Effect of *Eruca sativa* Seeds on Semen Quality in Lohmann Roosters

Abstract

Background and Objective: Poultry industry plays a very important role in improving the standard of life worldwide due to its concern to fight hunger. Improving the fertility, hence, productivity is a main goal in poultry production. This study was designed to investigate the effect of *Eruca sativa* (Rocket salad) on semen quality in cockerels' chicken. **Material and methods:** The *Eruca sativa* seeds were provided to fifteen cocks of Lohmann chicken breed (age seven month) at a concentration of 0.0% (control), 0.01%, and 0.02% as feed additive for nine weeks. The cocks' sperm were collected using an abdominal massage technique. The semen volume, concentration, motility, live/dead ratio and normal/abnormal ratio were determined weekly over the experiment period. Data were statistically analyzed by one-way ANOVA test using Statistical Package for Social Science (SPSS version 16). **Results:** Concentration of 0.02% *Eruca sativa* significantly ($p < 0.05$) increased semen volume, motility, live/dead ratio. The other characteristics were also numerically increased in response to this concentration. Although, it was not significantly different from control group, 0.01% of *Eruca sativa* improve semen characteristics. **Conclusion:** *Eruca sativa* improved the reproductive potentiality in cock by optimizing the semen quality. Generally, 0.02% *Eruca sativa* improved semen quality better than 0.01%.

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Key words: cockerels, *Eruca sativa*, feed, fertility, powder seeds, semen quality

Introduction

Eruca sativa is one of the medicinal plants commonly known as salad rocket, originated in the mediterranean region and western Asia. It is also found in tropical Africa and South Africa. It has several antioxidant constituents including glucosinolates, flavonoids, carotenoids, vitamin C and volatile oils like myristicin and apiole [1]. *Eruca sativa* seeds restore normal blood value, improve semen motility in rabbits, and reduce the toxic pathological effects of toxins on liver and kidneys [2]. The extracts of leaves and seeds of *Eruca sativa* have diuretic effects and increase excretion of Na^+, K^+ and Cl^- in dogs [3]. The antihepatotoxic properties of *Eruca sativa* has been also confirmed in rats; liver function improves under a diet supplemented with its ethanolic extract [4]. In central Asia, *Eruca sativa* seed oil, called "taramira or jamba oil", and it is used for massage [5], hair treatment, and as a medicine against influenza [6]. Historically, *Eruca sativa* has been used in Egyptian folk medicine as a lactagogue, aphrodisiac, diuretic, antimicrobial and to disintegrate renal calculi and induce vomiting for long time[7]. Noteworthy, *Eruca sativa* has been shown to have anti-cancer activities [8].

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On the other hands, the administration of low dose of *Eruca sativa* seed oil causes dilatation of the seminiferous tubules, proliferation of spermatogenic cell with an increase in its mitotic activity, increase number of sperms and epididymis weight, elevation of testosterone hormone level and hyperplasia of interstitial Leydig cells in rats [7, 8]. Also, it is found to increase the percentage of haploid and decreases diploid and tetraploid cells [7]. In addition, Eruca sativa causes a considerable rise in three important sexual activity indices in rats: penis licking, attempt

to mount the female, and successful mounting and ejaculation [9]. Recently, the poultry production companies in the Sudan start to import breeders, and some of them use artificial insemination to ensure fertilization from less numbers of cockerels. So, they try to enhance the fertility as production improvement policy. Although, *Eruca sativa* has been shown to improve the fertility in mammals, its effects on poultry fertility need excessive studies due to lack of information. Therefore, this study has been designed to investigate the effects of *Eruca sativa* on semen quality in cocks as part of the attempts for improving the productivity in poultry.

Materials and Methods

Experimental Birds and Management

This study was conducted at the University of Khartoum's poultry production unit in Sudan. The experiment was conducted in a disinfected open poultry barn divided into three identical $1m^2$ pens. In this study, fifteen Lohmann cocks (aged seven months) were used. The Eruca sativa seeds were collected from a local market, crushed, and added to a basal diet as a feed supplement to create three experimental diets containing 0.00 percent, 0.01 percent, and 0.02 percent *Eruca sativa* seeds, respectively. As a litter, wood shavings were used, and the pens were equipped with clean feeders and drinkers, as well as 24 hours of light. The three experimental diets were distributed evenly among the three prepared pens at random (5 cocks per each treatment).The experiment continued for 9 weeks. Cocks were trained by massage technique for 4 weeks to get semen before starting semen evaluation. Feed and water were provided *ad libitum* consumption and semen was collected once a week.

