Research Article

Effect of Dietary Antioxidant Supplementation on the Fertility Parameters of Turkeys

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Abstract | Twenty four turkey toms, selected at 32 weeks of age were randomized into four treatment groups (TI, T2, T3 and T4). T1 received basal male breeder turkey diet containing 2,700 k.cal ME per kg of diet and 16 % CP without supplemented Vit-E, T2 received basal diet supplemented with 300 IU Vit-E/kg diet, T3 received basal diet supplemented with 0.3 mg of inorganic Selenium/kg diet and T4 received basal diet supplemented with 150 IU Vit-E and 0.15mg Selenium /kg diet. Hens were fed with standard female turkey breeder diet containing 2,900 k.cal ME per kg of diet and 16 % CP. Pooled semen samples were collected from each treatment group was diluted with BPSE diluent at 1:4 ratio and were artificially inseminated into the hens of respective groups with a dose 0.2 ml of diluted semen per hen. Hatching eggs were collected, incubated and fertility parameters were recorded. All the three antioxidant supplemented groups shown a significant (P≤0.01) improvement in the total fertility and total hatchability percentage of turkey eggs with the combination group giving the better fertility and total hatchability percentages comparing to the Vit-E supplemented group or Selenium supplemented group. Fertile hatchability percentage and late embryonic mortality percentage did not shown (P>0.05) any significant effect due to dietary addition of antioxidants. The study thus revealed better reproductive performance with the antioxidant supplemented group which can be implemented in commercial turkey breeding programmes for better reproductive performance.

Keywords | Vitamin E, Selenium, Turkey tom, Fertility

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INTRODUCTION

One of the main objectives in turkey breeder production is to increase the number of poults per tom. Egg yield in turkeys are lower than that of other poultry species. In addition to low egg yield, unsatisfactory egg fertility and hatchability constitute a major problem for turkey breeding enterprises (Ozcelik et al., 2009). Avian spermatozoa are rich in long chain polyunsaturated fatty acids (PUFA), in particular the docosatetraenoic acid and arachidonic acid. This high proportion of PUFA provides membrane flexibility, which is very essential for spermatozoa motility and sperm-egg fusion. However, because of high level of PUFA, avian sperms are very sensitive to reactive oxygen species, a major cause for male infertility. The resulting adverse effect may be partially controlled by dietary supplementation of certain beneficiary compounds, including vitamins and antioxidants. Therefore, an increased antioxidant status in semen or spermatozoa is a pre-requisite for the prevention of male infertility.

Selenium, a trace mineral is considered as an integral part of at least 25 selenoproteins including glutathione peroxidase, which protects the spermatozoa against oxidative

damage. Selenium can be found in common feed ingredients, but its concentration varies substantially. Selenium concentration of grains is less than 0.1 ppm. According to National Research Council (NRC), 1997; breeder turkey requires 0.2 mg of Selenium per kg of diet for their optimum performance. But, the Selenium requirement is quite low as per NRC, 1994. However those data are not related to commercial conditions and it seems that the Selenium requirement of commercial poultry is much higher for optimum performance. The dietary requirement of Vit-E in the poultry diet is highly variable. The NRC, 1994, recommended 25 IU Vit-E/kg of diet for breeder turkeys for optimum performance. However, because oil refining process removes most of the Vit-E, commercially used oils like soya, maize and sunflower oils are deficient in Vit-E. In the poultry industry Vit-E concentration in feed ingredients is not analyzed and supplemental Vit-E is the main source of the same for poultry. Vit-E interacts with Selenium and both plays a vital role in the maintenance of reproductive function as well as in the reduction of reactive oxygen species and free radical generation. Recently, it has been shown that Vit-E can be effective only in an optimal balance with Selenium. Vit-E performs only half the job of preventing lipid peroxidation by scavenging free radicals and forming hydro peroxides.

Keeping this in view, this study was therefore aimed at evaluating the effect of dietary supplementation of Selenium and Vit-E on the fertility parameters of male breeder turkey following Artificial Insemination.

MATERIALS AND METHODS

The study was conducted using 24 male and 48 female Belts-ville small white cross variety of turkeys to find the effect of dietary supplementation of either Selenium or Vit-E alone and their combination on the fertility and hatchability following insemination of turkey hens using the semen collected from the toms of respective groups. This study was conducted at Poultry Research Station, Madhavaram Milk Colony, TANUVAS, Chennai- 600 051.

