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Effects of vitamin E supplement and semen collection time on sperm quality of locally Noi crossbred cocks

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Abstract Artificial insemination has been used recently in chickens to improve fertilization rates in local breeds; however, one of the factors affecting semen quality of chickens is peroxidation lipid in sperm because it contains many long-chain polyunsaturated fatty acids, which cause damage to sperm and reduce fertility in chickens. Therefore, the objective of this study was to find out the best level of vitamin E supplementation and the best time of semen collection as a basis for the improvement and improvement of sperm quality of Noi crossbred cocks. This study was designed completely randomized with 20 Noi crossbred cocks (10-12 months of age, 2037.6 ± 134.6 g of body weight) were fed 5 dietaries of vitamin E (0.75 and 125 mg with/without germinated rice [Gr] and 2 semen collection times (3 and 7 days) with 10 replicates/9 collection times. Semen samples were stored at 5 ^{o}C and sperm quality was analyzed at 0, 4, 6, and 12 hours of time storage. Vitamin E supplementation had no effect on semen weight and semen collection time nor on sperm volume of roosters ($P > 0.05$). However, E75Gr gave the largest volume of semen (0.34 ml), the highest pH, and the lowest sperm concentration. The time of collection at 7 days had the heaviest semen weight (0.43 g), while in 3 days had the highest pH and lowest sperm concentration. The interaction between vitamin E supplementation and the collection time was statistically significant in terms of semen pH and percentage of sperm abnormality ($P < 0.05$). There was a significant difference in pH among treatments supplemented with vitamin E at 0 and 4 hours after semen storage ($P < 0.05$). At 4 hours and 6 hours of storage, the percentage of sperm abnormality had a statistically significant difference among treatments ($P < 0.05$), E75Gr had the lowest value compared to the peak of E75 ($P < 0.05$). The research results recommended supplementing with E75Gr diet and collecting semen at 7 days for the best sperm quality.

Keywords: alpha tocopherol, breed rooster, semen characteristics, semen storage

1. Introduction

Noi chicken, a local breed of chicken in Vietnam, is popularly raised in the Mekong Delta. The advantages of the local chicken breeds are that they are well adapted to the local environmental conditions, find their own feed well, and have delicious meat quality but very low growth and reproductive performance (Quyen and Son 2008; Padhi 2016). Beside, the major problem is the low fertility rate due to the cock to female ratio (1:5-8), which results from natural mating and, of course, leads to poor breeding performance.

Currently, artificial insemination (AI) is one of the most optimal methods of the conservation of genetic resources of local livestock breeds because of its low cost and high profit, as semen from a single breeder cock can be used for multiple hens. However, the key to success in using AI is the quality of sperm (Mohann et al 2018). There are many factors that affect sperm quality, such as the breed and strains of chickens (Peters et al 2008; Murugesan et al 2013; Tarif et al 2013), age (Cerolini et al 1997; Shanmugam et al 2012), light time/day (Almahdi et al 2014), season (Saeid and Al-Soudi 1975; McDaniel et al 1995), body weight (Soeparno et al 2005) as well as semen collector (Benoff et al 1981), and diet (Hocking and Bernard 1997; Omeje and Ude 1998; Kabir et al 2007). Also, the frequency of semen collection has an important effect on the quantity and quality of semen of cock (Riaz et al 2004; Khongsen et al 2015). In addition to the above factors, it should also be recalled that the semen of roosters itself is rich in long-chain polyunsaturated fatty acids, which are responsible for increased free radical production and lipid oxidation in sperm (Adabi et al 2011; Alizadeh et al 2016), not only leads to damage to the sperm due to lipid oxidation but also to their low fertilization rate (Long and Kramer 2003).

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Vitamin E, an essential dietary factor for male and female reproduction, the addition of moderate amounts of vitamin E to poultry diets not only provides significant protection semen/sperm quality in male birds but also improves egg quality in female birds due to lipid reduction peroxidation in semen/sperm and egg (Rengaraj and Hong 2015).