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Feeding

The basal diet's formula is described in Table 1, and its chemical composition is shown in Table 2. The chemical composition of *Eruca sativa* is presented in Table 3.

Semen Collection

The semen collection was accomplished by abdominal massage technique [10]. Each cock was massaged at the back and stroked close to its tail. Meanwhile, the inseminator applied a slight finger pressure around the base of the tail. Pressure applied around the cloacae and the tail flattened towards the back of the bird, causing the phallus protrudes from the cloacae. The inseminator's thump was then pressed just behind the bird's vent on the bird's abdomen. Semen was released from the ductus deferens as a result of this. The inseminator gently squeezed the semen into a conical graded collection tube from the enlarged papillae at the base of the phallus.

Semen Volume

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Semen volume was measured with the use of a collection tube graduated in ml. The volume of the semen was recorded to the nearest 0.1ml.

Semen motility

A drop of semen with the aid of a micropipette was placed on a microscope slide and placed on a microscope for examination. The percentage of cells that motile under their own power in a semen sample is known as motility. **Semen concentration**

The semen concentration was measured using the direct cell count method; the loaded hemocytometer was then placed on the microscope with 40 X objective. The spermatozoa's head that falls within the subdivided smaller squares at the four edges and center of the hemocytometer was counted and the average per replicate was found.

Semen live/dead ratio

The percentage of dead spermatozoa was estimated by preparing stained film from semen sample. The staining solution used was Eosin-Nigrosin stain. The smears were examined microscopically using the oil immersion lens (×100), the spermatozoa with red stained head were classified as dead, while unstained spermatozoa were considered as viable.

Semen normal/abnormal ratio

The same stained slide used to calculate the dead and live sperms was examined for abnormal morphology. Data were presented as percentage of the number of the spermatozoa.

Statistical Analysis

The experimentation work of this study was carried out under complete randomized design. The values of the measured parameters were analyzed with one-way ANOVA, using the computer program SPSS, version (16.0). The results were given in terms of mean ± standard error, after the differences between means were statistically assessed, using Duncan multiple range test. $P<0.05$ was considered as significant difference.

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RESULTS

Semen volume

As shown in Figure 1, 0.02% *Eruca sativa* significantly ($p < 0.05$) increased semen volume at 2nd and 4th weeks of the experimental period. In 1st, 3rd, 5th, 6th and 7th weeks there were no significant ($p < 0.05$) differences among experimental groups.

At 8th and 9th weeks of the experimental period there was clear increase in semen volume in 0.02% *Eruca sativa* experimental group, however, this increase was not significant.

Semen Concentration

Date in Figure 2 reveals that the semen concentration did not show any significant difference in response to *Eruca sativa* treatment during the experimental period. Nevertheless, 0.02% *Eruca sativa* increased the semen concentration during the 2^{nd}, 4^{th} and 8^{th} weeks but this increase was not statistically significant.

Semen Motility

The effect of *Eruca sativa* on semen motility has been shown in Fig. 3. Although it reflected that 0.02% *Eruca sativa* increased significantly ($p < 0.05$) the semen motility only at the 8^{th} week, the general pattern of the figure showed that 0.02% group exhibited numerical increase in semen motility in comparison to the other experimental groups over the period extended from the 2^{nd} week till the end of the experimental period. Moreover, the motility in 0.01% group was numerically higher than that of the control one.

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Semen normal/abnormal ratio

The normal/abnormal sperms ratio increased significantly ($p < 0.05$) during the 5^{th} week using 0.02% *Eruca sativa* concentration. However, the ratio increased numerically in 0.02% *Eruca sativa* group over the whole experimentation period (Fig.4)

Semen live/dead ratio

Fig.5 shows that 0.02% *Eruca sativa* significantly ($p<0.05$) increased the live/dead semen ratio during the 1^{st} and 9^{th} weeks. Although it was not significant during the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 8^{th} weeks, it was numerically higher in this group compared to the other experimental groups. Noteworthy, there was a sharp increase in the life/dead ratio during the 7^{th} week.

