

The addition of selenium and vitamin E to the diet and its effect on body weight, semen characteristics and testosterone in V-line breed rabbits

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ABSTRACT:

This research aims to study the effect of dietary of selenium and vitamin E on body weight and reproductive performance in rabbit. A total of 80 male rabbits at two months age and average of initial live body weight was 2.27 kg. Animals were separately housed in wire-cages. The light-dark period was controlled to be 16 h of light and 8 h of darkness. The animals were randomly assigned to the following: four experimental treatments (n=20 each): T1: Control group that fed basal diet, T2: Fed basal diet + 0.1 mg organic Se / kg diet, T3: Fed basal diet + 40 mg α -tocopherol acetate / kg diet and T4: Fed basal diet + 40 mg α -tocopherol acetate / kg diet + 0.1 mg organic Se/kg diet, The diet contains 18% protein, 2850 kcal. These measurements recorded body weight, semen collection and evaluation, ejaculate volume, mass motility, percentage of progressive sperm motility, percentage of dead sperm (%), percentage of abnormal spermatozoa (%), acrosomal damages, hypo-osmotic swelling (HOS-test) reacted spermatozoa (%), and testosterone hormone concentration. The results can summarize the changes of averages body weight that were not significant among treatment, while means of ejaculate volume, the percentage of mass motility, acrosome damages, progressive motility, and testosterone levels were significantly the highest ($P < 0.05$). The percentage of dead sperms, percentage of abnormalities, HOS-test, was significantly the lowest in treatment group. From this study, it is recommended to add selenium and vitamin E to the diet of rabbits.

Keywords: Rabbits, Body weight, Semen characteristics, Testosterone, Reproductive performance.

INTRODUCTION

Rabbit breeding is one of the most successful investment projects, especially in recent years because rabbits are characterized by abundant production and rapid growth than other animals. Rabbits can be relied upon to fill the shortage of meat due to their rapid growth rate and short generation period (Eman 2019 and Ayyat et al. 2021). The V-line rabbit strain is a modern Spanish breed that was imported and bred in Egypt due to its high growth rate. This strain was selected in 1982 in the Department of Animal Sciences, University of Politecnica, Valencia, Spain. based on the litter size (Estany et al. 1989). In recent years, Egyptian farms have shown increasing interest in using foreign rabbits such as the V-line breed to increase rabbit meat production (Abdel-Khalek et al. 2019).

The addition of micro-mineral elements in animal diets is important for growth, reproductive and productive performance, in addition to its importance in improving the function of the immune system (Kassim et al. 2022). Some researchers reported that no effect of selenium on growth, thus, there is no effect on body weight in rabbits (Dokoupilová et al. 2007 and Marounek et al. 2009). However, other studies indicated that dietary supplementation of vitamin E organic,

selenium and their combination resulted in higher body weight (Ebeid et al. 2013 and Safan 2016). These differences may be due to the type of breed used in the research or to differences in the conditions in which the research was conducted.

Vitamin E is a nutrient that helps improve sperm condition and development of the male reproductive system in mice and goats (Rao and Sharma 2001 and Hong et al. 2009), and the diameter of the seminiferous tubules in rats (Momeni et al. 2012). Moreover, previous studies had showed that vitamin E has a good influence on sperm motility and the volume of semen (Yousef et al. 2003 and Yue et al. 2010). Selenium is important as it works to increase testosterone levels and sperm maturity and formation (Flohé 2007). Selenium plays major roles for spermatogenesis and maintaining sufficient viability of spermatozoa and had ability to diminishing abnormalities of spermatozoa through direct effect on raise antioxidant status (Ebeid 2009) and its deficiency results in deterioration sperm motility and morphological abnormalities in rodents (Kehr et al. 2009). The present study aims to evaluate effect of dietary that includes organic selenium, vitamin E and their combination on body weight, physical of semen characteristics and testosterone.

MATERIALS AND METHODS

Duration and location of study:

The herein experimental work was carried out in the experimental rabbit flock maintained by the Department of Animal Production, Faculty of Agriculture, Al-Azhar University in Nasr City, Cairo, Egypt. This work started from mid of November 2020 to near the end of March 2021.

Animals housing and management:

Rabbit V-line breed was used in this study. This purebred V-line was obtained from one of the private sector companies (Doctor Rabbit Farms), Gharbia Governorate, Egypt. A total of 80 male rabbits at two months age and average of initial live body weight was 2.27 kg. All animals were healthy and clinically free of external and internal parasites. Rabbits males were raised in a semi-closed place of 160 m² (8 m width and 20 m length) with wire-netted windows opened on the eastern and western sides to receive natural ventilation. Animals were separately housed in wire-cage with standard dimension of 60 × 35 × 35 cm: long, width, and height in respectively. The design of cages was arranged in double tier batteries. The light-dark period was controlled to be 16 h of light and 8 h of darkness, The maximum and minimum values of ambient temperature and relative humidity were recorded weekly at mid-day and inside the rabbit farm building throughout the experimental periods (Table 1).

Animals feeding:

Following the National Research Council (NRC 1977) and (De Blas and Wisewan 2020) recommendations, pelleted basal diets were formulated to support nutrients' requirements (Table 2). Granular commercial rabbit feed has been obtained from one of the private sector companies (Doctor Rabbit Farms), Gharbia Governorate, Egypt.

Experimental design:

The animals were randomly assigned to the following four experimental treatments (n=20 each):

T1: Control group that fed basal diet

T2: Fed basal diet + 0.1 mg organic Se / kg diet.

T3: Fed basal diet + 40 mg α -tocopherol acetate / kg diet.

T4: Fed basal diet + 40 mg α -tocopherol acetate / kg diet + 0.1 mg organic Se/kg diet).

