Effect of vitamin E supplementation on semen quantity and quality of Local Kampong roosters

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Abstract

This experiment was conducted to investigate the effect of increasing dietary levels of vitamin E on semen quantity and quality characteristics of local kampong roosters. Forty-five roosters were randomly distributed equally to 3 treatment groups with each group consisting of 5 replicates of 3 roosters per replicate. The 3 treatment groups were no vitamin E (T0), 200 IU vitamin E (T1) and 400 IU vitamin E (T2) supplementations. DL a-tocopherol acetate was used as the source of vitamin E. Commercial broiler feed, crushed corn and water were given ad libitum. Semen quantity and quality were evaluated at week 2, 3 and 4 after the initial vitamin E supplementation. Improvement in semen production characteristics was only noticeable after 4 wk of vitamin E supplementation. Semen characteristics (sperm gross motility score, percentage of live sperm and colour score) at week 4 after supplementation were significantly higher (6.20 ± 1.10, 81.68 ± 5.39% and 2.80 ± 0.30, respectively) in T2 group ($P < 0.05$) compared to control group. Percentages of abnormal tail spermatozoa were significantly ($P < 0.05$) lower (3.84 ± 2.55) T2 group compared to control group. From this study, it can be concluded that higher supplementation of dietary vitamin E is beneficial in improving the semen characteristics of local kampong chicken after 4 wk of supplementation.

Keywords: Vitamin E; sperm and rooster

Introduction

The poultry sector is part of the livestock industry in Malaysia. The incredible growth of the sector has been largely pushed by private sector enterprise and it has evolved into a progressive, organized and developed industry with annual production of meat and eggs. Poultry farming contributed 55.4% to livestock value added in 2003, while beef contributed 5.8%. pork 16.7% and eggs 20.4% (Arshad and Kaur, 2007).

Indigenous chicken also known as village chicken (ayam kampong) is gaining popularity in production due to its disease resistance, able to utilize low quality feed and their products are preferred by consumers (Mengesha, 2012). Most indigenous chicken production is conducted in small scale mostly as hobby or back yard production. So that, there is still lack of knowledge in some areas regarding nutrition and reproduction especially on fertility of indigenous chicken when compared to that of commercial chicken. Indigenous chicken has many advantages and benefits compared to the commercial chicken. The indigenous chicken are well adapted to the local climate, cost less on maintenance and feed, resistant to diseases and hardy (Ramlah, 1999).

The commercial production of poultry utilizing the efficient commercial lines of broilers and layers has become very

successful and highly competitive in the South-east Asian countries, and backyard chicken production in rural areas would still continue to contribute towards the domestic chicken meat consumption. In Malaysia, the contribution is very small but a large contribution of village based production occurs in Indonesia, Thailand and the Philippines (Aini, 1990).

The avian semen contains high concentrations of polyunsaturated fatty acids (PUFAs) (Golzar Adabi *et al.*, 2011; Parks and Lynch, 1992). PUFAs had been associated with increased relative oxygen damage (ROS) and lipid peroxidation in chicken sperm. When ROS is higher than the natural antioxidant defense mechanisms, the sperm will be damaged by lipid peroxidation and later can result in lower fertility (Long and Kramer, 2003). Normally, spermatozoa are protected from ROS and lipid peroxidation by various antioxidants and enzymes present in seminal plasma (Min *et al.*, 2016a).

The antioxidants have important roles in avian reproduction. Vitamin E is a natural antioxidant capable of enhancing semen quality and fertilizing capacity (Cerolini *et al.*, 2006). Vitamin E protects the sperm from ROS and lipid peroxidation which help to maintain optimum fertilizing ability (Biswas *et al.*, 2007). Vitamin E is a natural antioxidant capable of enhancing semen quality and fertilizing ability of chickens, when it is provided at a level some 500 times greater than the NRC requirements (15 IU/kg diet) (Tabatabaei *et al.*, 2011). Recently, vitamin E supplements have been widely used in poultry diets to enhance production and reproductive performance several folds (Surai, 1999).