Selection of Breeder Toms and Hens

Twenty four Belts-ville small white cross toms belonging to the same hatch with uniform body weight were selected at 32 weeks of age. Selection was done based on their physical appearance by recognizing the bird's vigor and health. Similarly, fourty eight turkey hens belonging to the same hatch were selected at 20 weeks of age based on the phenotypic characters by measuring the distance between the pelvic bones and the distance from the end of breast bone to the pelvic bone.

Management of Experimental Birds and Experimental Design

The selected toms were reared under deep litter system with a floor space of 5 sq.ft/tom. They were provided with *ad-libidum* water and turkey breeder mash containing 2,700 k.cal/kg metabolizable energy (ME) and 16 per cent crude protein (CP). The birds were subjected to 12 hours of lighting period per day with good ventilation. Turkey hens were also housed under deep litter system with a floor space of 4 sq.ft/bird and fed with a turkey breeder layer mash containing 2,900 k.cal. ME and 16 per cent CP per kg of diet. Lighting period of 14 hrs/day was maintained throughout the study period. The design of experiment was presented in Table 1.

Table 1: Experimental Design

Treatment number	Turkey toms		No. of Tukey hens
	Number	Feed	
T1	6	Standard male turkey breeder diet with 2,700 k.cal. ME and 16 per cent of CP per kg of diet.	12
T2	6	Standard male turkey breeder diet added with 300 IU of Vit-E/kg of the diet.	12
T3	6	Standard male turkey breeder diet added with 0.3 mg inorganic Selenium/kg of diet.	12
T4	6	Standard male turkey breeder diet added with 150 IU of Vit-E+0.15 mg inorganic Selenium/kg of diet.	12

Mixing of Nutrients in the Feed

In this study, Selenium and Vit-E are supplemented in the standard male turkey breeder ration in the form of sodium selenite and alpha (α)-tocopherol acetate respectively. Mixing of the above nutrients was done slowly and thoroughly by hand mixing after grinding it with a small quantity of sample feed. The inclusion level of each feed ingredients among the treatment diets were shown in the Table 2.

Semen Collection

All the twenty four toms were trained individually in the early morning by abdominal massage technique (Burrows and Quinn, 1937). The first semen collection was initiated two weeks after the commencement of feeding experimental diet to the toms. The feathers around the vent were trimmed off periodically for easy collection of semen. The vent area was cleaned with normal saline, wiped with sterile cotton and kept dry before semen collection to prevent the contamination of semen by the adhered dirt or droppings. Semen was collected during early hours of the day with a

frequency of twice a week. Pearly white drop ofejaculate was found to ooze out of the phallus after gentle pressure at the sides of the phallus using the thumb and the index finger. The semen was then immediately aspirated by using a sterile glass tuberculin syringe and then transferred into a sterile micro-centrifuge tube kept in a chill water bath at 18-20° C for further dilution and insemination.

Table 2: The inclusion level of feed ingredients and nutrients among thevarious treatment diets

S. No.	Feed Ingredients	Inclusion Level(%)			
		Treatment-1	Treatment-2	Treatment-3	Treatment-4
1	Maize	35	35	35	35
2	Bajra	7	7	7	7
3	Wheat	10	10	10	10
4	Oats	5	5	5	5
5	Deoiled rice bran	18.75	18.75	18.75	18.75
6	Sunflower oil cake	4	4	4	4
7	Soyabean meal	15.25	15.25	15.25	15.25
8	Min mix	3.5	3.5	3.5	3.5
9	Grit	0.5	0.5	0.5	0.5
10	Salt	0.25	0.25	0.25	0.25
11	Vitamin Premix	0.75	0.75	0.75	0.75
12	Total	100	100	100	100
13	**Vit E/ kg (IU)**	-	**300**	-	**150**
14	**Selenium (mg/ kg)**	-	-	**0.3**	**0.15**

Semen Dilution

Belts-ville Poultry Semen Extender (BPSE) diluent was used to extend the raw semen before insemination. The composition of the BPSE diluent (Sexton and Giesen, 1982) was presented in Table 3. Dilution was done by mixing one part of raw semen with three part of diluent to obtain a dilution ratio of 1:4.

Artificial insemination of turkey hens

The pooled semen from the toms of each treatment group was utilized to inseminate the hens of respective group. The insemination was carried out during the evening hours after 5.00 P.M within an hour following collection and dilution of semen. Forty eight hens were totally inseminated with 0.2 ml of diluted semen intra vaginally using a sterile graduated glass tuberculin syringe, after manual eversion of the vagina. The semen was deposited slowly into the vagina at a depth of about 3 cms following release of pressure from the pelvic region, without stress to the bird. Insemination was done twice in a week in all the four groups. Artificial insemination (AI) was carried out for a period of 12 weeks in each group of hens.