Obtained of vitamin E (α -tocopherol) acetate from Adisseo company, Antony,

France. Organic selenium in the form of selenomethionine produced by Saccharomyces cerevisiae (Selenized yeast inactivated) from Lallemand company, Blagnac, France.

Body Weight:

Changes in live body weight were recorded weekly individually using a digital scale before the morning feeding throughout the experimental period.

Semen collection and evaluation:

Semen samples were collected once a week for 8 successive weeks where 160 ejaculations from all treatments were randomly collected at a rate of five samples from each group and that using artificial vagina (AV). The collected semen samples were immediately assessed microscopically after removing gel clot (without any gel-mass). To measure physical semen characteristics (ejaculate volume, wave motion, progressive liner motility, sperm concentration, live and abnormal spermatozoa and total sperm output) according to El-Gaafary (1987).

Ejaculate volume:

Semen volume was determined in milliliter directly after collection using a measuring cylinder, with gradations of 0.1 mL-15 mL units attached to the artificial vagina (AV), after removal of the gel portion. (Carrillo-González and Hernández 2016). The reading was taken by direct observation of the transparent semen collection tube

Sperm concentration ($\times 10^6/\text{mL}$):

The sperm concentration count using by hemocytometer were estimated according to (Khadr et al. 2015).

Mass motility:

Mass activity was scored subjectively according to the intensity of the wave motion seen in the medium by the collective activities of spermatozoa, from the absence of wave motion to very turbulent motions according to Plasson (1975) using the following scales as the guideline:

Scales	Motility
5 Above 80 %	Motile spermatozoa (Excellent)
4 60 to 80 %	Motile spermatozoa (Good)
3 40 to 60 %	Motile spermatozoa (Fair)
2 20 to 40 %	Motile spermatozoa (Oscillating)
0 0 to 10%	Motile spermatozoa (non-motile)

Percentage of progressive sperm motility:

The individual motility of the sperm was measured as indicated by Ajam et al (1990).

Percentage of dead sperm (%):

The eosin/nigrosine staining procedure was carried out by dissolving 1.67 gm eosin and 10.0 g nigrosine in 100 mL distilled water according to Hackett and Macpherson (1965).

Percentage of abnormal spermatozoa (%):

The morphological abnormalities of spermatozoa (%) were determined in the same smear prepared for live/dead spermatozoa ratio under oil immersion using x1000 objective of a light microscope.

Acrosomal damages:

Acrosome integrity was assessed using Giemsa staining procedure (Watson 1975).

Hypo-osmotic swelling (HOS-test) reacted spermatozoa (%):

Spermatozoa with intact membranes were identified by changes in the shape of the cell, as indicated by coiled tail according to (Zeidan et al. 2008), and only those sperm having a curling tail were HOS-test positive.

Testosterone hormone concentration:

It is intended to use immunoassay for the in vitro quantitative determination of testosterone in serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and Cobas® immunoassay analyzers (Roch Diagnostics®, E 170 electrochemiluminescence immunoassay (ECLIA) Mannheim, Germany) according to (Wheeler 1995).

Statistical analysis:

All results were statistically analyzed by General Linear Models (GLM), one way analysis of variance, using SAS software (SAS, 2003). Following model: $Y_{ij} = \mu + T_i + e_{ij}$ Where:

Y_{ij} = Performance traits measured on the V-line rabbit in the treatment.

μ = Overall mean.

T_i = Effect of treatments (i= 1, 2, 3 and 4).

e_{ij} = Random error effect.

Differences among means were separated using Duncan's multiple range Test (Duncan 1955).

RESULTS AND DISCUSSION**Effect of selenium and vitamin E on body weight:**

Data in Table (3) showed that the changes of averages initial and final body weight were nonsignificant between control group and rabbits in different treatments.

The results in the present study showed that dietary supplementation of selenium and vitamin E and a mixture had no positive effect on male rabbits' growth. Similar results have been observed by Marounek et al. (2009) and Dorra et al. (2014) they found that body weight gain of growing rabbits did not significantly affect by selenium supplemented diets at level ranged from 0.8 to 0.1 mg/ kg diet. Similarly, in rabbits, Albuquerque et al. (2017) and Hosny et al. (2020) reported that there was no effect of interaction between the levels of supplementation of selenium and vitamin E on weight gain.

In contrast, these results conflict with results gained by Eiben et al. (2011) and Ebeid et al. (2013) who reported that vitamin E addition to diet of animals lead to higher body weight in rabbits. Also, Safan (2016), found that dietary supplementation of selenium and vitamin E and their combination had positive effect on growing rabbits.

Effect of selenium and vitamin E on physical of semen characteristics:**Semen ejaculate volume (ml):**

Data of the Table (4) showed that averages of ejaculate volume were 0.78, 0.79 and 0.85 ml for control, selenium and mixture of selenium with vitamin E groups respectively, compared to control group (0.66 ml). These results showed a significant ($P < 0.05$) improvement in the physical properties of semen, including semen volume of rabbits fed a basal diet fortified with vitamin E or selenium or both.

These results obtained in the present study are agreement with Hosny et al. (2020); Enrique et al. (2022) and Mohamady (2022) where ejaculation volume showed differences among treatments ($p < 0.05$). An increase in semen volume may be a reason for the increase in fluid seminal. In rams' secretion of testosterone that affects the secretory activity of the late sex glands (Bearden and Fuquay 1997). There is a contribution of some minerals, including selenium, to increasing the activity of the sexual glands following the male reproductive system, including the prostate gland and the vesicular gland (Underwood

and Suttle 1999). The highest concentration of selenium in the later sex glands is in the prostate, follicular gland and Cooper's gland and its effect on GSH-px enzyme activity and its ability to eliminate free radicals produced endogenously due to metabolic processes, thus, the secretory activity of these glands increases (Smith et al. 1979). The increase in both semen volume and sperm concentration in the current study appears to be due to the improvement in semen oxidative stability (Wang and Wang 2008), possibly due to the addition of vitamin E (Asl et al. 2018) in the roosters' diet.

