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Influence of Dietary Protein, Calcium and Vitamin-E on the Semen Qualities of Broiler Breeder Males in an Organised Farm

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ABSTRACT: Dietary levels of Protein, Calcium and Vitamin E are known to have significant effect on the production and reproduction characteristics of adult breeder birds. The current study was conducted on thirty-two broiler breeder males (Nandanam Broiler) to assess their semen qualities as influenced by dietary protein, calcium and vitamin-E levels. They were fed *ad libitum* breeder mash containing 12 or 18 percent protein, 1 or 3 percent calcium and 10 or 40 mg/kg of vitamin-E from 24 week onwards. The experimental design conducted was 2 × 2 × 2 on factorial basis. The individual semen analysis was carried out in triplicates during 26 and 36 weeks of age. Semen analysis like volume, color and consistency, sperm motility, semen pH, Sperm concentration, live sperms, abnormal sperms and MBRT were carried out. Cockerels fed 12 percent protein evinced consistently superior semen qualities compared to the birds fed with 18% protein. Most of the semen traits were slightly in favour of cockerels fed with higher calcium level (3%). However, dietary vitamin-E levels did not show significant variations in semen quality traits.

Keywords: Semen quality, semen volume, broiler breeder, protein, calcium, vitamin E.

INTRODUCTION

High environmental temperature negatively affects spermatogenesis in both mammalian (Bah *et al.*, 2001; Ordas *et al.* 2015; Garcia-Oliveros *et al.*, 2020) and avian species (Karaca *et al.*, 2002; Attia *et al.*, 2019). Among the various nutrients, the requirement of protein, calcium and vitamin-E play a major role in the reproduction of males (Arscott and Parker 1963). It is well known that the requirements of various nutrients are different in adult male and adult female breeder birds. Some practice sex separate feeding and few of them do not practice separate feeding regime. The requirement of protein and calcium are higher in female birds than male birds due to egg production. Wilson *et al.* (1987) reported that a greater percentage of caged males produced semen when fed 12-14% CP, rather than 16 or 18%. Rakphongphairoj *et al*. (1988) reported that broiler breeders fed with 7 or 16 per cent crude protein, had no significant variation in their semen volume. Silviera *et al.* (2014) reported 1.5% and 4.1% improvements in fertility and hatchability, respectively, when males were provided a male feed (13.5% CP, 0.95% Ca, 2750 kcal/kg) after 27 weeks instead of a series of hen feeds (14.0-15.5% CP, 3.1-3.7% Ca, 2740-2850 kcal/kg). Prolonged feeding of diets, deficient in vitamin-E decreased the reproductive efficiency of the roosters. Dietary calcium levels exert only a negligible effect on the semen quality and testicular function (Elthohamy and Takahara 1985). Availability of protein to broiler breeders above the requirement has shown to result in a shorter fertile period (Tyler and Bekker 2012) and a reduction in sperm concentration and testicular function (Hocking and Bernard 1997). Even though much work has been carried out on the individual effects of dietary protein, calcium and vitamin-E on the reproduction performance in males, very little work has been reported so far, on their additive effect. It would also be worth to understand whether there is an interaction (positive or negative) of Crude Protein, Calcium and Vitamin. E when these nutrients are provided in excess to broiler breeder males. Hence, a study has been conducted to study the effects of Crude Protein, Calcium and Vitamin E on the reproductive performance (semen quality) in broiler breeder males.

MATERIALS AND METHODS

The study was carried out at the Poultry Research Station, Nandanam, a premier institute of Tamil Nadu Veterinary and Animal Sciences University. The work was carried out in Nandanam Broiler, a variety released by the University. 2 × 2 × 2 factorial design experiment involving two levels of dietary crude protein (12 and 18 per cent), two levels of calcium (1 and 3 per cent) and two levels of vitamin-E (10 and 40mg/kg) were carried out using 32 meat-type Nandanam broiler breeder males. Wide ranges of nutrient levels have been selected based on the maximum and minimum values**.** The ingredient composition and chemical composition of the experimental feed are given in Table 1.

