



## Effect of dietary supplementation of vitamin E on semen quality in White Pekin drakes<sup>#</sup>

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### Abstract

*The present study was conducted to evaluate the effect of dietary supplementation of vitamin E on semen quality in White Pekin ducks. The drakes were randomly assigned to three experimental groups at 20 weeks of age based on the treatments followed, as T1 (Basal diet), T2 (Basal diet + 100 mg vitamin E/kg diet), and T3 (Basal diet + 200 mg vitamin E/kg diet). Semen was collected once in four days and was subjected to various microscopic and macroscopic parameters. There was no significant difference in semen volume and appearance score among the experimental treatments. The pH of semen was found slightly alkaline in all three treatments. Most samples were white in colour and were medium thick or thick in consistency. The major contaminants observed were faeces and uric acid crystals. Significantly ( $p < 0.05$ ) higher progressive motility per cent and motility score was observed in T3 compared to control group and the values of T2 were comparable. There was no significant difference in concentration, viability and sperm abnormality among the three treatments. The sperm HOS response per cent was significantly ( $p < 0.05$ ) higher in T3 and T2 compared to control group. Dietary addition of vitamin E enhanced the semen quality by eliminating the free radicals.*

**Keywords:** Semen, ducks, Vitamin E

Duck rearing is an emerging animal husbandry sector that occupies a key position next to chicken farming in India. Exotic breeds of ducks, mainly White Pekin and its crosses are commonly used for meat production. Semen quality is a crucial factor in predicting the fertility rates and the reproductive performance of males. Avian spermatozoa are abundant in polyunsaturated fatty acids (PUFAs), mainly docosatetraenoic acid and arachidonic acid. The high proportions of PUFAs are essential for sperm mobility and sperm-egg fusion in female reproductive system. However, because of their high levels of polyunsaturated fatty acids, avian sperms are very sensitive to reactive oxygen species (ROS), causing oxidative stress (Ebeid, 2012).

Oxidative stress affects the gamete numbers, reduces sperm motility and reduces the percentage of live spermatozoa (Surai *et al.*, 1998). Studies suggest that an increased antioxidant status of semen or sperm can be achieved by increasing the antioxidant content of the diet. Vitamin E is considered as the main chain-breaking antioxidant present in the biological membranes and its supplementation has been found to reduce the harmful effects of lipid peroxidation

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on semen quality and fertility. However, the information on vitamin E supplementation on semen quality in drakes is very scanty. Hence the present study was conducted to assess the effect of supplementation of various levels of vitamin E on various semen quality parameters in White Pekin drakes.

## Materials and methods

Twenty-four White Pekin drakes of 20 weeks of age were utilised for the study. These drakes were randomly assigned to three experimental groups at 20 weeks of age as T1, T2 and T3. The drakes of the group T1 was fed with basal diet, T2 and T3 with basal diet supplemented with 100 and 200 mg vitamin E/kg diet, respectively. The birds were maintained on the experimental diet for a period of two weeks before the trial. Semen from the experimental drakes was collected once in four days using abdominal massage technique from 26 weeks of age.

The semen was collected from drakes by abdominal massage method (Lake, 1985) and the semen volume was measured immediately after collection. Based on colour, the semen samples were classified into yellowish white, white and chalky white and the semen samples were categorized into watery, medium thick, thick and very viscous based on consistency and the per cent of samples belonging to each group was calculated. The appearance of semen was scored from 1 to 5 by visual examination as described by Mc Daniel and Craig (1959). The pH of fresh semen was measured using narrow range pH paper [Merck pH indicator paper with colour scale (pH 6.5-9)]. The semen samples were examined for the presence of possible contaminants like blood, faecal matter or uric acid crystals by visual examination and the per cent of samples with the different contaminants were calculated.

The progressive motility per cent was estimated on the basis of swirling oscillation of semen and was scored as per Wheeler and Andrews (1943). The concentration of spermatozoa was estimated as per Raghavendra *et al.* (2022) and Sutiyono *et al.* (2021) by using Neubauer's hemocytometer. The sperm viability was estimated as per Raghavendra *et al.* (2022) after eosin-nigrosine staining and the per cent of live spermatozoa was calculated (Fig 1). The smear prepared for sperm viability was used to estimate sperm abnormality and the abnormality percentage was calculated (Fig. 2).