By contrast, other studies have shown different results (Mohamed and Abd al-Rasheed (2017) that observed that supplementing rations with high levels of vitamin E did not affect both semen volume and sperm concentration. Also, Castellini et al. (2003) in chickens, and Gliozzi et al. (2009) reported that supplementation of vitamin E in the diet of rabbits did not affect both semen volume and sperm concentration.

Sperm concentration ($\times 10^6/\text{mL}$):

Data available in Table (4) showed that the average sperm concentration were 366.48, 474.65, 451.48 and $514.55 \times 10^6/\text{mL}$ for control, selenium, vitamin E and they're its combinations, respectively. The lowest values were noticed for control group than other treatment groups. The highest results were recorded by the T4 group. Statistically there were significant difference among treatment groups except for rabbits treated with selenium or vitamin E only, they did not differ significantly.

In this study, the findings on (T2, T3 and T4) treatments sperm concentration in rabbits were like findings by (Hosny et al. (2020) and Baker et al. (2021), in that selenium and vitamin E increased sperm number per ejaculate. Also, the results of this study agree with El-Sheshtawy et al. (2014); Sharaf et al. (2019) and Mohamad (2022).

The increased concentration of sperm in animals supplemented with both vitamin E and selenium may be due to this, but both vitamin E and selenium act against oxidation from free radicals that damage sperm (Brzezińska-Ślebodzińska et al. 1995). Selenium is involved in the growth and development of testicular tissue resulting in improved spermatogenesis (Behne et al.1996). Abundant sperm production also depends on the availability of selenium needed for testicular development, and selenium

deficiency leads to a decrease in sperm concentration (Liu et al., 1982). Vitamin E and selenium stimulates Leydig cells to testosterone biosynthesis of testes by stimulating the anterior pituitary hormones secretion and increase testicular cholesterol content (Abdel-Hasseb et al. 2004) Vitamin E and selenium are involved in the synthesis and production of prostaglandins (Ahmed et al. 2001), prostaglandin $F_{2\alpha}$ administration has increased the number and motility of spermatozoa in farm animals (Hafs et al. 1974).

Total and progressive motility (%):

The data in table (4) explained the percentage of mass motility were 79.00, 76.63 and 81.88% for animals' supplantation with selenium, vitamin E and their combinations, respectively compared to control group (72.38%). Also, the present study showed the same trend of the progressive motility which were 82.62, 81.12 and 85.87% for animals' supplantation with selenium, vitamin E and their combinations, respectively compared to control group (78.12%). In the present study, the differences in total and progressive motility that were significantly ($P < 0.05$) affected by the treatments, are agreement with that obtained by Mohamad and Abdul Rashid (2017); Butt et al. (2019); Raouf and Taha (2021); Baker et al. (2021) and Shabani et al. (2022).

Many studies agreed with this study which showed the importance of vitamin E and selenium in increasing both total and individual motility, as vitamin E plays an important role in protecting the cell membrane from peroxides, and from unsaturated fatty acid peroxides (Baker et al. 2021). It has been confirmed that vitamin E plays an important role in providing energy for sperm motility by improving the efficiency of mitochondria located in the midsection of the sperm (Ener et al. 2016).

Dead sperms (%):

The results in the present study Table (4) showed that the differences of dead spermatozoa percentage are significant among treatment groups. The lowest percentage of dead sperms was found in rabbits of treatment by selenium plus vitamin E (12.20%) compared by other treatments, while there was no significant difference between rabbits of treatment by only selenium or vitamin E (16.60 and 17.23% respectively). However, all treatments were significantly compared to the control group (20.13%).

The percentage of dead sperm in this study were consistent with archived by (Domosławska et al. 2018; Hosny et al. 2020 and Mohamady 2022). They explained that this decrease in the percentage of dead spermatozoa is due to the function of vitamin E and selenium in increasing sperm vitality and may also be due to the function of vitamin E in protecting the sperm plasma membrane from the harmful effects of unsaturated fatty acid peroxides. They also noted that selenium has a role in maintaining the integrity of sperm (Baker et al. 2021).

On the contrary, there was no difference between treatments regarding the percentage of live sperm (Enrique et al. 2022). Similarly, (Daramola et al. 2016) did not notice any effect of vitamin E and selenium in improving the quality of semen, and the reason for this may be due to the conditions of the experiment on the one hand and to the experimental animals on the other.

Abnormal Sperms (%):

The averages percentage of abnormalities sperm are shown in Table (4). These results shows that treatment by selenium plus vitamin E had lower significant ($p < 0.05$) percentage of abnormalities (14.88%) compared to control group (18.28%), but there were no significant differences in case of comparison to treatment by only selenium (16.60%) or vitamin E (15.63%).

The percentage of abnormalities sperm in the present study are closed with those reported by (Hosny et al. 2020; Baker et al 2021; Mohamady 2022 and Sabzian-Melei et al. 2022). Flohé (2007) indicated that selenium acts as a cofactor for the enzyme glutathione peroxidase (GSH-Px), which leads to the elimination of peroxides of unsaturated fatty acids. Therefore, selenium deficiency leads to abnormalities in the function of testicular tissues, resulting in abnormalities of spermatozoa. Selenium deficiency has been associated with decreased reproductive performance and decreased sperm quality as produces abnormal sperm shapes, structural abnormalities (Baiomy et al. 2009) in rams.