Management of Birds. From a flock of Nandaman broiler breeders, 32 males belonging to the same hatch with good degree of uniformity were selected at 8 weeks of age and reared in deep litter pens. From 9 to 24 weeks, they were fed *adlibitum* with grower mash containing 2500 Kcal/kg of ME and 16% of protein.

Water was provided *adlibitum*. Around 20 weeks of age, the feathers around the vent of the cockerels were plucked and the area was trimmed and cleaned. The cockerels were trained for artificial collection of semen according to the "One Man technique" of Wheeler (1948). At 24 weeks of age, the birds were randomly divided into 8 groups of 4 each and were fed with one of the eight experimental mashes (T1, T2, T3, T4, T5, T6, T7 and T8). All the birds were fed with 135g of any one experiment feed. Except for the feed all other managemental conditions remain unchanged.

Semen Collection and Evaluation. Semen from individual bird were collected as per the method described above. Semen from individual cockerels were evaluated in triplicate at 26 and 36 weeks of age. The semen was subjected to semen evaluation for the traits like volume, color and consistency, as per the methods described by McDaniel and Craig (1960). Spermatozoa motility was assessed by "Hanging drop method" as described by Parker *et al.* (1942). The pH of neat semen was measured by Wheeler and Andrew's (1943) method. The sperm concentration was measured as per Allen and Champion's (1955) method. Live and dead spermatozoa count and the percentage of abnormal spermatozoa were estimated as per the method described by Parker *et al.* (1942) using nigrosin eosin stain and it is expressed in percentage of total sperm count. The percentage of The Methylene Blue Reduction Time (MBRT) test was done according to the method described by Beck and Salisbury (1943). All the data recorded was subjected to analysis as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The results of the work done are presented in Table 2.

Semen Volume. Higher semen volume was recorded in males fed with lower Protein level (12%), higher Calcium levels (35) and higher Vitamin. E levels (40mg/kg) at 26 weeks of age. Similar trend was seen even at 36 weeks of age. The results revealed highly significant variations ($P<0.01$). Similar to our findings, Zhang *et al.* (1999); Hocking (1989) recorded greater semen volume at 12% protein level. Abioja *et al.* (2023) observed that male breeder birds fed with higher levels of Vitamin. E (0, 50, 100, 150 mg/kg) results in significantly higher semen volume (0.71 ml to 0.94 ml)

Semen Color and Consistency. The study revealed that none of the dietary factors influenced either the semen colour or its consistency.

Sperm Motility. Significantly higher sperm motility was recorded in lower crude protein level (12%) at 26 weeks of age compared to higher protein level (18%). Other dietary factors also did not bring about any changes in the sperm motility. The results agreed with the reports of Wicker *et al.* (1972); Eltohamy and Takahara (1985) who concluded that calcium and vitamin. E did not influence sperm motility. Contrary to our findings, Tyler *et al.* (2021) observed that Crude protein and calcium had no significant effect on the sperm mobility at any age. Monsuru Oladimeji Abioja *et al.* (2023) observed that male breeder birds fed with higher levels of Vitamin. E (0, 50, 100, 150 mg/kg) results in significantly higher semen motility (79.1 % to 84%).

Semen pH. The results revealed that only calcium levels 1% and 3%) of the diet had significantly ($P<0.05$) influenced the semen pH at 36 weeks of age. However, irrespective of other dietary treatments, hydrogen ion concentration (pH) was within the normal range.

Sperm Concentration. Sperm concentration was significantly influenced by dietary protein ($P<0.01$) and calcium ($P<0.05$) at 26 weeks of age. At 26 weeks of age, highest sperm concentration was observed at 26 weeks of age ($3.20 \times 10^6/mm^3$). Irrespective of dietary treatment, sperm concentration was consistently higher at 36 weeks of age. El-Din *et al.* (1988); Hocking (1989) also recorded increased sperm concentration with decrease in dietary protein levels. Sperm concentration was positively influenced when the birds were fed with 3 % Calcium. The results were in agreement with the findings of Naumenko *et al.* (1984) who noticed significant variation in the sperm concentration based on dietary calcium level. Inclusion of vitamin E in the diet did not influence the sperm concentration. Unlike the results obtained in this study, Masliev and Davtjan (1967); Kuhns and Arscott (1969) noticed increased sperm concentration with increased dietary vitamin-E levels. But, Degtyar (1974) observed higher sperm concentration at lower levels of vitamin-E (5 and 10 mg/kg vs 20mg/kg). Tyler *et al* (2021) recorded no significant differences when the males are provided with either 12 or 14% CP or 1 and 3% calcium. However, the findings revealed that the sperm concentration increases with age. Monsuru Oladimeji Abioja *et al.* (2023) observed that male breeder birds fed with higher levels of Vitamin. E (0, 50, 100, 150 mg/kg) results in significantly higher sperm concentration (2×10^9 ml to 2.68^9 ml).