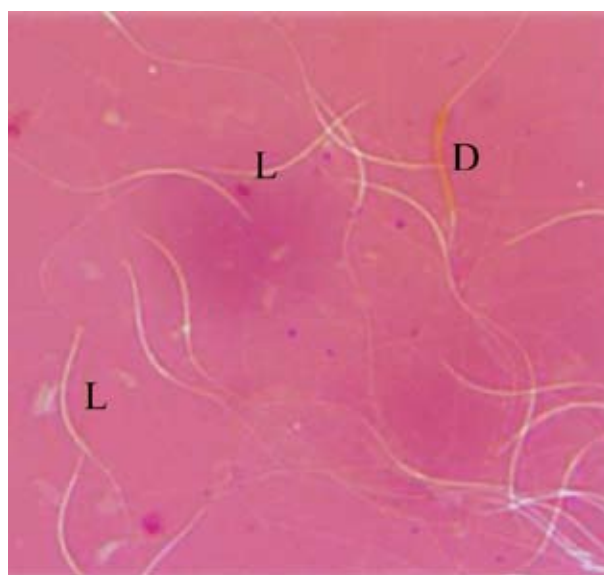
Sperm hypo-osmotic swelling (HOS) response as described by Chauhan *et al.* (2017) was performed to assess the functional status of sperm membrane. A volume of 100  $\mu$ L semen was incubated at a temperature of 37 °C for one hour in hypo-osmotic test solution and iso-osmotic (control) solution. The per cent of reacted spermatozoa was calculated from the smears prepared from test and control solutions and the total per cent of hypo osmotic reactive spermatozoa was calculated by subtracting the

per cent of reacted spermatozoa in the control from that in the test (Fig. 3). Three collections were made from each drake at four day intervals. The mean of the three observations was calculated for each trait in each drake and the mean values in each treatment were calculated. Data collected on various parameters were analysed using SPSS (version 24.0).

## Results and discussion

### Macroscopic evaluation

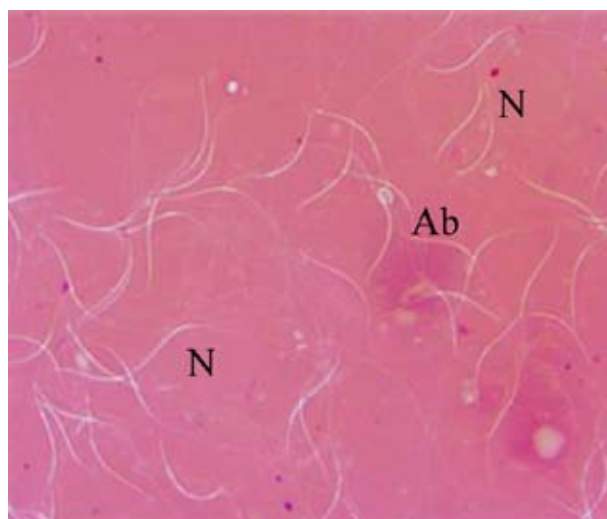
The mean semen volume of drakes in T1, T2 and T3 was 0.25, 0.24 and 0.27 mL, respectively. The mean semen volume observed in the present study agrees with the findings of Surai and Wishart (1996) in White Pekin drakes and Etuk *et al.* (2006) in Muscovy ducks reared



**Fig. 1.** Eosin-nigrosine stained smear of White Pekin drake - Live (L) and Dead (D) spermatozoa (1000  $\times$ )



**Fig. 2.** Eosin-nigrosine stained smear of White Pekin drake - HOS responsive (coiled-C) spermatozoa (1000  $\times$ )



**Fig. 3.** Eosin-nigrosine-stained smear of White Pekin drake Abnormal (Ab) and Normal (N) spermatozoa (1000 ×)

under intensive system. However, the volume observed in the present study is lower than the findings of Nahak *et al.* (2015) and is higher than that reported by Stella (2011) in White Pekin drakes.

