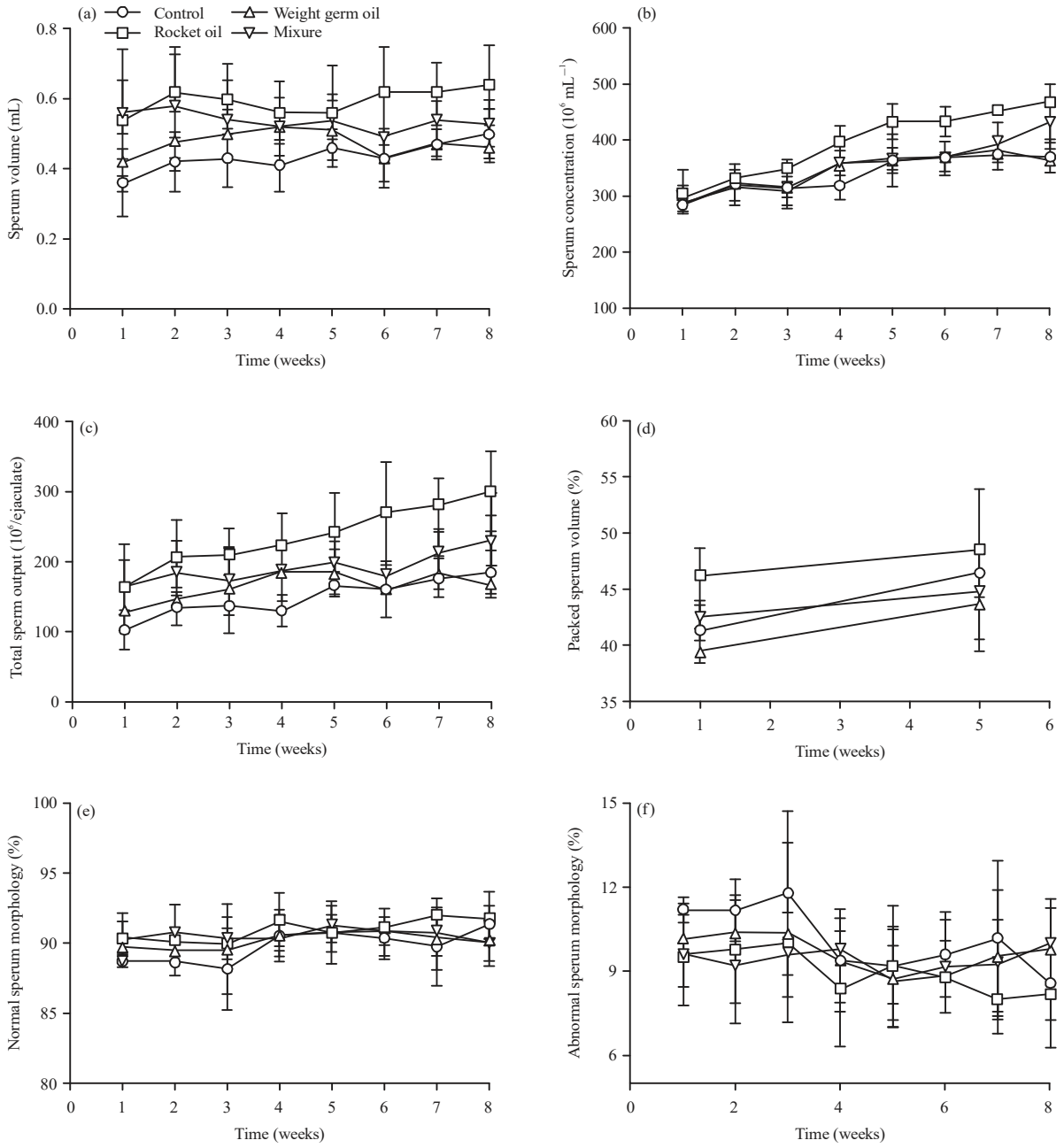

Fig. 1(a-f): Evaluation parameters of V-line rabbit bucks, (a) Sperm volume, (b) Sperm concentration, (c) Total sperm output, (d) Packed sperm volume, (e) Normal sperm morphology and (f) Abnormal sperm morphology