Supplemented with moderate amounts in the poultry diet, it significantly protects semen/sperm qualities in male birds and egg qualities in female birds via decreasing the lipid peroxidation in semen/sperms and eggs (Rengaraj and Hong 2015). This was demonstrated by Biswas et al (2007, 2009) when supplementation with 150 IU of vitamin E/kg not only improved semen characteristics in male Japanese quail, but also improved physical and biochemical characteristics of Kadaknath cockerels A report by Biswas et al (2007) concluded that 150 IU vitamin E/kg was not only beneficial for foam and gland production, but also improved semen characteristics in male Japanese quail Besides, improving fertility by enhancing semen volume, sperm concentration, viability, forward mobility, and protection against oxidative damage has been investigated by several authors (Biswas et al 2009; Deivendran and Hong 2015; Fouad et al 2020). Furthermore, germinated raw rice is also a good source of protein, amino acids, and bioactive components, such as α-tocopherol, γ-oryzanol, thiamine, niacin, pyridoxine (Moongngarm and Saetung 2010), and γ-amino butyric acid (GABA) (Oh et al 2010), which enhances its antioxidant activities. Therefore, germinated raw rice can be a good alternative feed ingredient for poultry (Likittrakulwong et al 2020). In the Mekong delta, farmers use it as a feed supplement for poultry, especially Noi roosters, to increase their aggression and fighting behavior. However, to date, there has been no or very little research on the effects of Gr in combination with vitamin E on sperm quality.

The objective of this study was to find out the best level of vitamin E supplementation and the best time of semen collection as a basis for the improvement and improvement of sperm quality of Noi crossbred cocks, contributing to improving AI efficiency in these chicken breeds.

2. Materials and Methods

2.1. Materials

Chicken semen was obtained from 20 Noi crossbred cocks 10-12 months old, with an initial body weight of 2300-3100 ± 263.8 g/bird. Cocks were fully vaccinated against common diseases according to local veterinary vaccination guidelines and dewormed before experimentation. Each cock was raised in an individual cage with dimensions of 60 x 40 x 40 (cm), an open and ventilated house system with a roof of corrugated tole and canvas. The basal diet in pellet form had a ME value of 3050 kcal/kg and a CP of 19%, Ca, and P were 1.15 % and 0.85 %, respectively. The care and experimental use of animals were approved by the Animal Ethics Committee of Can Tho University (CTU-AEC).

Vitamin E powder was milky white, odorless, and tasteless. Germinated round rice (Gr) bought at the market was incubated in warm water (55 ^{o}C) for about 2 days, the germ length was no more than 5 mm, and drained before feeding the chickens. The chemical composition of germinated rice as the fed form was 11.55 %CP, 7.88 %CF, 4.98 %EE, 0.13 %Ca, and 0.85 %P, respectively.

The semen extender was a white powder with a net weight of 6.9 g, containing the components of NaCl (50.72%), $MnSO_4$ (0.72%), $NaHCO_3$ (0.72%), KCl (7.25%), $CaCl_2$ (3.62%), Na_2HPO_4 (0.72%), and glucose (36.23%). It was diluted with 500 ml of distilled water, dissolved completely, adjusted to pH 6.8, then filtered by Advantec qualitative filter paper, diameter 185 mm and stored at 5°C until use.

2.2. Experimental design

A total of 20 Noi crossbred cocks were completely randomized and designed with 5 levels of vitamin E supplementation and 2 different semen collection times. Five levels of vitamin E were as followed as: Control (Con) - a basal diet without any supplementation; E75 - basal diet plus 75 mg vitamin E/kg feed; E125 - basal diet plus 125 mg vitamin E/kg feed; E75Gr - basal diet plus 75 mg vitamin E/kg feed with 10 g germinated rice (Gr); and E125Gr - basal diet plus 125 mg vitamin E/kg feed with 10 g Gr.

There were 4 replicates with 1 cock per replicate; and 2 collection times were at every 3 days and every 7 days with 10 replicates. Each cock was collected 9 times.

2.3. Experimental management and semen collection

Experimental cocks were weighed at 40 and 47 weeks of age. Feed intake was provided 2 times per day at 7 a.m and 14 p.m daily with the amount of 40% and 60% feed, respectively. Vitamin E was mixed directly into the basal diet in a 3-day supplemental dosage, and the geminated rice was fed with 10 g per cock. Feed intake and leftovers refuse were recorded daily, and drinking water was freely provided. Feeding and drinking troughs were cleaned daily. Feed and germinated rice samples were collected for nutrient composition analysis according to AOAC (1986).