The major semen colours of drakes in T1, T2 and T3 were white (54.17, 62.50 and 70.83 per cent, respectively), chalky white (33.33, 20.83 and 12.50 per cent, respectively) and yellowish white (12.50, 16.67 and 16.67 per cent, respectively). The semen colour observed in the present study is in line with the reports of Stella (2011) in White Pekin drakes, Penfold *et al.* (2001) in Northern Pintail ducks, Nair (2003) in Austra-white chicken and Nahak *et al.* (2015) in White Pekin drakes. White and chalky white colour is indicative of good quality semen while yellowish white colour of semen might be due to faecal contamination.

The consistency of raw semen in T1, T2 and T3 was viscous (20.83, 16.00 and 8.33 per cent, respectively), thick (41.17, 25.00 and 25.00 per cent, respectively), medium thick (29.17, 37.50 and 50.00 per cent, respectively) and watery (8.33, 20.83 and 16.67 per cent, respectively). This is in agreement with the findings of Humphreys (1972), Nair (2003) and Stella (2011). The

semen samples with medium thick or thick consistency can be considered as good quality semen.

The mean semen appearance score of drakes in T1, T2 and T3 was 3.53, 3.39 and 3.29, respectively. The present findings on appearance score are in agreement with that the reports Stella (2011) in White Pekin drakes and Kundu and Panda (1990) in White Leghorn cocks reared under hot and humid environment. Contrary to the present findings, Stunden (1996) reported a lower appearance score in Mallard drakes and Haunshi *et al.* (2011) reported higher appearance score in Aseel and Kadaknath birds. Stella (2011) reported that the appearance of semen depends on the concentration of spermatozoa as well as quantity of the seminal fluid and lower values in appearance score might be due to the addition of lymphatic exudates from phallic folds.

The mean semen pH of drakes in T1, T2 and T3 was 7.53, 7.54 and 7.53, respectively. The semen pH observed in the present study is similar to the findings of Nahak *et al.* (2015) and Stella (2011) in White Pekin drakes. The semen pH values observed in supplemented treatments of present study agrees with the findings of Danikowski *et al.* (2002) in cockerels fed with vitamin E. The reports of Akshay (2022) revealed that acidic pH was detrimental to sperm viability. The pH of semen from White Pekin drakes observed in all the treatments in the present study was slightly alkaline and hence could be considered as ideal. No significant difference was observed in semen volume, appearance score and semen pH between the treated and control group.

The major semen contaminant in all three treatments was faeces. In T1, 20.83 per cent of the ejaculates were contaminated with faeces. The predominant semen contaminants in T2 were faeces (45.83 per cent) followed by 12.50 per cent of uric acid crystals. In T3, 29.17 per cent of the ejaculates were contaminated with faeces and 4.17 per cent ejaculates were contaminated with uric acid crystals. The results of present study are in agreement with the findings of Stella (2011), Kasai *et al.* (2001) and Penfold *et al.* (2001). The contamination of semen with faeces, uric acid crystals and blood could decrease

**Table 1.** Microscopic semen quality attributes of White Pekin drakes supplemented with vitamin E

Parameters	Treatments			P-value
	T1 (Control)	T2 (100 mg Vit E)	T3 (200 mg Vit E)	
Progressive motility (per cent)	81.17 <sup>b</sup> ± 1.22	85.42 <sup>ab</sup> ± 1.76	86.35 <sup>a</sup> ± 1.70	0.05*
Motility score	3.48 <sup>b</sup> ± 0.07	3.88 <sup>ab</sup> ± 0.11	3.98 <sup>a</sup> ± 0.12	0.05*
Sperm concentration (billions/mL)	2.10± 0.04	2.23± 0.05	2.17± 0.06	0.19 <sup>ns</sup>
Sperm viability (per cent)	87.88± 1.08	88.97 ± 0.72	88.99 ± 1.36	0.71 <sup>ns</sup>
Sperm abnormality (per cent)	7.18 ± 0.60	7.17± 0.66	8.43 ± 1.56	0.61 <sup>ns</sup>
Sperm HOS response (per cent)	85.20 <sup>b</sup> ± 0.83	87.87 <sup>a</sup> ± 0.93	88.17 <sup>a</sup> ± 0.83	0.03*

Mean values bearing different superscripts in the same row differ significantly

\* significant ( $p < 0.05$ ) ns-non-significant

the fertilising capacity of spermatozoa in drakes. The increased water content in the droppings of ducks might contribute to the increased chance of contamination. Feed withholding for at least 12h prior to semen collection might help to minimise faecal contamination.