These results agree with Mahmoud et al. (2013) who reported that vitamin E has a role in reducing the rate of sperm abnormalities. Mohamady (2022) found that the lowest values of abnormal spermatozoa percentages were obtained with NZW buck rabbits fed basal diet and treated with Nano-selenium plus vitamin E when compared with the other treatment groups. These results agree with those

obtained by El-Sheshtawy et al. (2014) who reported that 0.10 mg selenium/kg live body weight and / or 1.35 IU vitamin E /kg Live body weight administration significantly improved semen characteristics with advance of time as indicated by decreased incidence of sperm abnormalities. The improved characteristics are more obvious for those bucks injected simultaneously with selenium and vitamin E.

Acrosome damages (%):

The results in the present investigation revealed that acrosome damages influenced significantly ($P < 0.05$) on rabbits which were treated by selenium coupled vitamin E (14.88%) compared to other treatments where acrosome damage was 18.73, 17.35 and 16.40% in control groups and rabbits treated by only of selenium or vitamin E, respectively (Table 4).

This study is consistent with that reported by Zhu et al. (2015) and Hosny et al. (2020) in rabbits. It found a decrease in the percentage of spermatozoa with damaged acrosomes in rabbits whose diets were supplemented with selenium. This decrease in sperm with damaged acrosomes may be since both vitamin E and selenium have a function as antioxidants, and therefore could eliminate free radicals that deform spermatozoa (Baker et al. 2021).

Hypo-osmotic swelling (HOS-test) test:

Statistically, HOS-test was significantly different among groups of treatment rabbits (Table 4). The lowest significantly percentage was obtained in control group (78.08%) than in treatment animals by selenium and by selenium plus vitamin E (81.78, and 84.83%), respectively, while no significant different between control group and rabbits treated with vitamin E (80.30%) or between of rabbits treated only with selenium or vitamin E.

It is worth noting that the HOS-test is not rigorous in predicting sperm fertility, but the test helps in identifying the percentage of mature spermatozoa. Hypo-osmotic swelling test is a simple and method that provides useful information on the functionality of plasma membranes and determine the ability of the sperm membrane to maintain equilibrium between the sperm cells and its environment (Neild et al. 2000 and Pajovic et al. 2016).

The percentages of sperms with swollen head and coiled trails in response to HOS-test are in good agreement with those observed in

white New Zealand rabbit (Daader and Seleem 2005 and Safaa et al. 2008b). It also agrees with that observed in rabbit A-line and R-line (Safaa et al. 2008a) and in V-line rabbits (Hosny et al. 2020).

Worse HOS-test results correlated with lower levels of carnitine and selenium, and vice versa (Pajovic et al. 2016). It was also mentioned that selenium and carnitine play an important role in improving fertility by improving the integrity and morphology of sperm membranes, which can be presented as a positive HOS-test. Easa et al. (2013) they concluded that the response of rabbit sperm to the hypo-osmotic swelling test was a good indicator of the reproductive capacity of rabbit males under which the sperm that shows better motility results can be used or survive as a good indicator of ability to fertilize.

Effect of selenium and vitamin E on plasma testosterone hormone concentrations:

The values of Table (4) showed a significant sudden increase ($P < 0.05$) in testosterone levels where it was the lowest level in the control group (1.35 ng / ml) and the highest concentration in the group which diet was added as a mixture of selenium in addition to vitamin E (5.74 ng / ml), while there was no significant difference between the group of rabbits fed only vitamin E (2.99 ng/ml) or selenium (3.67 ng/ml).

These results agreed with Majeed and Al-Khashab (2019); Hosny et al. (2020) and Mohamady (2022) as they found vitamin E and selenium supplementation significantly improved testosterone levels in the blood. Also, the result in the present study is close to similar response detected by El-Sisy et al. (2008) and Ibrahim and Mohamed (2018) in male Baladi goats, and Abdel-Wareth et al. (2019) and Mohamady (2022) in rabbit bucks.

That improvement in the level of testosterone hormone in the blood plasma of V-line rabbits fed with vitamin E and selenium as antioxidants. To protect the Leydig cells responsible to produce testosterone from oxidative stress due to oxidants (Swathy et al. 2006) confirmed that there is a high and significant correlation between selenium deficiency and low testosterone levels. And the lack of vitamin E and selenium leads to a weakening of the ability of testicular tissues to synthesize steroid hormones from cholesterol.

CONCLUSION

It is concluded that a diet containing supplements such as selenium and vitamin E, and in particular a mixture of them could improve the health status of rabbit bucks, leading to substantial improvements in physical of semen characteristics then subsequent improving in the reproductive performance.

REFERENCES

- Abdel-Hasseb, A., El-Ashary, K., Abdullah, M. 2004: Testicular physiological response to administration of selenium and Vitamin E in rabbits. *Alex. J. Vet. Med*, 21, 162-167.
- Abdel-Khalek, A.E., Khalil, W.A., El-Sayed, R.E. 2019: Effect of Different Dietary Sources of Oils on Growth Performance and Profile of Lipid, Testosterone and Fatty Acids in Rabbit Bucks. *J. Anim. Poult. Prod.*, 10(9): 297-304.
- Abdel-Wareth, A.A.A., Ahmed, A.E., Hassan, H.A., Abd El-Sadek, M.S., Ghazalah, A.A., Lohakare, J. 2019: Nutritional impact of nano-selenium, garlic oil, and their combination on growth and reproductive performance of male Californian rabbits. *Animal Feed Science and Technology*, 249, 37-45.
- Ahmed, W.M., Sabra, H.A., Hamam, A.M. 2001: Effect of administration of vitamin A, D, E, and selenium during prepartum period on the reproductive function of native cows in Egypt. *Beni-Suef Vet. Med. J*, 11, 81-91.
- Ajam, I.K., Al-Saadi, H.A., AlHakim, M.K. 1990: Reproductive physiology, artificial insemination and reproductive care. College of Agriculture and Forestry. House of Books for Printing and Publishing - University of Mosul.
- Albuquerque, D., Lopes, J.B., Ferraz, M.S., Ribeiro, M.N., Silva, S.R., Costa, E., Lopes, J.C. 2017: Vitamin E and organic selenium for broilers from 22 to 42 days old: performance and carcass traits. *Anais da Academia Brasileira de Ciências*, 89, 1259-1268.
- Asl, R.S., Shariatmadari, F., Sharafi, M., Torshizi, M.A.K., Shahverdi, A. 2018: Improvements in semen quality, sperm fatty acids, and reproductive performance in aged Ross breeder roosters fed a diet supplemented with a moderate ratio of n-3: n-6 fatty acids. *Poultry science*, 97(11), 4113-4121.
- Ayyat, M.S., Abd El-Latif, K.M., Helal, A.A., Al-Sagheer, A.A. 2021: New Zealand White rabbits tolerance to chronic thermal stress at different dietary energy/protein levels. *Animal Feed Science and Technology*, 278: 114992.
- Baiomy, A.A., Mohamed, A.E.A., Mottelib, A.A. 2009: Effect of dietary selenium and vitamin E supplementation on productive and reproductive performance in rams. *Journal of Veterinary Medical Research*, 19(1), 39-43.