Semen samples from Noi crossbred cocks were taken at 8-9 a.m. The cocks were cleaned in the anal area with 0.9‰ NaCl before semen collection (Figure 1). Semen was collected by abdominal massage, as described by Peters et al (2008), with modification: Place the chicken breast on the right leg of the collector so that the chicken body was parallel to the collector's body, the right hand grasped and shaked the cock's tail gently to create excitement for the cock, avoiding the cock's movement.

After that, the collector's left hand held the chicken's leg while holding the Eppendorf tube, using the right hand to gently massage from the back to the chicken's tail for about 30 seconds to 1 minute to stimulate the ejaculate reflex of the chicken. If mucus appeared in the chicken's anus, the 2 ml Eppendorf tube was used to collect semen when this sign of the chickens having begun to ejaculate was observed. The following parameters were examined: volume, the weight of pooled ejaculates (Mini Electronic Balance Powder Scale), and color and then filled with 1.5 ml of semen extender, pH (Hi 99163, Rumani), concentration, motility, survival rate, and sperm morphology. The semen of crossbred cocks was stored in an ice bucket and transferred to the refrigerator at 5 ^{o}C before assessing quality at 4, 6, and 12 hours after semen collection.

Figure 1 Clean the anal area of Noi crossbred cock with 0.9‰ NaCl before semen collection (A) and mucus after massage (B).

Sperm concentration was determined by the method of direct cell count using an improved Neubauer hemocytometer. The viability of spermatozoa was evaluated using an eosin-nigrosin staining technique (Adabi et al 2007), taking 5 µL drop of semen from each group was mixed with 20 µL of eosin/nigrosin solution. The prepared semen samples were smeared on microscope slides and fixed by air-drying at room temperature for 10 min before observation. Based on the color of the sperm after staining to distinguish, the surviving sperm remained unstained and the completely or partially dead cells were pink to red/brown in color. The percentage of live, dead, and abnormal spermatozoa (Figure 2) was evaluated after nigrosin-eosin staining (Adabi et al 2007) and observed under the microscope at 100× magnification. Dead sperm were dark in color, while living sperm were unstained in light color. Sperm viability was assessed microscopically by evaluating at least 300 sperm per group under the microscope. and bright field microscopy at 100× magnification. Dead sperm will absorb the stain and appear the same colour as the background and live sperm will resist the stain and appear clear, therefore, unstained spermatozoa were regarded as live whereas stained or partially stained spermatozoa were counted as dead. Sperm viability was microscopically evaluated by assessing at least 300 sperms per group for each breed under a microscope. Five microscopic fields were examined. Each slide was evaluated twice.

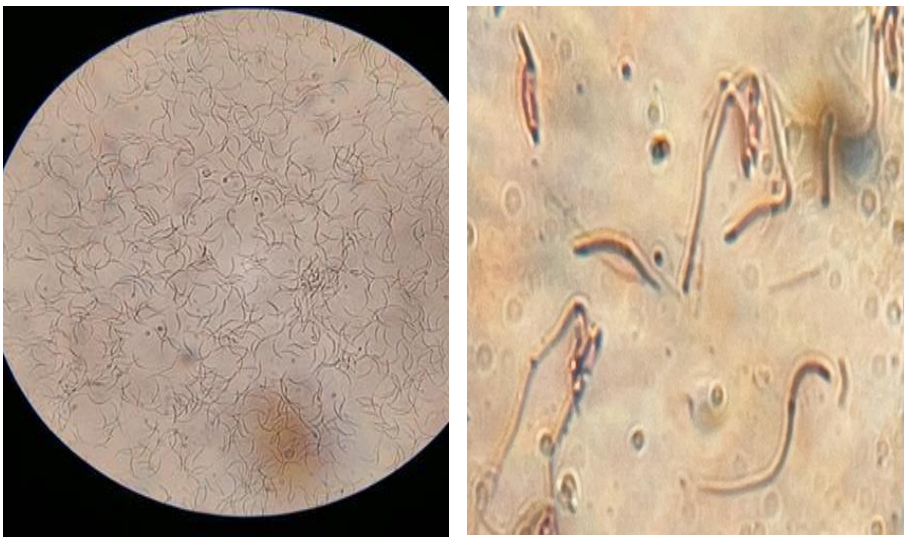

Figure 2 Noi crossbred cock's sperm and sperm abnormality.