Figure 1-2 showed the interaction effect between treatments and weeks on semen evaluation parameters. There was a time-dependent increase in semen ejaculate volume, sperm concentration, percentage of packed sperm volume, total sperm output and normal sperm morphology during the 7 weeks of the experiment by oral administration of RSO, WGO and their mixture (Fig. 1a-e). While there was a time-dependent decrease in the percentage of abnormal sperm morphology (Fig. 1f). Moreover, there was a time-dependent increase in percentages of mass and progressive motility, total motile sperm, total functional sperm fraction and live sperm during the 7 weeks of the experiment by oral administration of RSO, WGO and their mixture (Fig. 2a-e). While there was a time-dependent decrease in percentage of dead sperm (Fig. 2f).

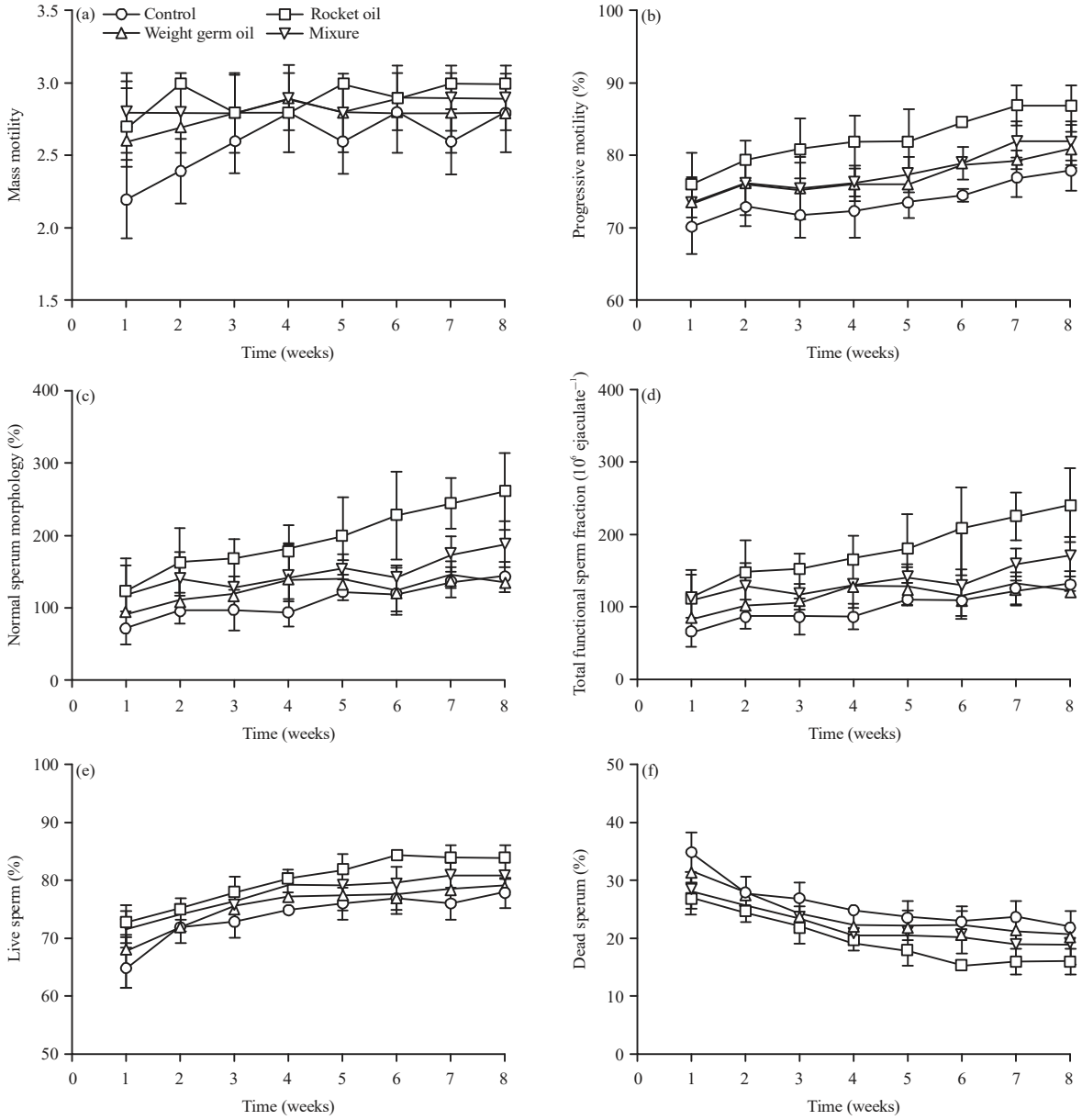

Fig. 2(a-f): Evaluation parameters of V-line rabbit bucks, (a) Mass motility, (b) Progressive motility, (c) Total motile sperm, (d) Total functional sperm fraction, (e) Live sperm and (f) Dead sperm

DISCUSSION

The present results showed that feed intake significantly decreased with the oral incorporation of 0.3 mL of RSO per kilogram BW in rabbit bucks compared with other groups. An important implication of these findings is that RSO has large amounts of erucic acid, which has unpalatable flavor, thus reducing feed intake[11].

Contrary to current findings, Ibrahim[42] and Abdel-Azeem *et al.*[43] found that a diet supplemented with 1% rocket seed caused an increase of feed intake compared to the control group. This inconsistency with the present results may be due to reducing the active components in 1% rocket seed compared to 0.3 mL of rocket seed oil. Body weight of rabbit bucks treated with RSO and WGO decreased ($p = 0.03$) in comparison with the control group. Body weight change was ($p = 0.05$) higher in rabbits received RSO than the rabbits orally administrated with the mixture of RSO and WGO, while it didn't differ compared with WGO and control groups. There is a lack of information in the literature on the effects of RSO and WGO supplementation on body weight and body weight change of rabbit bucks.