- Baker, A.G., Alssadi, S.A.R.A., Mohammad, A.K. 2021: The effect of selenium and vitamin E addition on semen quality of Awassi rams. *Zanco Journal of Pure and Applied Sciences*, 33(s1), 127-134.
- Bearden, H.J., Fuquay, J.W. 1997: Semen evaluation. *Applied animal reproduction*. 4th ed. New Jersey: Prentice Hall, 158-169.
- Behne, D., Weiler, H., Kyriakopoulos, A. 1996: Effects of selenium deficiency on testicular morphology and function in rats. *Reproduction*, 106(2), 291-297.
- Brzezińska-Ślebodzińska, E., Ślebodziński, A.B., Pietras, B., Wieczorek, G. 1995: Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. *Biological trace element research*, 47(1), 69-74.
- Butt, M.A., Shahid, M.Q., Bhatti, J.A., Khalique, A. 2019: Effect of dietary vitamin e and selenium supplementation on physiological responses and reproductive performance in holstein friesian bulls during humid hot summer. *Pak. Vet. J*, 39(4), 593-597.
- Carrillo-González, D., Hernández, H.D. 2016: Caracterización seminal de individuos ovinos criollos colombianos de pelo en el departamento de Sucre. *Revista Colombiana de Ciencia Animal*, 8(2), 197-203.
- Castellini, C., Lattaioli, P., Dal Bosco, A., Minelli, A., Mugnai, C. 2003: Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. *Reproduction Nutrition Development*, 43(1), 91-103.
- Daader, A.H., Seleem, T.S.T. 2005: Response of spermatozoa of different breeds of rabbits to hypo-osmotic swelling test. *Proc.: The 4th Inter. Con. on Rabbit Prod. in Hot Clim., Sharm El-Sheikh, Egypt*, 177, 181.
- Daramola, J.O., Adekunle, E.O., Oke, O.E., Ogundele, O., Saanu, E.O.A., Odeyemi, A.J. 2016: Effect of vitamin E on sperm and oxidative stress parameters of West African Dwarf goat bucks. *Tropical and Subtropical Agroecosystems*, 19(2), 151-158.
- Dokoupilová, A., Marounek, M., Skřivanová, V., Březina, P. 2007: Selenium content in tissues and meat quality in rabbits fed selenium yeast. *Czech Journal of Animal Science*, 52: 165-169.
- Domosławska, A., Zdunczyk, S., Franczyk, M., Kankofer, M., Janowski, T. 2018: Selenium and vitamin E supplementation enhances the antioxidant status of spermatozoa and improves semen quality in male dogs with lowered fertility. *Andrologia*, 50(6), e13023.
- Dorra, T.M., El-Moghazy, G.M., Abo El-Maaty, H.M., Eid, R.H. 2014: effect of dietary supplementation with nano-selenium or glutamine on growth performance and carcass characteristics of growing rabbits fed diets containing two crude protein levels. *Journal of Animal and Poultry Production, Mansoura University*, 5(9), 549-562.
- Duncan, D.B. 1955: Multiple range and multiple F tests. *biometrics*, 11(1), 1-42.
- Easa, F.M., Hekal, A.M., Seleem, T.S.T. 2013: Injection effect of gibberellic and/or boric acid to rabbits on response of spermatozoa to hypo-osmotic swelling test. *Journal of Animal and Poultry Production, Mansoura Univ.*, 4(10), 571-581.
- Ebeid, T.A. 2009: Organic selenium enhances the antioxidative status and quality of cock semen under high ambient temperature. *British Poultry Science*, 50 (5), 641-647.
- Ebeid, T.A., Zeweil, H.S., Basyony, M.M., Dosoky, W.M., Badry, H. 2013: Fortification of rabbit diets with vitamin E or selenium affects growth performance, lipid peroxidation, oxidative status, and immune response in growing rabbits. *Livestock Science*, 155(2-3): 323-331.
- Eiben, C., Végi, B., Virág, G., Gódor-Surmann, K., Kustos, K., Maró, A., Fébel, H. 2011: Effect of level and source of vitamin E addition of a diet enriched with sunflower and linseed oils on growth and slaughter traits of rabbits. *Livestock Science*, 139(3), 196-205.
- El-Gaafary, M.N.S.A. 1987: The characteristics of semen from Welsh Mountain and Cambridge rams (Doctoral dissertation, University of Wales (UCNW, Bangor: Agriculture), 233-257.
- El-Sheshtawy, R.I., Ahmed, W.M., Zaabal, M.M., Ali, G.A., Shalaby, S.I. 2014: Effect of Selenium and /or Vitamin E Administration on Semen Characteristics, Plasma Testosterone Level and some Immunogenetic Constituents in seminal plasma proteins of Baladi Bucks. *Global Veterinaria*. 12(6), 878-884.
- El-Sisy, G.A., Abdel-Razek, A.M.A., Younis, A.A., Ghallab, A.M., Abdou, M.S.S. 2008: Effect of dietary zinc or selenium supplementation on some reproductive hormone levels in male Baladi goats. *Global Vet*, 2(2), 46-50.
- Eman, H.A.H. 2019: Study on Some Reproductive Traits in Rabbits. *Msc. Thesis, Fac. Agrc., Benha Univ., Egypt*.
- Ener, K., Aldemir, M., Işık, E., Okulu, E.M.R.A.H., Özcan, M.F., Uğurlu, M., Özayar, A. 2016: The impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocele: a randomised controlled study. *Andrologia*, 48(7), 829-834.
- Enrique Hernández-Carrillo., [Héctor Sánchez-Pineda.](#), [Jorge Tórtora-Pérez.](#), [Francisco A. Cigarroa Vazquez.](#), [María Reyes-García.](#), [Marisela Peralta-Lailson.](#), [José Antonio Hernández-Marín.](#) 2022: Effect of selenium on