2.4. Statistical analysis

Data were recorded and preliminarily processed using Microsoft Excel 2019 software, statistically analyzed by Minitab version 16 with GLM ANOVA model, and then compared each pair when the difference was statistically significant by Tukey's test with 95% confidence intervals. Pearson correlation was used to analyze sperm quality parameters.

The statistical model used was yijk = μ+ai+bj+(a×b)ij+eijk, where yijk is any observation; μ is the overall mean; ai is the effect of the vitamin E supplementation; bj is the effect of the semen collection time, (a × b)ij is the interactions; and eijk = errors.

3. Results

3.1 Effect of vitamin E supplementation and semen collection time on semen volume and weight of Noi crossbred cocks

Diets supplemented with vitamin E in terms of semen volume did not have a statistically significant difference (P > 0.05), but there was a statistically significant difference in semen weight among treatments (P < 0.05) (Table 1). In addition, time of semen collection did not affect semen volume (P > 0.05), but semen weight at every 7 days collection (0.48 g) was statistically significantly higher than at every 3 days collection (0.30 g) (P < 0.05). No interaction was found between vitamin E supplements and semen collection time on semen volume and weight (P > 0.05).

Table 1 Semen volume and weight of Noi crossbred cocks.

Criteria	Semen volume (ml)	Semen weight (g)
Vitamin E		
Con	0.21^{c}	0.30
E75	0.34^{a}	0.40
E125	0.23^{bc}	0.33
E75Gr	0.32^{ab}	0.41
E125Gr	0.29^{ab}	0.38
SEM/P	0.03/0.00	0.04/0.27
Semen collection time		
3 days	0.3	0.3
7 days	0.26	0.43
SEM/P	0.02/0.09	0.03/0.00
Vitamin × Semen collection time		
Con × 3 days	0.19	0.20
Con × 7 days	0.23	0.40
E75 × 3 days	0.35	0.31
E75 × 7 days	0.34	0.50
E125 × 3 days	0.26	0.27
E125 × 7 days	0.20	0.39
E75Gr × 3 days	0.37	0.37
E75Gr × 7 days	0.27	0.45
125Gr × 3 days	0.32	0.34
125Gr × 7 days	0.25	0.43
SEM/P	0.04/0.37	0.06/0.78

Con: control, E75: con + 75mg vitE /1kg feed; E125: con + 125mg vitE /1kg feed; E75Gr: con + 75mg vitE /1kg feed + 10g Gr, and E125Gr: con + 125mg vitE /1kg feed + 10g Gr, 3 days: Semen was collected every 3 days, 7 days: Semen was collected every 7 days.

3.2 Effect of vitamin E supplementation and semen collection time on semen quality parameters of crossbred cocks

Results of semen quality of Noi crossbred roosters are presented in Table 2. No statistically significant differences in sperm motility, survival, and sperm abnormality were found among vitamin E supplementation treatments (P > 0.05). Similarly, the time of semen collection did not affect these above criteria (P > 0.05). However, sperm concentration and pH were affected by vitamin E supplementation and the effect of semen collection time (P < 0.05), the highest pH was E75Gr (7.26), and the highest sperm concentration was in control (1.25 ×10^{9} sperm/ml) and E125Gr (1.22 × 10^{9} sperm/ml). Meanwhile, the time of semen collection every 3 days had the highest pH (7.22) and the highest sperm concentration at 7 days of semen collection (1.22 × 10^{9} sperm/ml). The interaction between vitamin E supplementation and semen collection time also did not affect survival, motility, and sperm concentration (P > 0.05). However, it affected the pH and sperm abnormalities between treatments (P < 0.05), the percentage of sperm abnormalities was highest in control × 7 days and E75 × 3 days compared with control × 3 days and E75Gr × 3 days. However, it did affect pH and sperm abnormality among treatments (P < 0.05), highest sperm abnormality rate in control × 7 days and E75 × 3 days compared to control × 3 days and E75Gr × 3 days.