Live Spermatozoa. The diet with 12 % protein had consistently recorded significantly ($P<0.01$) higher percentage of live spermatozoa than those with 18% (88.12 % vs 81.71%). Live spermatozoa remained unaffected by other dietary treatments. Proudfoot (1980) concluded that 13.6% of protein in the diet was adequate to maintain optimum live sperm percentage. The percentage of live spermatozoa was not influenced by the inclusion of either calcium or vitamin E. Kuhns and Arscott (1969) stated that vitamin-E level had no significant effect on live sperm percentage. Abioja *et al.* (2023) observed that male breeder birds fed with higher levels of Vitamin. E (0, 50, 100, 150 mg/kg) results in significantly higher live sperm (86.8% to 96.9%).

Abnormal Spermatozoa. The results of our study indicated higher percentage of abnormal spermatozoa ($P<0.05$) in the cockerels fed with 18 percent protein diet (5.75% vs 3.40%) at 26 weeks of age and similar findings were repeated at 36 weeks of age (4.62% vs 3.09%). No significant differences were observed in other dietary treatment groups. However, Dobrescu (1986b) recorded highest abnormal spermatozoa with low protein diet. Abioja *et al.* (2023) observed that male breeder birds fed with higher levels of Vitamin. E (0, 50, 100, 150 mg/kg) results in significantly lower sperm abnormalities (11.0 % to 5.6 %).

Methylene Blue Reduction Test. Our study revealed that dietary protein level (12%) had significantly influenced (P<0.01) the Methylene Blue Reduction Test (20.1 min vs 23.9 min). Due to higher concentration of live spermatozoa in the 12% group, the reduction in the MBRT time was observed. The finding was contradictory to the results of Dobrescu (1986a) and Hocking (1989) who concluded that roosters' semen remained unaffected by dietary protein levels. In accordance with the findings, Degtyar (1974) observed no significant variations in MBRT based on the dietary vitamin-E levels.

Table 1: Ingredient and Chemical Composition of the diet.

Ingredients (%)	T1	T2	T3	T4	T5	T6	T7	T8
Yellow Maize	34.4	34.4	42.0	42.0	35.0	35.0	38.4	38.4
Cumbu	36.0	36.0	34.0	34.0	22.0	22.0	24.8	24.8
Deoiled GNC	—	—	3.2	3.2	14.4	14.4	16.0	16.0
Deoiled rice bran	23.0	23.0	9.2	9.2	20.0	20.0	5.4	5.4
Fish Meal	4.0	4.0	3.6	3.6	6.4	6.4	8.0	8.0
Shell grit	—	—	5.6	5.6	—	—	5.2	5.2
Min. mix &Vit[1]	2.6	2.6	2.4	2.4	2.2	2.2	2.2	2.2
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Vitamin. E 50% (g/q)[2]	—	6.0	—	6.0	—	6.0	—	6.0
Chemical Composition								
Crude Protein %	12.1	12.1	12.1	12.1	18.0	18.0	18.0	18.0
ME Kcal/ kg	2631	2631	2622	2622	2603	2603	2572	2572
Ca %	1.0	1.0	3.0	3.0	1.0	1.0	3.0	3.0
P %	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4
Vit. E mg/kg	9.7	40.4	9.7	40.4	9.5	40.3	9.5	40.3

[1.] Mineral mixture containing Ca 28%, P 5%, Nacl 23%, I 10ppm, Cu 100ppm, Mn 2000ppm and Co 50 ppm. Each g of vitamin contains Vitamin A 82,000 IU, Vitamin B2 50mg, Vitamin D3 12,000 IU and Vitamin –K 10 mg , [2.]Vitamin E was supplemented as alpha toco pherol acetate (50% w/w)

Table 2: Effect of Protein Calcium and Vitamin-E levels on the Semen quality parameters.