Discussion

Recently, the world experiences a very strong revolution in poultry industry, and it has been used to challenge poverty and hunger. By all means, poultry is considered as the cheapest source of protein. So, a lot of efforts have been paid recently to improve the fertility and hatchability. Enhancing the traits of the semen and increasing the reproductive life of cocks could be one of these efforts. Semen evaluation could be considered as an important tool for clarifying the effect of internal agents affecting male reproductive capacity, so it can be used as a means for evaluation. Data of the present study revealed that 0.02% of *Eruca sativa* seeds added to the basal diet gave the best results in most of the evaluated semen characteristics. Mclennan and Dallinore[11]. found that the supportive valuable effects of the *Eruca sativa* seeds have been related to its fatty acids content, specially the erucic, oleic and linolenic acids. The essential fatty acid linolenic acid and oleic acid preserve the mitochondrial integrity and the rate of formation of acetyl Co-A that is necessary for the activity and motility of spermatozoa. Blesbois *et al.*[12] stated that dietary fatty acids represent an important factor in male fertility because of their incorporation in both the seminal fluid and spermatozoa. The presence of antioxidant and other stimulant materials in the *Eruca sativa* seeds could have the power to ameliorate semen characteristics. The *Eruca sativa* seed constituents, glucoerucin and polyphenols, have been shown to possess such activity[13]. The low dose of *Eruca sativa* seeds oil has been shown to produce a high rate of proliferation of the haploid cells resulting in stimulation of spermatogenesis and increase sperm count. Nevertheless, the large dose has been shown to inhibit DNA synthesis and decreases cell division resulting in hypo-spermatogenesis that has

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been diagnosed by the diploid peak and a decrease in sperm count[14]. This might be due to the high erucic acid content of *Eruca sativa* seed oil which decreases the level of testosterone hormone. The rate of spermatogenesis could be affected by the high erucic acid content either directly through its action on the membrane of spermatogenesis cells or indirectly through its action on Sertoli cells or Leydig cells[15,12,7]. Hussein[8] proved that the *Eruca sativa* leaves extract causes a significant increase in testosterone level and sperm activity with a significant decrease in sperm mortality and abnormalities. Interestingly, Alcoholic/hexane and alcoholic/acetontrile fractions of *Eruca sativa* significantly enhance the sexual activity of male rats in both behavioral parameters, by the significant increase in the sexual activity index and by increasing the intracavernosal pressure[9].

Conclusion and Recommendation

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The present data showed that *Eruca sativa* treated groups are superior to the control one in semen quality in cockerels. Generally, 0.02% *Eruca sativa* was better than 0.01%. Although *Eruca sativa* was effectively improved the studied semen characteristics, much more information is still needed to evaluate the effect of *Eruca sativa* seeds on sexual hormones levels, blood constituents, histological changes of testis, which will give a better opportunity for results interpretation.

Ethical Approval

Animal Ethic committee approval has been taken to carry out this study.

References

1. Martinez-Sanchez A, Gil-Izquierdo M I G and Ferreres. A comparative study of flavonoid compounds, vitamin C and antioxidant properties of baby leaf Brassicaceae species. J. Agric. Food Chem. 2008; 56: 2330–2340. https://pubs.acs.org/doi/full/10.1021/jf072975+.

2. Hanafi E M,. Hegazy E M, Riad RM, Amer H A. Bio-protective effect of *Eruca sativa* seed oil against the hazardous effect of aflatoxin B1 in male rabbits. Int. J. Acad. Res. 2010; 2(2):67–74. https://www.researchgate.net/publication/267196100_BIO-PROTECTIVE_EFFECT_OF_ERUCA_SATIVA_SEED_OIL_AGAINST_THE_HAZARDUS_EFFECT_OF_AFLATOXIN_B1_IN_MALE_-_RABBITS.

3. Mahran, G H, H Kadry A, Thabet C K, El-Olemy M M, Al-Azizi M M, Schiff P L and Wong L K. Gc/Ms analysis of volatile oil from Eruca sativa seeds. Int. J. Pharmacol. 1992; 30:135-7.

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4. Hussein J, Salah A, Oraby F, Nour El-Deen A and El-Khayat Z. Antihepatotoxic effect of *Eruca sativa* extract on alcohol induced liver injury in rats. J. Am. Sci., 2010; 6:381-389. https://www.researchgate.net/publication/228500685_Antihepatotoxic_Effect_of_Eruca_Sativa_Extracts_on_Alcohol_Induced_Liver_Injury_in_Rats.