3.3 Effect of storage time on semen quality parameters of Noi crossbred cocks

The storage time at 0 and 4 hours had a statistically significant effect on semen pH among treatments (P < 0.05) and E75 had the lowest pH (7.07 and 7.13) compared to the highest E75Gr (7.20 and 7.32) (Figure 3). At 4 hours and 6 hours after storage, the percentage of sperm abnormality had a statistically significant difference among treatments (P<0.05) E75Gr (0.51 %) and E125Gr (0.29 %), had the lowest value at 4 hours after storage (Figure 4). At 6 hours after storage, control (1.37 %), E75Gr (1.49 %), and E125 (1.92 %) had the lowest value compared to the peak of E75 (P < 0.05). Sperm survival, motility, and concentration were not affected by semen storage time (P > 0.05). In addition, after 4 and 6 hours after storage, there was an interaction between vitamin E and semen collection time to the sperm abnormality rate was found (P < 0.05), E75Gr and control at every 3 days and 7 days gave the lowest percentage of abnormal sperm rate, while E75 × 3 days gave the highest.

Table 2 Vitamin E supplementation and semen collection time affect semen quality parameters of Noi crossbred cocks.

Criteria	pH	Survival rate (%)	Motility (%)	Sperm abnormality (%)	Concentration, $\times 10^9$
Vitamin E					
Con	7.20^{ab}	94.19	94.02	2.14	1.25^{a}
E75	7.16^{b}	95.07	94.92	2.73	0.79^{b}
E125	7.16^{b}	95.38	95.23	2.04	1.08^{ab}
E75Gr	7.26^{a}	95.60	95.50	1.41	0.84^{b}
E125Gr	7.19^{ab}	94.52	94.30	2.39	1.22^{a}
SEM/P	0.02/0.00	0.79/0.60	0.80/0.66	0.42/0.24	0.10/0.00
Semen collection time					
3 days	7.22^{a}	95.32	95.16	1.89	0.85^{b}
7 days	7.16^{b}	94.59	94.43	2.39	1.22^{a}
SEM/P	0.01/0.00	0.50/0.30	0.50/0.30	0.26/0.19	0.06/0.00
Vitamin × Semen collection					
Con × 3 days	7.22^{ab}	93.81	93.63	1.05^{b}	1.03
Con × 7 days	7.19^{b}	94.57	94.41	3.24^{a}	1.47
E75 × 3 days	7.13^{b}	96.70	96.54	3.61^{a}	0.56
E75 × 7 days	7.18^{b}	93.45	93.30	1.85	1.04
E125 × 3 days	7.20^{b}	96.33	96.19	1.44	0.97
E125 × 7 days	7.12^{b}	94.43	94.27	2.65^{ab}	1.18
E75Gr × 3 days	7.33^{a}	96.11	96.09	1.22^{b}	0.7
E75Gr × 7 days	7.19^{b}	95.08	94.92	1.60^{b}	0.98
125Gr × 3 days	7.23^{ab}	93.63	93.36	2.16^{ab}	1.00
125Gr × 7 days	7.16^{b}	95.40	95.24	2.62^{ab}	1.45
SEM/P	0.03/0.02	1.12/1.17	1.13/0.30	0.59/0.02	0.14/0.82

Con: control, E75: con + 75mg vitE /1kg feed; E125: con + 125mg vitE /1kg feed; E75Gr: con + 75mg vitE /1kg feed + 10g Gr, and E125Gr: con + 125mg vitE /1kg feed + 10g Gr; 3 days: Semen was collected every 3 days; 7 days: Semen was collected every 7 days.

3.4 Pearson correlation between Noi crossbred cock body weight and sperm quality parameters

A positive correlation was found between sperm motility and sperm survival (0.993); between the initial body weight of the cock and the semen weight (0.565), and the semen volume (0.294); between semen volume and semen weight (0.699); between semen volume and pH (0.250) and sperm survival (0.222). There were negative correlations between sperm concentration and semen volume (-0.531), pH (-0.346), sperm abnormality (-0.327); between sperm motility and sperm abnormality (-0,350) (Table 3).

Table 3 Pearson correlation between Noi crossbred cock body weight and sperm quality parameters.

	BW initial	Semen volume	Semen weight	pH	Motility	Abnormality	Concentration	Survival
BW initial	1							
Semen volume	0.294**	1						
Semen weight	0.565***	0.699***	1					
pH	0.158	0.250*	0.176	1				
Motility	-0.061	0.188	0.090	0.068	1			
Abnormality	-0.074	-0.077	-0.136	-0.193	-0.350**	1		
Concentration	0.039	-0.531***	-0.189	-0.346**	0.006	-0.327**	1	
Survival	-0.048	0.222*	0.111	0.061	0.993***	-0.018	0.003	1

***Significant at P<0.001 for all correlation coefficients except where otherwise stated; **Significant at P<0.01, *Significant at P<0.05; BW: body weight.