### Microscopic evaluation

The results of microscopic evaluation of White Pekin drakes in experimental groups are presented in Table 1. Significantly ( $p < 0.05$ ) higher progressive motility per cent and motility score was observed in T3 (200 mg vitamin E/kg basal diet), when compared to control group (T1). The progressive motility per cent and motility score of T2 (100 mg vitamin E/kg basal diet) was comparable with that of T1 and T2. The progressive motility observed in the present study agrees with the findings of Fattah (2013) in White Pekin drakes. The present findings are in close agreement with the motility score reported by Ebeid (2012). Amevor *et al.* (2022) also reported that roosters supplemented with 200 mg vitamin E showed higher sperm motility score, which is similar to that of T3 in this study. The results of the present study revealed that supplementation of vitamin E produced a beneficial effect on the progressive motility and motility score of White Pekin drakes.

No significant difference was observed in sperm concentration, sperm viability and sperm abnormality between the treated and control group. The mean sperm concentration values obtained for White Pekin drakes in the control group of the present study are comparable with the findings of Surai and Wishart (1996) and Stella (2011) in White Pekin drakes. The present findings are in accordance with the results obtained by Biswas *et al.* (2009) in Kadaknath birds. Contrary to the present findings, Ebeid (2012) revealed increase in sperm concentration in roosters supplemented with vitamin E. The mean values of live spermatozoa in present study are similar to the findings of Kamar (1962) and Ghonim *et al.* (2009), while the values are lower than that reported by Stella (2011) in White Pekin drakes and Giri *et al.* (2016) in Muscovy drakes. The present findings are in line with the results obtained by Biswas *et al.* (2009) in Kadaknath birds. Contrary to the present findings, Ebeid (2012) revealed increase in live spermatozoa in roosters supplemented with vitamin E. The mean values of abnormal spermatozoa in present study are similar to the findings of Stella (2011) and Nahak *et al.* (2015) in White Pekin drakes. Contrary to the present findings, Biswas *et al.* (2009) and Ebeid (2012) revealed decrease in abnormal spermatozoa in roosters supplemented with vitamin E. From the results, it could be concluded that addition of vitamin E did not influence the sperm concentration, sperm viability and sperm abnormality of semen in White Pekin drakes.

Significantly ( $p < 0.05$ ) higher sperm HOS response per cent was observed in T3 (200 mg vitamin E/kg

basal diet) and T2 (100 mg vitamin E/kg basal diet), when compared to control group (T1). The mean percentage of HOS response observed in White Pekin drakes fed with basal diet in the present study is similar to that of Giri *et al.* (2016) in White Pekin drakes. The mean HOS response recorded in the present study is comparable with the values of Shanmugam *et al.* (2014), Chauhan *et al.* (2017) and Akshay (2022) in various chicken breeds, while the values are higher than the reports of Santiago *et al.* (2009) in broiler roosters. Sperm swelling under hypo-osmotic conditions indicates normal integrity and functional activity of sperm plasma membrane. From the results, it could be inferred that the sperm plasma membrane integrity was good in drakes in T2 and T3.

### Conclusion

The present study revealed that the supplementation of 200 mg vitamin E/kg basal diet produced a beneficial effect on the progressive motility, motility score and hypo-osmotic swelling response of spermatozoa in White Pekin drakes. The semen volume, appearance score, sperm concentration, sperm viability and per cent of abnormal sperms were comparable in all the treatments. It could be concluded that, dietary addition of vitamin E enhanced the semen quality by eliminating the free radicals. However, vitamin E supplementation alone could not completely eliminate all free radicals.

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### Conflict of interest

The authors declare that they have no conflict of interest

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