The present study showed that the oral administration of RSO, WGO or their mixture improved semen characteristics under summer condition. The current findings are in harmony with Ahmed *et al.*[44] who found that the addition of water-cress (*Nasturtium officinale*) in male rabbits significantly improved semen ejaculation volume compared to the control group. In addition, semen quality, including ejaculate volume, percentages of motility, live sperm, sperm concentration and total sperm output and sperm concentration was significantly improved by supplementation of RSO in rabbit bucks[21], in male goat[22] and in ram lambs[23].

Generally, rocket seed considered a good source for improving, semen quality and quantity and fertility[43,45,46]. Moreover, Hanafi *et al.*[47] investigated that RSO in rabbit ameliorates the harmful effect of aflatoxin, which disturbed blood biochemical constituents and semen characteristics and pathological changes of liver, kidney and testes.

Also, sperm ejaculation volume, pH, mass motility, individual motility and live sperm were significantly increased in rabbits fed diets supplemented with 4 g wheat germ/rabbit compared to those of rabbits fed a diet containing 2 g wheat germ and the control group[35]. Moreover, Okab *et al.*[48] found that buck rabbits fed diets containing vegetable oils (soybean oil, n-3, sunflower oil, n-6 and mixture with them) could improve sperm concentration, total sperm output, percentage of motile sperm, in addition, to depress percentages of dead sperm than the control group control bucks.

This improvement could be attributed to containing RSO and WGO on several active components. Whereas, RSO consists of total unsaturated fatty acids 82.16%, which contain α-linolenic acid 19.34%, oleic acid 15.53% and linoleic acid 13.67%, monounsaturated fatty acids 49.09% and polyunsaturated fatty acids 33.01%[10].