- semen quality and fertility of rams. *Agro Productividad*, 15(5), 33-39.
- Estany, J., Baselga, M., Blasco, A., Camacho, J. 1989: Mixed model methodology for the estimation of genetic response to selection in litter size of rabbits. *Livestock Production Science*, 21(1), 67-75.
- Flohé, L. 2007: Selenium in mammalian spermiogenesis. *Biol. Chem.* 338: 987- 995.
- Glozzi, T.M., Zaniboni, L., Maldjian, A., Luzi, F., Maertens, L., Cerolini, S. 2009: Quality and lipid composition of spermatozoa in rabbits fed DHA and vitamin E rich diets. *Theriogenology*, 71(6), 910-919.
- Hackett, A.J., Macpherson, J.W. 1965: Some staining procedures for spermatozoa. *The Canadian Veterinary Journal*, 6(3), 55.
- Hafs, H.D., Louis, T.M., Waters, R.J., Stellflug, J.N., Haynes, N.B. 1974: Increased sperm output of rabbits and bulls treated with prostaglandin F_{2α}. *Prostaglandins*, 8(5), 417-422.
- Hong, Z., Hailing, L., Hui, M., Guijie, Z. 2009: Effect of vitamin E supplementation on development of reproductive organs in Boer goat. *Animal reproduction science*, 113(1-4), 93-101.
- Hosny, N.S., Hashem, N.M., Morsy, A.S., Abo-Elezz, Z.R. 2020: Effects of organic selenium on the physiological response, blood metabolites, redox status, semen quality, and fertility of rabbit bucks kept under natural heat stress conditions. *Frontiers in Veterinary Science*, 7, 290.
- Ibrahim, E.M., Mohamed, M.Y. 2018: Effect of different dietary selenium sources supplementation on nutrient digestibility, productive performance, and some serum biochemical indices in sheep. *Egyptian Journal of Nutrition and Feeds*, 21 (1), 53-64.
- Kassim, A.S., Ali, A.H.H., Marwan, T.A., Abdel-Wareth, A.A.A. 2022: Selenium nanoparticles in rabbit nutrition. a review. *SVU-International Journal of Agricultural Sciences*, 4(1), 90-98.
- Kehr, S., Malinouski, M., Finney, L., Vogt, S., Labunsky, V.M., Kasaikina, M.V., Carlson A.B., YouZhou, Hatfield D.L., Gladyshev, V.N. 2009: X-ray fluorescence microscopy reveals the role of selenium in spermatogenesis. *Journal of molecular biology*, 389(5), 808-818.
- Khadr, A.H., Abdou, A., El-Sherbiny, A.M. 2015: Age of puberty and fertility of male new zealand white rabbits orally administered with royal jelly or/and bee honey. *Journal of Animal and Poultry Production*, 6(4), 201-217.
- Liu, C.H., Chen, Y.M., Zhang, J.Z., Huang, M.Y., Su, Q., Lu, Z.H., Yin, R.X., Shao, G.Z., Feng, D., Zheng, P.L. 1982: Preliminary studies on influence of selenium deficiency to the developments of genital organs and spermatogenesis of infancy boars. *Acta Vet. Zootech. Sin.* 13, 73-77.
- Mahmoud, G.B., Abdel-Raheem, S.M., Hussein, H.A. 2013: Effect of combination of vitamin E and selenium injections on reproductive performance and blood parameters of Ossimi rams. *Small Ruminant Research*, 113(1), 103-108.
- Majeed, M.M., Al-Khashab, A.T. 2019: Effect of vitamin E and selenium injection on semen quality and some blood parameters. *Iraqi Journal of Veterinary Sciences*, 33(1), 17-24.
- Marounek, M., Dokoupilová, A., Volek, Z., Hoza, I. 2009: Quality of meat and selenium content in tissues of rabbits fed diets supplemented with sodium selenite, selenized yeast and selenized algae. *World Rabbit Science*, 17(4), 207-212.
- Mohamad Asrol, K., Abdul Rashid, B. 2017: Effect of vitamin E supplementation on semen quantity and quality of Local Kampong roosters. *Malaysian Journal of Animal Science*, 20(1), 37-43.
- Mohamady N.H.G. 2022: Using nano - particles to improve productive and reproductive performance of rabbits under egyptian summer conditions. P.H. Thesis, Faculty of Technology and Development Zagazig University.
- Momeni, H.R., Oryan, S., Eskandari, N. 2012: Effect of vitamin E on sperm number and testis histopathology of sodium arsenite-treated rats. *Reproductive biology*, 12(2), 171-181.
- Neild, D.M., Chaves, M.G., Flores, M., Miragaya, M.H., Gonzalez, E., Agüero, A. 2000: The HOS test and its relationship to fertility in the stallion. *Andrologia*, 32(6), 351-355.
- Pajovic, B., Dimitrovski, A., Radojevic, N., Vukovic, M. 2016: A correlation between selenium and carnitine levels with hypo-osmotic swelling test for sperm membrane in low-grade varicocele patients. *European review for medical and pharmacological sciences*, 20(4), 598-604.
- Plasson, S.J. 1975: *Veterinary obstetries and gynecology*. William and Wilson Company, 4th (Ed.), New York, USA, 415-486.
- Rao, M.V., Sharma, P.S.N. 2001: Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. *Reproductive Toxicology*, 15(6), 705-712.
- Raouf, S.M., Taha, A.T. 2021: Effects of COQ10 with vitamin E supplementation on semen quality and seminal plasma parameters of broiler breeder males. *Iraqi Journal of Veterinary Sciences*, 35(1), 65-70.
- Sabzian-Melei, R., Zare-Shahneh, A., Zhandi, M., Yousefi, A.R., Rafieian-Naeini, H.R. 2022:

- Effects of dietary supplementation of different sources and levels of selenium on the semen quality and reproductive performance in aged broiler breeder roosters. *Poultry Science*, 101(10), 101908.
- Safaa, H.M., Emarah, M.E., Saleh, N.F.A. 2008b: Seasonal effects on semen quality in black Baladi and white New Zealand rabbit bucks. *World Rabbit Sci.*, 16(1), 13-20.
- Safaa, H.M., Vicente, J.S., Lavara, R., Viudes de Castro, M.P. 2008a: Semen evaluation of two selected lines of rabbit bucks. *World Rabbit Science*, 16(3), 141-148.
- Safan, W.M.M. 2016: Effect of selenium and vitamin e on the immune response, reproductive and productive performance of rabbits. MSc. Thesis, Fac. Agric., Al-Azhar. Univ., Egypt.
- Shabani, S., Mehri, M., Shirmohammad, F., Sharafi, M. 2022: Enhancement of sperm quality and fertility-related parameters in Hubbard grandparent rooster fed diets supplemented with soybean lecithin and vitamin E. *Poultry Science*, 101(3), 101635.
- Sharaf, A.K., El-Darawany, A.A., Nasr, A.S., Habeeb, A.A.M. 2019: Recent techniques for amelioration the effect of heat stress conditions on male rabbits. *Zagazig Journal of Agricultural Research*, 46(2), 501-514.
- Smith, D.G., Senger, P.L., McCutchan, J.F., Landa, C.A. 1979: Selenium and glutathione peroxidase distribution in bovine semen and selenium-75 retention by the tissues of the reproductive tract in the bull. *Biology of reproduction*, 20(2), 377-383.
- Swathy, S.S., Panicker, S., Indira, M. 2006: Effect of Hexogenous Selenium on the Testicular Toxicity Induced by Ethanol in Rats. *Indian journal of physiology and pharmacology*, 50(3), 215-224.
- Underwood, E.J. Suttle, N.F. 1999: *The Mineral Nutrition of Livestock*: 3rd edition Pages: 1-16
- Wang, G., Wang, T. 2008: Oxidative stability of egg and soy lecithin as affected by transition metal ions and pH in emulsion. *Journal of Agricultural and Food Chemistry*, 56(23), 11424-11431.
- Watson, P.F. 1975: Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *The Veterinary Record*, 97(1), 12-15.
- Wheeler, M.J. 1995: The determination of bio-available testosterone. *Annals of clinical biochemistry*, 32(4), 345-357.
- Yousef, M.I., Abdallah, G.A., Kamel, K.I. 2003: Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal reproduction science*, 76(1-2), 99-111.
- Yue, D., Yan, L., Luo, H., Xu, X., Jin, X. 2010: Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. *Animal Reproduction Science*, 118(2-4), 217-222.
- Zeidan, A.E.B., El Baz., M.M., Ahmadi, E.A., Abdel Aziz, N.A., Abdel Salaam, A.M. 2008: Use of hypoosmotic swelling test for evaluating camel spermatozoal membrane integrity in relation to different solutions. *Veterinary Medical Journal*, 56 (1): 109-122
- Zhu, Z., Fan, X., Lv, Y., Zhang, N., Fan, C., Zhang, P., Zeng, W. 2015: Vitamin E analogue improves rabbit sperm quality during the process of cryopreservation through its antioxidative action. *PloS one*, 10(12), e0145383.

Table 1: Ambient temperature, relative humidity during the experimental treatments.

Treatment period	AT (°C)	RH (%)
15 th November to 30 th November	21.7	65
1 st December to 31 st December	20.36	63
1 st January to 31 st January	21.33	57
1 st February to 28 th February	19.35	44
1 st March to 24 th March	22.5	61

Table 2: Chemical analysis of the components of the basic feed used in feeding male rabbits.

Items	Ingredient (g/kg)	Items	Chemical analysis (%)
Clover hay	400	Crude protein (CP)	17.00
Barley grain	125	Crude fiber (CF)	15.43
Wheat brain	145	Ether extract (EE)	2.60
Soybean meal (44% crude protein)	180	Nitrogen free extract (NFE)	57.10
Yellow corn	130	Ash	9.20
Di-calcium phosphate	8	Digestible energy (kcal/kg)	2490
Limestone	5	Calcium (Ca)	1.23
DL-Methionine	1	Total phosphorus (P)	0.60
Sodium chloride	3	Lysine	0.95
Vitamins + minerals premix ¹	3	Methionine	0.46
		Sodium	0.16

¹The vitamin and mineral premix/kg contained Vitamin A, 6,000 IU; Vitamin D3, 900 IU; Vitamin K3, 2 mg; Vitamin B1, 2 mg; Vitamin B2, 4 mg; Vitamin B6, 2 mg; Pantothenic acid, 10 mg; Vitamin B12, 0.01 mg; Niacin, 50 mg; Folic acid, 3 mg; Biotin, 0.05 mg; Choline, 250 mg; Fe, 50 mg; Mn, 85 mg; Cu, 5 mg; Co, 0.1 mg; I, 0.2 mg; and Zn, 50 mg pre/kg feed. The total experimental period was 7 days adaptation period, and 4-month experimental period.