Parameters	Age	Crude Protein (%)		Calcium (%)		Vitamin-E (mg/kg)	
		12	18	1	3	10	40
Semen volume (ml)	26 weeks	0.39^{m}±0.01	0.26^{n}±0.02	0.29^{o}±0.03	0.36^{p}±0.02	0.31±0.03	0.34±0.02
	36 weeks	0.42^{m}±0.01	0.36^{n}±0.01	0.38±0.01	0.39±0.01	0.38±0.01	0.39±0.01
Color and Consistency	26 weeks	4.16±0.08	3.93±0.09	4.06±0.11	4.06±0.07	4.06±0.12	4.06±0.05
	36 weeks	4.5±0.06	4.34±0.07	4.48±0.08	4.34±0.05	4.45±0.07	4.37±0.07
Sperm motility (%)	26 weeks	82.87^{m}±0.76	78.28^{n}±1.00	79.68±1.03	81.47±1.04	80.47±1.17	80.68±0.93
	36 weeks	85.31±0.85	82.81±1.18	84.37±1.02	83.75±1.12	84.37±0.81	83.75±1.28
Semen pH	26 weeks	7.13±0.03	7.07±0.02	7.12±0.03	7.08±0.02	7.06±0.03	7.15±0.02
	36 weeks	7.09±0.02	7.06±0.01	7.10^{o}±0.02	7.05^{p} ±0.02	7.06±0.01	7.09±0.03
Sperm concentration ($x\ 10^6/mm^3$)	26 weeks	3.20^{m}±0.03	2.79^{n}±0.09	2.88^{o}±0.10	3.1^{p}±0.06	2.96±0.99	3.02±0.08
	36 weeks	3.50±0.06	3.46±0.09	3.44±0.07	3.52±0.07	3.53±0.08	3.42±0.06
Live Spermatozoa (%)	26 weeks	88.12^{m}±0.90	81.71^{n}±1.31	84.12±1.55	85.81±1.03	84.90±1.65	85.00±0.91
	36 weeks	88.92^{m}±.72	85.51^{n}±1.05	86.83±1.08	87.61±0.89	87.12±1.11	87.40±0.85
Abnormal Spermatozoa (%)	26 weeks	3.40^{m}±0.21	5.75^{n}±0.36	4.62±0.49	4.53±0.33	4.59±0.39	4.56±0.44
	36 weeks	3.09^{m}±0.21	4.62^{n}±0.33	4.09±0.39	3.62±0.26	3.87±0.34	3.84±0.33
Methylene Blue Reduc -tion test (min)	26 weeks	20.1^{m}±0.63	23.9^{n}±0.91	21.2±1.01	22.7±0.77	22.3±1.04	21.7±0.78
	36 weeks	20.1^{m}±0.52	22.1^{n} ±1.47	20.5±0.65	21.5±0.54	20.9±0.55	21.1±0.66

Means within the column bearing at least one common superscript do not differ significantly

CONCLUSIONS

The findings of the study concludes to a point that dietary inclusion of Crude Protein, Calcium and Vitamin E has definite effect on the semen parameters of the male broiler breeders. Since, the protein part of the diet plays a major part in determining the cost of feed, lower protein diet (12%) can be sufficient for optimum reproductive performance irrespective availability of dietary Calcium and Vitamin E. However, higher Calcium and Higher Vitamin effects the semen qualities, but the lower levels of Vitamin E (10mg/kg) is sufficient for optimum semen characteristics.

FUTURE SCOPE

This study has been limited to broiler breeders, similar study on other chicken species (Layers), other species of poultry like Japanese Quail, turkey, Guinea Fowl, etc may be conducted to ascertain the actual requirement of dietary factors like Protein, Calcium, Vitamins, etc.

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Conflict of Interest. None.

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