3.5 The body weight and feed intake of Noi crossbred cocks

The initial and final body weight of Noi crossbred cocks at 40 and 47 weeks of age are presented in Table 4. No statistically significant differences were found among treatments in terms of chicken body weight (BW), weight gain, and feed intake (FI).

Table 4 Body weight and feed intake of Noi crossbred cocks at 40-47 weeks of age.

Parameters	Treatments					SEM/P
	Con	E75	E125	E75Gr	E125Gr	
BW initial (g)	2538	2550	2425	2675	2425	138.4/0.69
BW final (g)	2850	2650	2538	2875	2625	165.7/0.55
Weight gain (g/bird)	312.5	100.0	112.5	200	200	84.53/0.43
FI (g/bird/day)	84.74	84.34	89.90	93.68	90.89	3.27/0.24

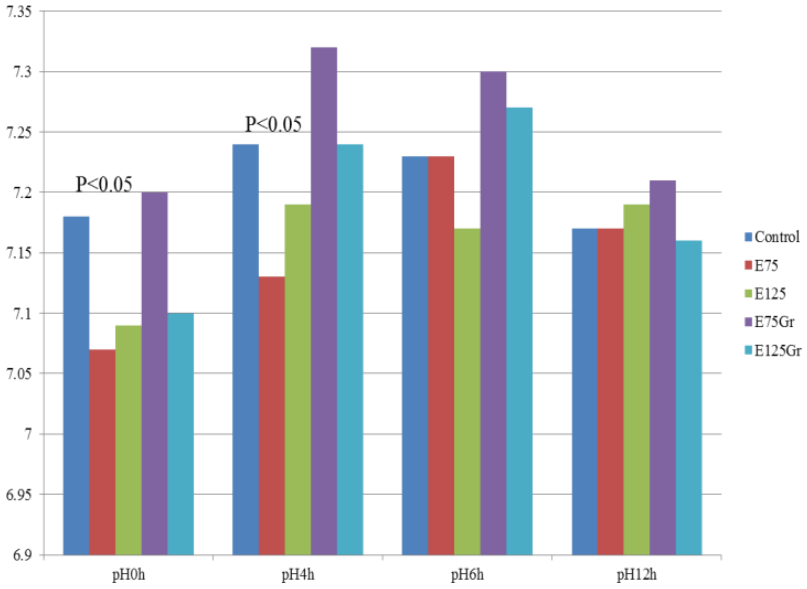

Figure 3 Effect of storage time on semen pH.

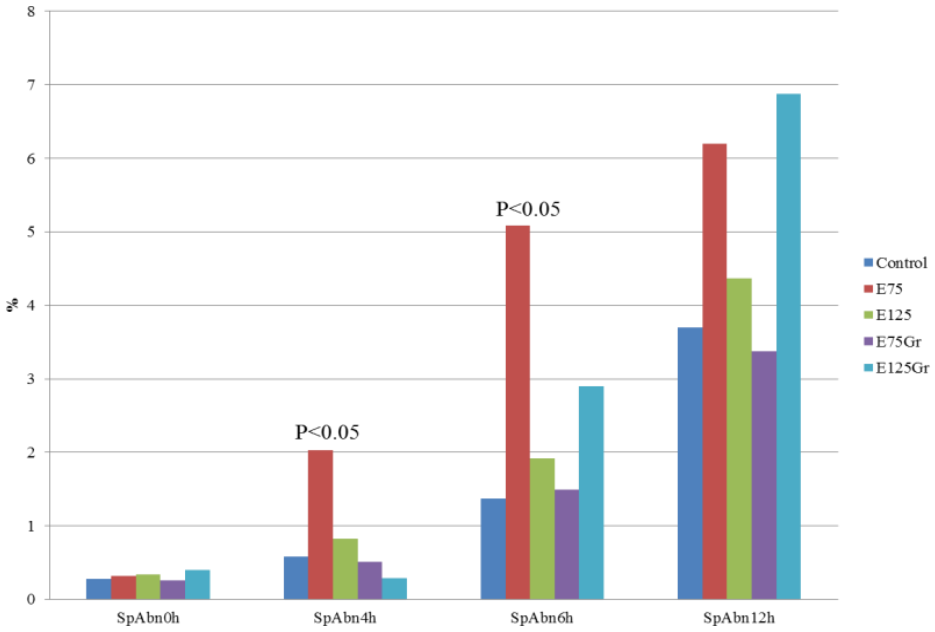

Figure 4 Effect of storage time on sperm abnormality.