5. Sastry M, Ahmed A, Khan M I and Kumar R. Biosynthesis of metal nanoparticles using fungi and actionmycete. Curr. Sci. 2003; 85: 162-170. https://www.researchgate.net/publication/228550063_Biosynthesis_of_metal_nanoparticles_using_fungi_and_actinomycete.

6. Ahmed M, Khan M A and Zafar M. Traditional herbal cosmetics used by local women communities in district Attock of Northern Pak. J. Traditi. Knowl.. 2008; 7:421-424. https://www.researchgate.net/publication/237675231_Traditional_herbal_cosmetics_used_by_local_women_communities_in_district_Attock_of_Northern_Pkistan.

7. Salem A R M and. Moustafa A N. Histological and quantitative study of the effect of *Eruca sativa* seed oil on the testis of Albino rat, Histology Dep., Egypt. J. Hosp. Med. 2001; 2: 148-162. https://ejhm.journals.ekb.eg/article_18936.html.

8. Hussein Z F. Study the effect of *Eruca sativa* leaves extract on male fertility in Albino mice. J. Al-Nahrain Uni. 2013; 16 (1):143-146. https://pdfs.semanticscholar.org/09a6/8412e2964fbae5ec5b7e1e74fff167a32f8a.pdf.
9. Tawfeq A A, Al-rehaily A J, Kamal E H, El Tahir A M and Al-Taweel. Molecular mechanisms that underline the sexual stimulant actions of ginger (Zingiber officinale Rosocoe) and garden rocket (*Eruca sativa* L.). J. Med. Plants Res. 2013; 7(32): 2370-2379. https://www.researchgate.net/publication/256454246_Molecular_mechanisms_that_underlie_the_sexual_stimulant_actions_of_ginger_Zingiber_officinale_Rosocoe_and_garden_rocket_Eruca_sativa_L.
10. Lake P E 1. Artificial insemination in poultry. In: Maile, J.P. (eds). The semen of animals and A.I. Commonwealth Agri. 962; Bureau, Bucks, England, pp:331-335.
11. Mclennan P I and Dallinore J A. Dietary canola oil modifie myocardial fatty acids and inhibits cardiac arrgythmias in rats. J. Nutr. 1995; 125:1005-1009. https://www.ncbi.nlm.nih.gov/pubmed/7722678.
12. Blesbois, E, Lessire M and Hernier D. Effect of dietary fat on fatty acid composition and fertilizing ability of semen. Biol. Reprod. 1997; 56: 1216-1220. https://academic.oup.com/biolreprod/article/56/5/1216/2760838.
13. Barillari J, Canistro D, Paolini M, Ferroni F, Pedulli G F, Iori R and Valgimigli L. Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca satriva Mill*) seeds and sprout. J. Agric. Food Chem. 2005; 6: 2475-82. https://www.ncbi.nlm.nih.gov/pubmed/15796582.

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14. Mona A R S and Nehal A M. Histological and Quantitative Study of the Effect of Eruca Sativa Seed Oil on The Testis of Albino Rat. The. Egy J. Hosp Med. 2001; 2: 148 – 162. https://ejhm.journals.ekb.eg/article_18936.html.

15. Sebokova E, Gargl L and Clandinim M T. Alteration in lipid composition of rat testicular plasma membrane by dietary fatty acids changes responsiveness of Leydig cells and testosterone synthesis. J. Nutrition. 1990; 120: 610-618. https://www.researchgate.net/publication/20804123_Alteration_of_the_Lipid_Composition_of_Rat_Testicular_Plasma_Membranes_by_Dietary_n-3_Fatty_Acids_Changes_the_Responsiveness_of_Leydig_Cells_and_Testosterone_Synthesis.

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Table 1: The Formula of the Basal Diet

Ingredient	(%)
Sorghum	59
Groundnut cake	15
Wheat bran	11.83
Super concentrate	5
Dicalcium	0.5
Lame stone	8

Nacl	0.3
Methyonine	0.02
Antifungal	0.1

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Table 2: Chemical analysis of the Basal Diet