Semen quality has a prominent role in reproduction of the chicken. The quality of semen will be revealed through its reproductive performance. Oxidative stress is of great concern to poultry industry with breeder roosters. The sperm of the rooster will be affected by oxidative stress and resulting infertility (Min *et al.*, 2016b). Nutrition has a strong impact on oxidative stress. Animal diets include natural molecules with antioxidant characteristics, such as carotene, flavonoids, and non-flavonoid phenols. Apart from these, the most important natural antioxidant is vitamin E. So, supplementing vitamin E will likely improve semen characteristics and then greatly help in the success rate of reproduction performance in the chicken production.

The present study was conducted to determine the effect of vitamin E on semen quantity and quality of local kampong roosters.

Materials and Methods

Housing and rearing

Local kampong roosters (n = 45; age = 60 to 64 wk), *Gallus gallus domesticus,* were obtained from around Sandakan and individually caged in a semi intensive poultry house located at the Faculty of Sustainable Agriculture Universiti Malaysia Sabah (UMS) Sandakan. Local kampong roosters used were as described by Azahan (1994). The local kampong roosters were randomly divided into 15 groups of 3 roosters each (3 dietary treatments x 5 replicates). The experiment was a completely randomized design (Snedeor and Cochran, 1994). The local kampong roosters were provided with mixed commercial poultry pellets (16% CP, 2700 kcal/kg, 0.75% calcium and 0.45% phosphorus) and crushed corn (1:1) and water ad libitum. All the groups were subjected to similar management practice (brooding, feeding and watering) throughout the experiment and kept under natural light.

One mo prior to start of semen collection, all local kampong roosters were kept in individual cages.

Feed preparation

The local kampong roosters were given basal diet of commercial poultry pellet and corn mixed (1:1). Rooster in T0, T1 and T2 groups were supplemented with 0 IU, 200 IU, and 400 IU of vitamin E, respectively. The roosters were fed orally by using a syringe. Liquid a-tocopherol acetate oil based was used as the source of Vitamin E (Nature's Bounty Inc, Bohemia, NY).

Semen collection

Semen was collected weekly using the method described by(Burrows and Quinn (1939). First, the cloacal area was cleaned. The back and tail feathers and the abdominal region were then stroked gently and repeatedly which resulted in the tumescence (erection) of the phallus. Semen was ejaculated after slight pressure was applied to the inverted cloaca. The semen carefully collected using a syringe and then transferred to 2-ml micro centrifuge tube before placed in a water bath maintained at 37°C prior to evaluation. Special care was taken to minimize contamination by faeces, foam and watery fluids from the cloacal region.

Semen characteristics

Immediately after collection of semen, the volume was determined using a 1-ml syringe, and sperm concentration was determined using a Neubaur haemocytometer. Semen colour was evaluated by using score: 1=watery, 2=cloudy and 3=creamy. The gross motility score was evaluated using Mass Motility (MMOT) score (Blesbois *et al.*, 2008) observed under a light microscope at 100x magnification. Then, 10 µL of fresh semen was diluted using 990 µL of modified Ringer's solution. Modified Ringer's solution (Helmenstine, 2016) (9.0 g sodium chloride, 0.4 potassium chloride, 0.3 g calcium chloride 1.3 g dextrose and 0,2 g sodium bicarbonate in 1 L distilled water) was used as a semen diluent. The percentage of individual sperm progressive motility was determined under a light microscope at 200x magnification after placing a coverslip over 10 µL drop of semen diluted with Modified Ringer's solution on a warm microscope slide. The percentages of live and dead and abnormal spermatozoa were determined using an eosin–nigrosin stained smear and observed under a light microscope at 400x magnification. Unstained spermatozoa were regarded as live whereas stained or partially stained spermatozoa were counted as dead. Abnormalities were observed at the head, mid –piece and tail of individual spermatozoa. The percentage of abnormal spermatozoa was determined over observations of about 200 spermatozoa per slide.