Furthermore, WGO consists of total unsaturated and polyunsaturated fatty acids (PUFA) about 64-81%[25,26]. Moreover, WGO has a high content of bioactive compounds, including α-tocopherol (vitamin E, 1300-2700 mg kg^{-1}), carotenoid (56 mg kg^{-1}), policosanols and phytosterols[29-31].

This hypothesis was confirmed by Al-Dain and Jarjeis[49] who reported that rocket seeds have some nutritional compounds, like vitamins E and C, linoleic and arachidonic acids, which are essential fatty acids playing as important roles for testosterone activity. Consequently, polyunsaturated fatty acids (PUFA) may improve animal reproduction and fertility[50]. These results agree with Adibmoradi *et al.*[51], who found that n-3 PUFA rich diet significantly boosts the growth of testis tissues of growing male kids. Also, the dietary supplementation of PUFA influences the testis development and hormonal secretion consequently, decreases the period of puberty in the male buffalo via improving the expression and affinity of gonadotropin receptors, the phospholipid composition of testis plasma membranes and the rate of testosterone synthesis[52] and also increasing plasma IGF-1 concentration[52-54]. As it's known that the increase in the synthesis of testosterone caused regulating in the spermatogenesis process[55].

Overview, the dietary supplementation of n-6: n-3 PUFA increases sperm concentration, total and progressive motility, velocity, viability and normality in male buffalo, boars, rabbit bucks and Friesian bulls[52,56-58]. This is associated to increase the fluidity and integrity of spermatozoa plasma membrane[52]. Nerveless, Tran *et al.*[52] have addressed not only the effect of dietary supplementation of omega 3 PUFA on fresh semen quality in male buffalo, but also on post-thawing. They found that the changing of fatty acid composition in the plasma membrane of sperm alleviates sperm damage through increasing the resistance of the sperm membrane against damage.

Supplementary vitamin E improves semen quality in poultry[59], rams[60] and rabbits[61-63]. This may due to vitamin E is the main component of the antioxidant system of spermatozoa and plays a crucial role in protecting sperm membrane, which contains high concentrations of PUFA within the phospholipids, against reactive oxygen species and lipid peroxidation[64,65].

High ambient temperature promotes the hypothalamus-pituitary-adrenal axis activity stimulating the sympathetic system functions, consequently, increasing levels of free radicals and disrupting the antioxidant-defence system[66,67]. Accumulations of the free radicals have been resulted in significant lack in sperm motility and increasing sperm plasma membrane disorder, sperm abnormality and DNA damage leading to infertility[68]. These effects were alleviated in the current study by oral administration of RSO, WGO and their mixture. It shows clearly through the improvement of semen quality during the 7 weeks of the experiment under summer conditions in Egypt.

CONCLUSION

The oral administration of RSO, WGO and their mixture improved semen evaluation parameters under heat stress conditions. The best values of semen evaluation were recorded in the group of bucks received 0.3 mL of RSO as compared to the control or the other experimental groups.

Further studies could be evaluated the effect of the studied oil on growth performance and feed utilization of rabbits as an anti-heat stress factor.

SIGNIFICANCE STATEMENT

This study discovered that the possible synergistic effect of RSO and WGO combination can be beneficial for improving the semen quality of rabbits under summer conditions. This study will also help the researchers to uncover the critical areas of affecting vegetable oils on growth performance and feed utilization of rabbits as an anti-heat stress factor that many researchers were not able to explore. Thus, a new theory of these vegetable oils combinations and possibly other combinations, may be arrived at.

ACKNOWLEDGMENT

The authors acknowledge the Rabbit Research Laboratory and Animal Nutrition Laboratory, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt for providing the infrastructure and staff necessary for this study.

Authors also thankful to the Asian Journal of Animal Sciences for publishing this article FREE of cost and to Karim Foundation for bearing the cost of article production, hosting as well as liaison with abstracting and indexing services and customer services.

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