DM (%)	**CP** (%)	**CF** (%)	**EE** (%)	**Ash** (%)	**NFE** (%)
92.06	18.23	3.99	3.84	8.18	57.85

Table 3: Chemical Analysis of *Eruca Sativa* Seeds

DM (%)	CP (%)	CF (%)	EE (%)	Ash (%)	NFE (%)
94.7	21.94	18.32	16.45	4.64	13.34

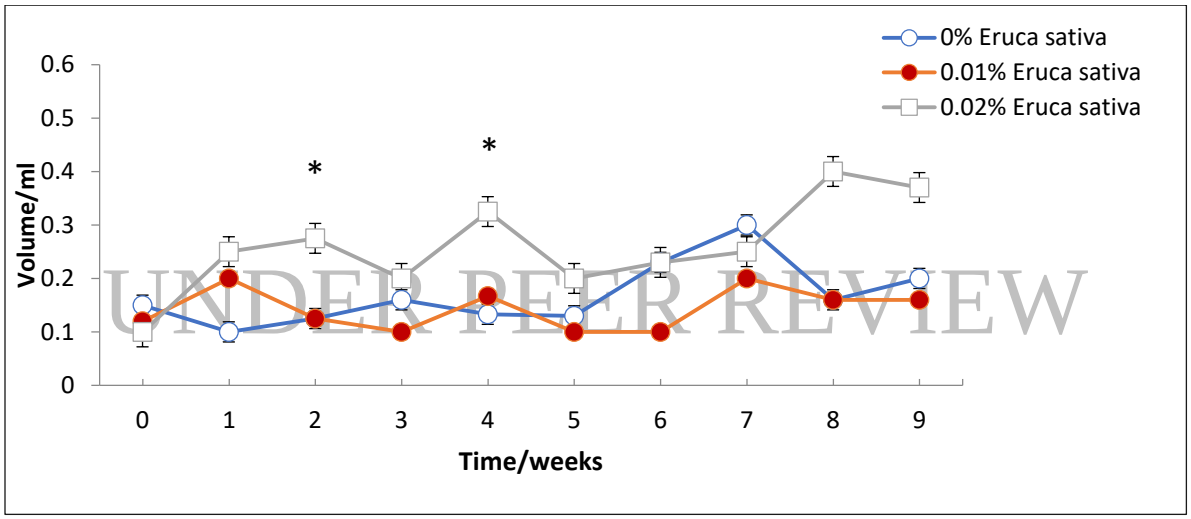

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Figure (1): Effect of the dietary *Eruca sativa* seeds on semen volume (ml)