Table 3: Composition of Diluents

Component	g/100 ml
Sodium glutamate (monohydrate)	0.867
Fructose	0.500
TES buffer	0.195
Potassium citrate (monohydrate)	0.064
Potassium diphosphate (trihydrate)	1.270
Potassium monophosphate	0.065
Sodium acetate (anhydrous)	0.430
Magnesium chloride (Octahydrate)	0.034
Deionised triple distilled water	100 ml
pH	6.5
Osmolarity (mos/kg H_2O)	353

Collection and Incubation of Hatching Eggs

Hatching eggs were collected from each group of hens after two weeks of first insemination and was continued till a week after last insemination. The egg was collected thrice in a day to prevent dirty eggs and was labeled appropriately to identify the treatment group and the date of lay. After collection, the eggs were fumigated using formaldehyde and potassium permanganate and were stored in the egg storage room at a temperature of 18° C and 75 per cent relative humidity for a maximum period of one week and were then collectively set in the incubator on the same day. Totally the eggs laid over a period of 10 weeks were utilized for the study.

Egg Break Open Study

On the 24th day, the eggs from the setter were candled and placed in the pedigree box treatment wise and were transferred to the hatcher. On the 28th day, the hatch was taken out and the percentage of live viable chicks was recorded. On the next day of hatching, unhatched eggs were subjected to break open study to record the early or late embryonic mortality.

Fertility and Hatchability

Fertility was determined by reducing the number of infertiles from the total number of egg set. Fertile hatchability was calculated as total poults hatched out of total fertile eggs set and the total hatchability was calculated as total poults hatched out of total eggs set.

Altogether, ten hatches were utilized for the hatchability study in which the number of infertiles, early embryonic mortality, late embryonic mortality and the live hatched chicks were recorded treatment wise and expressed in percentage.

Table 4: Effect of dietary supplementation of Vitamin E and Selenium on per cent fertility in turkey (Mean±SE)

Parameter	Treatment-1 (Control)	Treatment-2 (Vit-E diet)	Treatment-3 (Selenium)	Treatment-4 (Combination)	F Value
Fertility	65.61^{c}±1.24	73.96^{a}±1.37	69.91^{b}±1.14	74.96^{a}±1.16	11.42**
Total Hatchability	50.49^{c}±1.75	59.54^{a}±0.94	54.42^{b}±0.88	62.31^{a}±0.98	19.42**
Fertile Hatchability	77.10±2.68	80.71±1.75	77.99±1.59	83.29±1.84	2.01^{NS}
Early Embryonic Mortality	5.31^{b}±0.53	3.67^{ab}±0.82	5.70^{b}±0.41	3.05^{a}±0.95	3.452*
Late Embryonic Mortality	9.80±1.53	10.74±1.30	9.78±1.46	9.60±1.13	0.07^{NS}

Means bearing at least one common superscript in a row does not differ significantly ** Highly significant (P≤0.01), *Significant (P≤0.05), ^{NS} Non significant (P>0.05)

STATISTICAL ANALYSIS

Data were analyzed with analysis of variance (ANOVA) as per Snedecor and Cochran, 1994; in SPSS, version 20.0 for windows. When significant difference (P<0.05) was detected the Dunccan multiple range test was used to separate the mean value.

RESULTS

Effect of Dietary Vit- E and Selenium on Fertility Parameters

The dietary effect of Vit-E, Selenium and its combination on the per cent fertility, hatchability, fertile hatchability, early embryonic mortality and late embryonic mortality of turkey eggs are presented in the Table 4.

Percent Fertility

Dietary supplementation of Vit-E, Selenium and its combination to the turkey toms significantly (P≤0.01) affected the per cent fertility of turkey eggs. Maximum mean fertility percentage of 74.96±1.16 was observed in the group fed with the combination of Vit-E and Selenium. Comparable results were also observed in the group supplemented with Vit-E alone (73.96±1.37). However, the group fed with the dietary supplementation of Selenium shown significantly lower fertility percentage compared to other antioxidant supplemented groups (69.91±1.14). The control group recorded a significantly lower fertility percentage (65.61±1.24) than other groups.