4. Discussion

Male fertility is mainly related to semen and sperm quality, including semen volume, sperm concentration in semen, sperm viability, sperm motility, spermatogenesis, and sperm fertility. Vitamin E is one of the most powerful antioxidants and has been shown to improve semen quality, protect sperm from ROS and lipid peroxidation, and help maintain optimal fertility

(Cerolini et al 2006; Biswas et al 2007). This study, therefore, highlights the inclusion of feedback value of vitamin E levels or in combination with sprouted rice to optimize the semen quality parameters in Noi crossbred chickens. The results showed that vitamin E supplementation or combination with germinated rice significantly improved semen volume and weight compared with the control. The present result differed from that reported by Biswas et al (2007) and Mohamad and Abdul (2017), which showed that vitamin E supplementation did not increase semen volume in chickens. In general, Noi crossbred cocks treated with vitamin E supplements or in combination with germinated rice had semen volume between 0.23-0.34 ml, similar to the results reported by Hafez and Hafez (2000), which reported semen volume ranging from 0.2-0.5 mL in domestic cockerels. However, the semen volume of this experimental chicken is still lower than that of other local breeds of Vietnam, such as Ho cock (0.53-0.63 ml) (Hue et al 2015; Doan et al 2016) and Dong Tao cock (0.46 ml) (Tham et al 2017). This difference may be due to the influence of each breed and individual.

Moreover, the results of this study showed that E125Gr and control had the highest sperm concentration compared to E75 and E75Gr, which were the treatments with the lowest sperm concentration, and E75Gr had the highest pH compared to other treatments. In other words, there was a negative correlation between sperm concentration and semen pH in Noi crossbred cocks, as shown in Table 3. The pH results of the rooster semen in this experiment were in agreement with the recommendation of Bogdonoff and Shaffner (1953), whose pH of 7-7.25 was optimal for the endogenous respiration of cock spermatozoa and the alkaline condition is more harmful than slightly acid condition. Besides, Hafez and Hafez (2000) found semen pH at 7.2-7.6 in domesticated cockerels. Biswas et al (2007) found that 150 IU vitamin E/kg supplementation improved male reproductive performance. Asrole and Rashid (2017) reported that a diet supplemented with 400 IU of vitamin E resulted in higher sperm concentrations compared with controls ($P>0.05$). Moreover, these authors also found a statistically significant improvement in live sperm compared with the control.

Similar to vitamin E results, the frequency of semen collection in Noi crossbred cocks also affected the semen pH and concentration of sperm, with the highest sperm concentration and lowest pH at every 7 days compared to every 3 days. The present result is consistent with the conclusion of Partodihardjo (1982), cited by Mugiyono et al (2015), which claim that sperm concentration depends on age, food, breed, body weight, and frequency of semen collection. McDaniel and Sexton (1977) showed that the frequency of semen collection might affect the characteristics of semen, and they suggested that chicken semen should be collected at a 48-hour interval for the long-term. Khongsen et al. (2015) concluded that it could be performed daily without affecting the quantity and quality of sperm in 52-week-old Dang cocks and Betong cocks.

During the time of storage, sperm viability and motility decreased, and the percentage of abnormal sperm increased. The results of this experiment were similar to those of previously published studies (Siudzinska and Lukaszewics 2008). The percentage of abnormal sperm increased statistically significantly after 4 and 6 hours of storage at 5^{o}C, especially E75Gr at different storage times had a lower percentage of abnormal sperm and a higher percentage of live sperm compared with other treatments. In addition, the pH in the semen of E75Gr at the time of storage was always within the range recommended by Hafez and Hafez (2000).

5. Conclusions

The sperm quality of Noi crossbred cocks was improved when supplemented with vitamin E or combined with germinated rice, in which E75Gr was the best. The frequency of semen collection in this chicken breed can be applied every 3 days.

Conflict of Interest

The authors declare that they have no conflict of interest.

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