***Significantly different at ($p < 0.05$)**

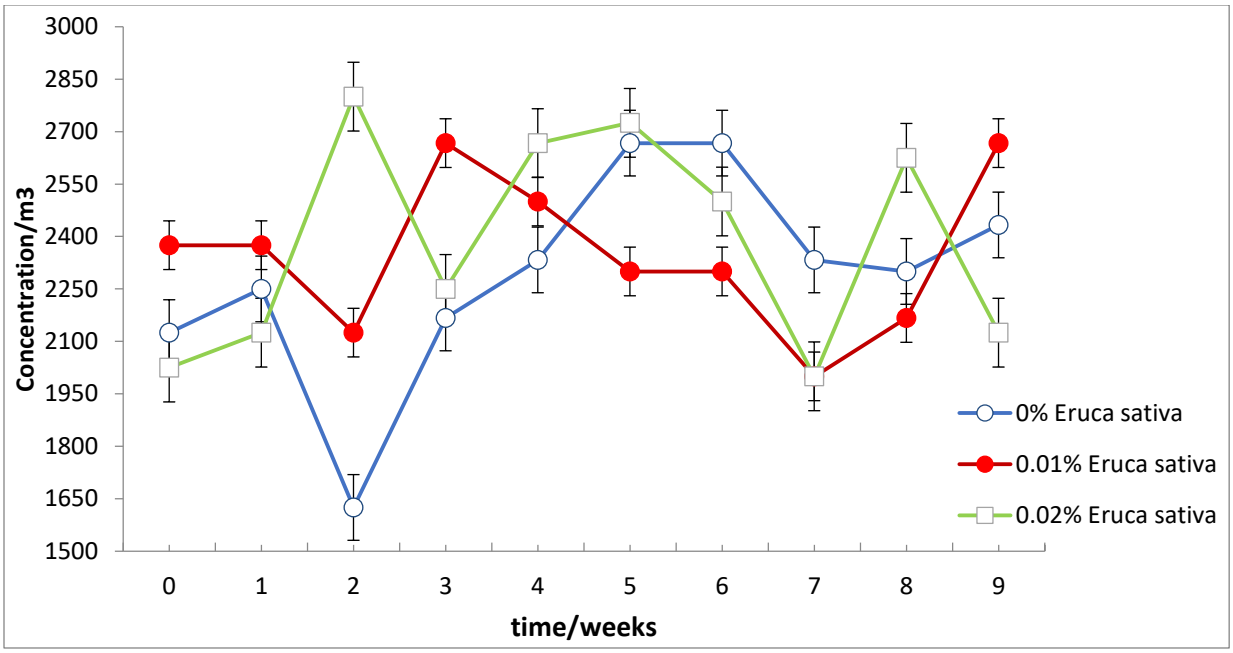

Figure 2: Effect of the dietary *Eruca sativa* seeds on semen concentration (sperm/mm^3)

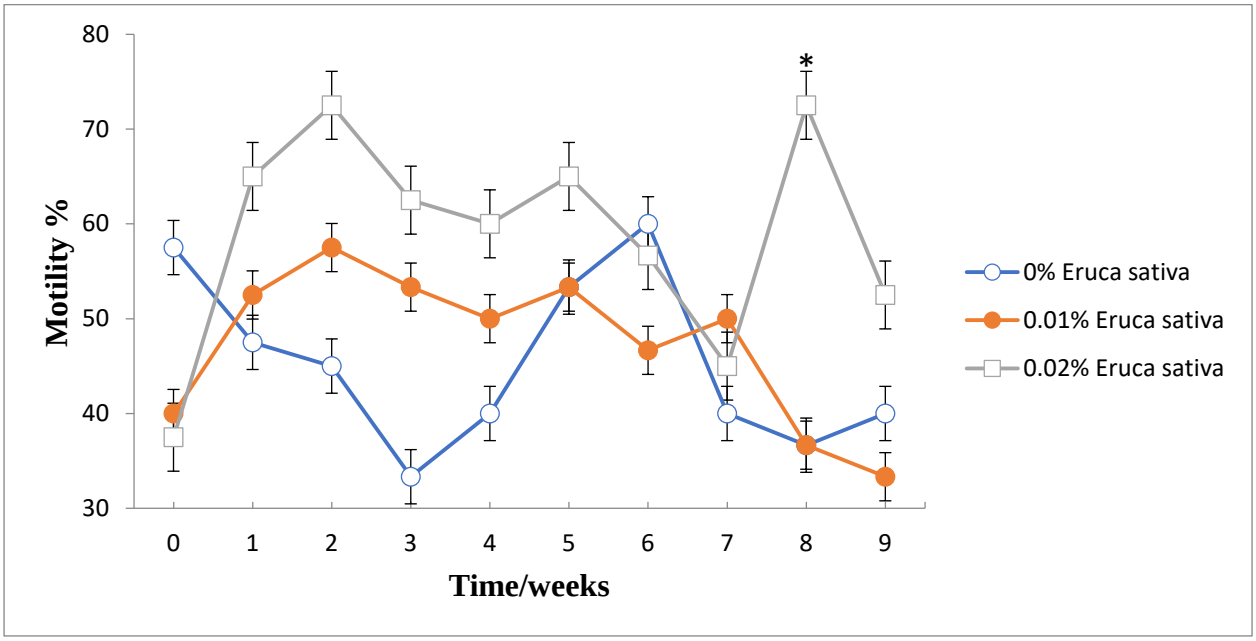

Figure (3): Effect of the dietary *Eruca sativa* seeds on Semen motility (%)