Artificial insemination

For artificial insemination, the 15 roosters from each treatment group were randomly divided into 3 groups of 5 roosters each. The pooled fresh semen from 5 roosters was used for inseminations into 15 healthy female Hisex Brown hens making 3 replicates per treatment group. A total of 45 hens were inseminated per treatment group. AI was performed by everting the hen's vaginal orifice by applying pressure on left side of abdomen (Burrows and Quinn, 1939). The semen collected was evaluated in terms of progressive motility and sperm concentration. After initial evaluation to

ensure more than 80% progressive motility about 200 to 300 million spermatozoa were loaded into a micropipette tip and then injected into the cloaca within 15 min of semen collection. The eggs were collected daily for 7 d and labeled according to treatment group.

An egg incubator was fumigated and all eggs were set in the incubator after 7 d of egg collection. The incubator temperature was set at 37.5^{o}C. Percent humidity was set at between 45 to 50% for the first 17 d and 55 to 65% from 18 d onwards until hatching. Egg turning was automatically set at every 2 hr. Candling of eggs was done at day 7 and 14 after beginning of incubation. The number of fertile eggs was recorded for each treatment and the infertile eggs were removed. Fertility was determined as the ratio of the number of fertile eggs to the number of total eggs set and hatchability was determined as the ratio of the number of eggs hatched to the number of fertile eggs (Biswas *et al.*, 2007). Non-hatched eggs from each replicate were analyzed at the end of the experiment for embryo mortality causes and period, which was divided in early (1-7 days), and late (14 - 21 days) mortality (Almeida *et al.*, 2008).

Statistical analysis

The results expressed as the mean ± SD. Means were analyzed using a one-way analysis of variance, followed by a Duncan's post hoc test to determine significant differences in all the parameters recorded using the general linear model (GLM) procedure of SAS 9.4 (Systems, 1999). The effects of the experimental diets on response variables were considered to be significant at $P<0.05$.

Results and Discussion

There was no significant difference among treatment groups in all observed parameters after 2 and 3 wk of supplementation of vitamin E as shown in Tables 1, 2, 3 and 4.

Table 1. Effect of vitamin E on semen characteristics of local kampong rooster 2 weeks after initial supplementation (mean ± S.D., n = 45)

Group	Volume (ml)	Conc. (10^9/ml)	Gross motility	Individual motility (%)	Live (%)	Dead (%)	Colour score
T0	0.20 ± 0.17	1.33 ± 0.89	3.80 ±1.30	72.00 ± 14.83	60.40 ± 18.06	39.60 ± 18.06	2.73 ± 0.28
T1	0.15 ± 0.03	0.91 ± 0.37	5.40 ± 0.89	80.00 ± 10.00	79.46 ± 10.65	20.54 ± 10.65	2.40 ± 0.44
T2	0.13 ± 0.05	0.80 ± 0.45	4.80 ± 1.30	74.00 ± 21.90	79.87 ± 20.44	20.13 ± 20.44	2.33 ± 0.62

Mean values bearing the same superscripts in a column do not differ significantly ($P \geq 0.05$).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

Table 2. Effect of vitamin E on semen morphological abnormalities of local kampong roosters 2 weeks after initial supplementation (mean ± S.D.)

Group	Head (%)	Mid-piece (%)	Tail (%)	Total (%)
T0	2.24 ± 1.80	12.16 ± 5.27	22.08 ± 6.50	12.16 ± 2.48
T1	1.92 ± 2.27	14.16 ± 8.30	12.54 ± 5.29	9.60 ± 3.98
T2	0.78 ± 0.92	11.14 ± 5.65	14.48 ± 6.73	8.35 ± 3.37

Mean values bearing the same superscripts in a column do not differ significantly ($P \geq 0.05$).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

Table 3. Effect of vitamin E on semen characteristics of local kampong rooster 3 weeks after initial supplementation (mean ± S.D., n = 45)