Percent Total Hatchability

Per cent total hatchability was significantly affected by different dietary treatment in the study. The group of toms supplemented with either Vit-E or the combination of Vit-E and Selenium had shown a significantly higher (P≤0.01) mean per cent total hatchability percentage of turkey eggs with corresponding values of 59.54±0.94 and 62.31±0.98, respectively. Significantly lower value was observed in the group supplemented with Selenium (54.42±0.88 per cent). The control group fed with the basal diet recorded the least per cent total hatchability (50.49±1.75).

Percent Fertile Hatchability

Dietary supplementation of Vit-E, Selenium or its combination did not show any significant effect (P>0.05) on the per cent hatchability of fertile eggs set in the study. However, maximum mean per cent fertile hatchability was observed in the group fed with the diet supplemented with the combination of Vit-E and Selenium (83.29±1.84) followed by the group supplemented with Vit-E alone (80.71±1.75 per cent). Lower per cent fertile hatchability was observed in the control group (77.10±2.68).

Percent Early Embryonic Mortality

Dietary enrichment of Vit-E and Selenium significantly (P≤0.05) affected the early embryonic mortality percentage of turkey eggs in this study. The group fed with the diet supplemented with the combination of Vit-E and Selenium had shown significantly (P≤0.05) lower average early embryonic mortality percentage (3.05±0.95). However, comparable results were observed in the group fed with the diet supplemented with Vit-E alone (3.67±0.82 per cent). No significant difference was observed between the control (5.31±0.53%) and the group supplemented with Selenium (5.7±0.41 per cent).

Percent Late Embryonic Mortality

The per cent late embryonic mortality of turkey eggs did not show (P>0.05) any significant effect due to dietary supplementation of Vit-E, Selenium or the combination of both. However numerically lower values were observed in the group fed with the combination of both the antioxidants (9.60±1.13).

DISCUSSION

Percent Fertility

Dietary supplementation of antioxidants significantly

(P≤0.01) improved the total fertility of turkey eggs in this study. Maximum mean fertility percentage of 74.96±1.16 was observed in the group fed with the combination of Vit-E and Selenium. Significant (P≤0.01) increase in the fertility percentage was also observed in the Vit-E supplemented group. Complimentary results were also recorded by (Biswas et al., 2009) where the authors observed an increase in the fertility percentage from 81.24±2.12 to 93.45±3.89 in Kadaknath cockerels by the dietary supplementation of Vit-E at 150 IU/kg of diet. Similar results were also observed by (Marzoni et al., 2000) in open air reared Pheasants. In contrast, (Lin et al., 2005; Andi et al., 2006; Zaniboni et al., 2006) observed non significant effect by dietary Vit-E supplementation on per cent fertility in Taiwan native cockerels, broiler breeders and BUT, respectively. Hence, the improved fertility in the Vit-E supplemented group may be derived from the intracellular antioxidant effect of the supplemented Vit-E in protecting the spermatozoa membrane and thus facilitated better sperm-egg fusion.

In this experimental study, dietary addition of in-organic Selenium to the toms significantly (P≤0.01) increased the fertility of turkey eggs compared to the control group. This result agrees with the previous findings of (Maysa et al., 2009) where the dietary enrichment of Selenium significantly increased the mean fertility percentage in chicken. The increase in mean fertility percentage in the current study may be attributed to the spermatozoa's membrane protective role of various seleno-proteins and enzymes through its antioxidant effect which improved the quality of tom's spermatozoa which would have increased the sperm-egg interaction that ultimately increased the fertility rate. But the efficiency of interaction was less when compared with the dietary supplementation of Vit-E alone or in combination with Selenium.

The group of toms supplemented with the dietary combination of Vit-E and Selenium significantly (P≤0.01) improved the mean fertility percentage in this trial. Similar result was also obtained by (Ali et al., 2013) in Japanese Quails where the authors observed an increase in per cent fertility from 82 to 88 by the supplementation of combination of Vit-E and Selenium above the recommended levels through drinking water. This underlines the beneficial effect of Vit-E in protecting the spermatozoa structures by forming a strong antioxidant bond against oxidative damage through its synergistic action with Selenium which would have increased the chance of spermatozoa being interacted with the turkey egg for fertilization.