Table 3: Effect of organic selenium and vitamin E and their combinations on body weight in V-line male rabbits.

Item	Treatments			
	Control (T1)	Selenium (T2)	Vitamin E (T3)	Se + Vit. E (T4)
Initial weight, (Kg)	2.27±0.04	2.27±0.05	2.27±0.04	2.27±0.06
Final weight, (Kg)	3.21±0.19	3.20±.14	3.12±0.04	3.22±0.10

Table 4: Effect of addition to organic selenium and vitamin E and on physical of semen characteristics and testosterone hormone concentration.

Items	Treatments			
	Control (T1)	Selenium (T2)	Vitamin E (T3)	Selenium + Vit. E (T4)
Ejaculate V. (ml)	0.66±0.10 ^c	0.78±0.02 ^b	0.79±0.01 ^b	0.85±0.01 ^a
Sperm Conc. (×10 ⁶ /mL)	366.46 ± 9.23 ^c	474.65 ± 11.25 ^b	451.46 ± 10.05 ^b	514.55 ± 9.55 ^a
Total Motility (%)	72.38 ± 1.03 ^c	79.00 ± 1.09 ^b	76.63 ± 1.36 ^{ab}	81.88 ± 1.05 ^a
Progressive Motility (%)	78.12 ± 1.05 ^c	82.62 ± 1.07 ^b	81.12 ± 1.22 ^{bc}	85.87 ± 1.17 ^a
Dead Sperms (%)	20.13 ± 0.34 ^c	16.60 ± 0.94 ^b	17.23 ± 0.43 ^b	12.20 ± 0.72 ^a
Abnormal Sperms (%)	18.28 ± 0.68 ^b	16.60 ± 0.61 ^{ab}	15.63 ± 0.52 ^a	14.88 ± 0.63 ^a
Acrosome Damages (%)	18.73 ± 0.53 ^c	17.35 ± 0.53 ^{bc}	16.40 ± 0.57 ^{ab}	14.88 ± 0.58 ^a
HOS-test (%)	78.08 ± 0.91 ^c	81.78 ± 1.34 ^b	80.30 ± 1.09 ^{bc}	84.83 ± 0.83 ^a
Testosterone H. (ng/mL)	1.35 ± 0.39 ^c	3.67 ± 0.49 ^b	2.99 ± 0.41 ^b	5.74 ± 0.55 ^a

a, b and c Means with the different superscripts in the same raw, differ significantly (P<0.05).

إضافة السيلينيوم وفيتامين هـ إلى النظام الغذائي وتأثيره على وزن الجسم وخصائص السائل المنوي وهرمون التستوستيرون في أرانب-السلالة V

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الملخص العربي

يهدف البحث إلى دراسة تأثير إضافة المكملات الغذائية من السيلينيوم وفيتامين هـ على وزن الجسم والأداء التناسلي في الأرانب. إجمالي عدد الأرانب الذكور المستخدمة 80 ذكر في عمر شهرين وكان متوسط وزن الجسم الحي في بداية التجربة 2.27 كجم. تم إيواء الحيوانات بشكل منفصل في أقفاص سلكية، تم التحكم في فترة الإضاءة للإظلام لتكون 16 ساعة من الضوء و8 ساعات من الإظلام. تم تقسيم الحيوانات بشكل عشوائي إلى ما يلي: أربع معاملات (ن = 20 لكل منها): المعاملة الأولى: مجموعة التحكم التي تتغذى على النظام الغذائي الأساسي، المجموعة الثانية (المعاملة): تتغذى على الغذاء الأساسي + 0.1 مجم سيلينيوم عضوي / كجم علف، المجموعة الثالثة (المعاملة): تتغذى على الغذاء الأساسي + 40 مجم من فيتامين هـ والمجموعة الرابعة (المعاملة): عبارة عن خليط ما بين المعاملة الثانية والثالثة. يحتوي الغذاء الأساسي على 18٪ بروتين و2850 طاقة. تم تسجيل وزن الجسم، جمع وتقييم السائل المنوي، حجم السائل المنوي، الحركة الكلية للحيوانات المنوية، النسبة المئوية للحركة التقدمية للحيوانات المنوية، النسبة المئوية للحيوانات المنوية الميتة (٪)، النسبة المئوية للحيوانات المنوية غير الطبيعية (الشاذة) (٪)، النسبة المئوية لتلف الأروسوم، النسبة المئوية لاختبار انتفاخ ذيل الحيوان المنوي (HOS-test) (٪)، وتركيز هرمون التستوستيرون. يمكن تلخيص النتائج كالتالي: التغيرات في متوسطات وزن الجسم لم تكن معنوية ما بين المعاملات. بينما كانت متوسطات حجم السائل المنوي أعلى معنوياً ($P < 0.05$). كانت النسبة المئوية للحيوانات المنوية الميتة، والنسبة المئوية للحيوانات المنوية الشاذة، واختبار HOS، أقل معنوياً في مجموعات الأرانب المضاف إليها المكملات الغذائية. نستخلص من هذه الدراسة، إضافة السيلينيوم وفيتامين هـ إلى علائق الأرانب.

الكلمات الاسترشادية: الأرانب، وزن الجسم، خصائص السائل المنوي، التستوستيرون، الأداء التناسلي.