Group	Volume (ml)	Concentration (10^9/ml)	Gross motility	Individual motility (%)	Live (%)	Dead (%)	Colour score
T0	0.16 ± 0.04	0.93 ± 0.43	4.20 ± 1.64	60.00 ± 24.49	74.53 ± 9.79	25.47 ± 9.79	2.27 ± 0.43
T1	0.11 ± 0.01	1.26 ± 0.62	5.40 ± 0.55	78.00 ± 4.47	85.20 ± 10.03	14.80 ± 10.03	2.33 ± 0.47
T2	0.26 ± 0.32	1.17 ± 0.69	5.60 ± 0.89	82.00 ± 10.95	71.33 ± 16.08	28.67 ± 16.08	2.47 ± 0.38

Mean values bearing the same superscripts in a column do not differ significantly ($P \geq 0.05$).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

Table 4. Effect of vitamin E on semen morphological abnormalities of local kampong rooster after 3 weeks of initial supplementation (mean ± S.D.)

Group	Head (%)	Mid-piece (%)	Tail (%)	Total (%)
T0	2.88 ± 3.32	4.24 ± 2.34	12.72 ± 5.39	6.61 ± 1.84
T1	2.00 ± 2.07	6.80 ± 5.82	8.64 ± 3.38	5.81 ± 2.77
T2	2.96 ± 2.79	9.28 ± 3.43	13.52 ± 5.21	8.59 ± 2.28

Mean values bearing the same superscripts in a column do not differ significantly ($P \geq 0.05$).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

Table 5 shows some of the semen characteristics after 4 wk of vitamin E supplementation. The semen volume, sperm concentration and individual motility did not differ significantly among the different treatment groups. However, T2 group (400 IU of vitamin E) showed significantly higher (P<0.05) gross motility, percentage of live sperm and colour score than T1 (200 IU of vitamin E) and control group T0 (0 IU of Vitamin E) (P<0.05). After 4 wk of vitamin E supplementation, there was no significant difference in all observed parameters in term of sperm abnormalities of the local kampong rooster (Table 6).

Table 5. Effect of vitamin E on some semen characteristics of local kampong rooster after 4 weeks of supplementation (mean ± S.D., n = 45)

Gp	Volume (ml)	Concentration (10^9/ml)	Gross Motility	Individual Motility (%)	Live (%)	Dead (%)	Colour Score
T0	0.14 ± 0.04	0.93 ± 0.52	3.80 ± 1.64^{b}	82.00 ± 4.47	70.72 ± 7.70^{b}	29.20 ± 7.66^{a}	2.40 ± 0.44^{ab}
T1	0.14 ± 0.05	0.91 ± 0.59	4.80 ± 1.30^{ab}	78.00 ± 17.89	74.32 ± 7.81^{ab}	25.60 ± 7.96^{ab}	2.23 ± 0.22^{b}
T2	0.12 ± 0.03	1.82 ± 1.37	6.20 ± 1.10^{a}	90.00 ± 0.00	81.68 ± 5.39^{a}	18.40 ± 5.41^{b}	2.80 ± 0.30^{a}

Mean values bearing the same superscripts in a column do not differ significantly (P ≥ 0.05).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

Table 6. Effect of vitamin E on semen morphological abnormalities in local kampong roosters after 4 weeks of supplementation (mean ± S.D.)

Group	Head (%)	Mid-Piece (%)	Tail (%)	Total (%)
T0	2.88 ± 1.51	6.80 ± 3.72	6.64 ± 2.57	5.44 ± 1.69
T1	1.60 ± 1.10	3.36 ± 2.81	4.72 ± 4.57	3.23 ± 2.56
T2	3.44 ± 2.41	2.72 ± 2.79	3.84 ± 2.55	3.33 ± 1.49

Mean values bearing the same superscripts in a column do not differ significantly (P ≥ 0.05).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

Table 7 indicates the egg fertility in T1 (200 IU vitamin E) and T2 (400 IU vitamin E) was significantly higher than the control group T0 (0 IU vitamin E) (P<0.05). The egg hatchability was significantly higher in T2 group as compared with the other two groups. Hatchability in T1 was also significantly higher than control group. There were no significance differences in percentage of early and late embryonic dead among the treatment groups.