Percent Total Hatchability

Dietary supplementation of antioxidant to the toms significantly (P≤0.01) influenced the per cent hatchability of the total eggs set in this feeding experiment. The group enriched with dietary Vit-E significantly (P≤0.01) increased the per cent total hatchability (59.94±0.94) of the turkey eggs compared to the control group (50.49±1.75). This finding agrees with the results of (Atkinsonet al., 1955) where the authors observed an increase in the per cent hatchability from 57.1 to 88 per cent in Belts-ville small white turkey following dietary addition of Vit-E. However, (Andi et al., 2006; Zaniboni et al., 2006) found a non significant effect due to dietary addition of Vit-E on the hatchability percentage in broiler breeder and BUT variety (B6) of turkey, respectively. The result obtained in the study explains the positive effect of Vit-E in maintaining the antioxidant status of semen which reduced the spermatozoa abnormality thereby improved the total hatchability of turkey eggs.

The group of toms supplemented with dietary inorganic Selenium shown a significant (P≤0.01) increase (54.42±0.88) in the total hatchability percentage than control (50.49±1.75). This finding coincides with the results of (Davtyan et al., 2006; Maysa et al., 2009) where a significant increase in the hatchability percentage was obtained by dietary enrichment of Selenium in Radonezh local breed of chicken and Bandarah strain of local chicken, respectively. The increase in mean fertility percentage in the current study may be attributed to the spermatozoa's membrane protective role of various seleno-proteins and enzymes through its antioxidant effect which improved the quality of tom's spermatozoa which would have increased the sperm-egg interaction that ultimately increased the hatchability rate.

In this study, maximum mean hatchability percentage was observed in the group supplemented with the combination of Vit-E and Selenium which was significantly higher than the control group (62.31±0.98 Vs 50.49±1.75). Complimentary results were also observed by (Ali et al., 2013) and (Adebukolaet al., 2014) where total hatchability increased from 68 to 71 per cent and 39.44 to 91.67 per cent by the dietary supplementation of combination of Vit-E and Selenium in Japanese Quails and breeder turkey, respectively. This may be attributed to the better antioxidant function of Vit-E and Selenium combination which increased the chance for the spermatozoa to fertilize the ovum which subsequently improved the hatchability in the combination group.

Significantly higher hatchability percentage recorded in the antioxidant supplemented group may be correlated with improved fertility percentage in respective groups thereby improved the hatchability of turkey eggs.

Percent Fertile Hatchability

Dietary supplementation of either Vit-E or Selenium alone or their combination did not show any effect (P>0.05) on

the per cent hatchability of fertile turkey eggs set in this experimental study. However, a numerical increase in the per cent fertile hatchability was observed in the antioxidant supplemented groups compared to the control group. The findings of the current study is supported by (Andi et al., 2006) where the authors observed a significant improvement in fertile hatchability in broiler breeder by the dietary supplementation of Vit-E. Since lower fertile hatchability is mainly attributed to higher number of abnormal spermatozoa, numerical improvement in the per cent fertile hatchability observed in the study may be due to the effect of dietary supplementation of antioxidants to the toms which reduced the spermatozoa injury thereby maintaining the normal spermatozoa morphology. This can also be substantiated by the reduced activity of seminal plasma transaminase level in the antioxidant supplemented groups.

The earlier works regarding the effect of dietary enrichment of Selenium alone or in combination with Vit-E on the hatchability of the fertile eggs were scarce for comparing the findings.

Percent Embryonic Mortality

The dietary combination of Vit-E and Selenium to turkey tom had shown a significantly (P≤0.05) lower early embryonic mortality (3.05±0.95 per cent) and numerically lower late embryonic mortality (9.60±1.13 per cent) compared to the control group. The result of the study concurs with the finding of (Ipek and Dikmen, 2014) where the authors observed a significant reduction in the early embryonic mortality in Japanese Quails following dietary supplementation of Vit-E at varying doses from 60 to 240 mg/kg of diet in combination with Vitamin C. The lower embryonic mortality in the combination group may be attributed to better seminal parameters observed in the current study. This may also be attributed to the increase in the proportion of fit sperms that approach the infundibulum of hen which prevented the defective fertilization thus reflected by the reduction in embryonic mortality. But previous research on the effect of dietary Selenium on the embryonic mortality is scarce to compare with the result of the current study.

The study concluded that the dietary addition of antioxidants in combination of vitamin E and Selenium will be beneficial in improving the fertility parameters in commercial turkey farming thereby increasing the profitability by increasing the number of salable chicks per dam.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

AUTHORS CONTRIBUTION

The experiment was designed by G. Srinivasan, A.V. Omprakash and Karu. pasupathi. J. Sandhanu and G.H. Hudson conducted experiment. All the authors have read and approved the final manuscript.

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