*** Significantly different at ($p < 0.05$)**

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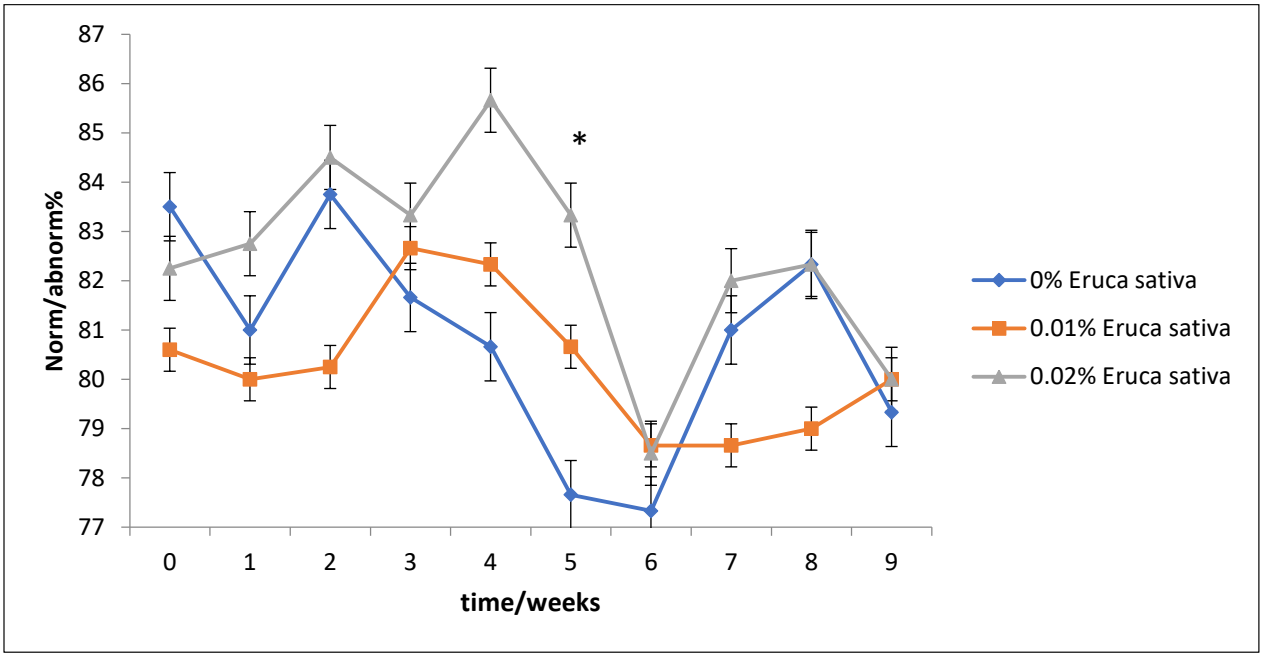

Figure (4): Effect of the dietary *Eruca sativa* seeds on semen Normal/abnormal ratio (%)

*** Significantly different at ($p < 0.05$)**

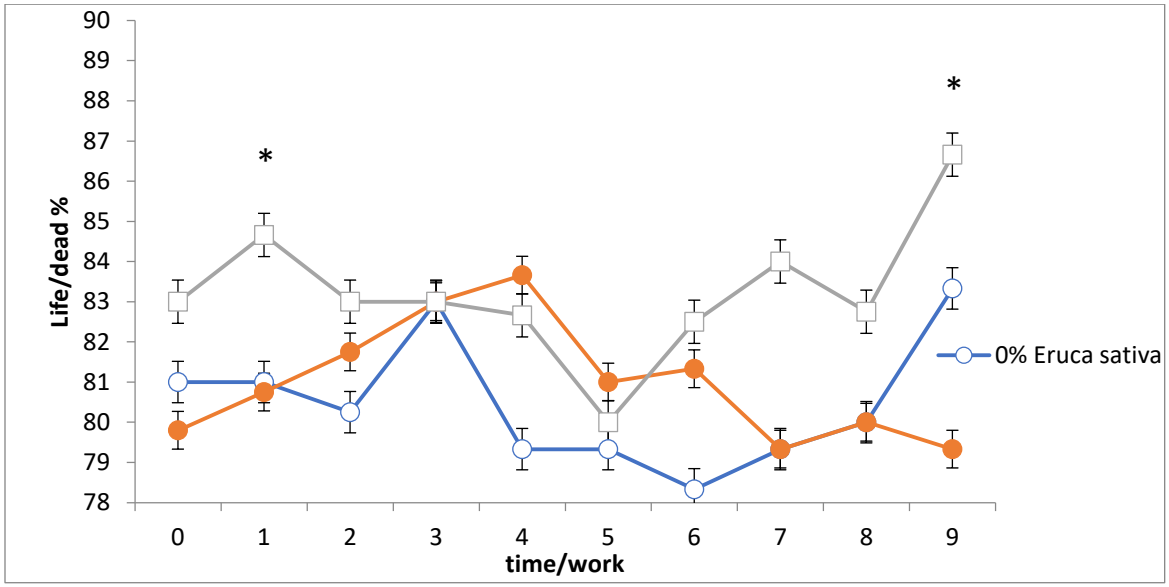

Figure (5): Effect of the dietary *Eruca sativa* seeds on semen live/dead ratio (%)

*** Significantly different at ($p < 0.05$)**