Table 7. The effect of vitamin E on fertility and hatchability of local kampong roosters

GP	Fertility (%)	Hatchability (%)	Early dead (%)	Late dead (%)
T0	28.97 ± 4.18[b]	27.78 ± 9.62[c]	22.22 ± 25.46	38.87 ± 9.62
T1	59.79 ± 16.83[a]	47.62 ± 4.12[b]	12.50 ± 12.50	39.88 ± 16.20
T2	60.42 ± 14.35[a]	63.44 ± 3.34[a]	6.11 ± 5.36	30.45 ± 5.69

Mean values bearing the same superscripts in a column do not differ significantly ($P \geq 0.05$).
T0 – 0 IU vitamin E; T1 – 200 IU vitamin E; T2 – 400 IU vitamin E

No significant differences were observed in semen volume, sperm concentration, and individual sperm motility – a similar trend as reported by Biswas *et al.* (2007). In contrast to Biswas *et al.* (2007) finding that supplementing the diet with a moderate level of Vitamin E (150 IU/kg) improved male reproductive performance, in this study percentage of live sperm was significantly different in T2 supplemented with 400 IU of vitamin E compared to control group (0 IU). The morphological abnormalities were not significantly different among the treatment groups. In this study, sperm individual motility and sperm concentration were numerically greater in T2 group but this was not statistically significant. Fertility was significantly ($P < 0.05$) improved after providing 200 IU and 400 IU of vitamin E in the diet. Khan *et al.* (2012) also reported that chicken fertility was higher when the diet was added with vitamin E. Biswas *et al.* (2007) also stated that fertility of quails that were supplemented with vitamin E was improved. Hatchability was also significantly ($P < 0.05$) improved after providing 400 IU of vitamin E in the diet. In contrast with this finding Biswas *et al.* (2007) reported that there was no significant difference in hatchability in quails after been supplemented with vitamin E compared to the control group. Zaniboni *et al.* (2006) also reported that there was no significant difference in turkey group that were supplemented with vitamin E in the diet compared to the control group. However Biswas *et al.* (2007) also stated that, although no significant differences were observed with hatchability, supplementing the diet with 300 IU/kg of vitamin E tended to increase hatchability compared with the other treatment groups. Latshaw and Osman (1974) and Wilson *et al.* (1962) were also consistent in their findings that dietary vitamin E increased hatchability in the chickens and quails. No significant differences were observed in semen volume, sperm concentration, and individual sperm motility because vitamin E is naturally present in chicken and turkey spermatozoa where it helps to maintain membrane integrity and sperm motility (Biswas *et al.*, 2007; Donoghue and Wishart, 2000) and a variation in sperm concentration is reflected in the degree of motility of spermatozoa (Blesbois *et al.*, 1993). There was no significant result of abnormal sperm because the short term effect of vitamin E on rooster morphological abnormalities is not obvious. They may need a longer time to decrease the negative effect on sperm morphological abnormalities (Min *et al.*, 2016b). Maybe due to small sample size, the variation of data was very wide that might have contributed to

non-significant result in semen volume, sperm concentration and individual sperm motility.

Conclusion

Semen characteristics in terms of gross motility, percentage of sperm viability and semen colour were improved with the supplementation of 400 IU vitamin E in the diet of local kampong roosters in 4 wk. Fertility and hatchability were also increased when the local kampong roosters were supplemented with 200 IU and 400 IU of vitamin E in their diet in 4 wk. From this study, it can be concluded that supplementation of dietary vitamin E (400 IU) can be beneficial in improving the semen characteristics in local kampong chicken roosters after 4 wk of supplementation.

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