

## Egg's vitamin E deposition of Kedu breeder chicken fed improved diets

H.I. Wahyuni\*, N. Suthama, I. Mangisah and L. Krismiyanto  
Faculty of Animal and Agricultural Sciences, Diponegoro University,  
Tembalang Campus, Semarang 50275 - Indonesia  
\*Corresponding E-mail: hihannyiw123@gmail.com

Received June 04, 2018; Accepted September 06, 2018

### ABSTRAK

Penelitian bertujuan untuk mengevaluasi deposisi vitamin E ke dalam telur ayam Kedu pembibit yang dipelihara *in situ* diberi ransum dengan perbaikan nutrisi dan penambahan vitamin E. Sejumlah 75 ekor ayam Kedu betina dan 15 ekor jantan berumur  $\pm$  12 bulan, masing-masing dengan rerata bobot badan  $1667 \pm 237$  dan  $2295 \pm 250$  g/ekor, didistribusikan ke dalam 15 kandang semi intensif dengan umbaran. Rancangan acak lengkap (RAL) digunakan pada penelitian ini dengan 3 perlakuan yaitu ransum formula peternak (T0), ransum perbaikan (T1) dengan peningkatan nutrisi, dan ransum T1 ditambah 6,28 mg/kg vitamin E (T2). Parameter yang diamati yaitu konsumsi ransum, konsumsi dan retensi vitamin E, deposisi vitamin E dalam telur, persentase deposisi vitamin E dalam telur, *hen day production* (HDP) dan berat telur. Data dianalisis ragam dan bila perlakuan berpengaruh nyata dilanjutkan dengan uji beda Duncan. Hasil penelitian menunjukkan bahwa parameter nyata ( $P < 0,05$ ) dipengaruhi oleh perlakuan, kecuali konsumsi ransum, HDP dan berat telur. Konsumsi, retensi dan deposisi vitamin E nyata meningkat karena perbaikan nutrisi ransum dan penambahan vitamin E. Vitamin E ransum formula peternak yang dideposisikan ke dalam telur menunjukkan nilai tertinggi (229,23%), diikuti ransum perbaikan (51,85%) dan ransum perbaikan ditambah vitamin E (5,38%). Kesimpulan, ayam Kedu pembibit mempunyai keterbatasan mendeposisikan vitamin E ke dalam telur bila diberi ransum perbaikan dengan tambahan vitamin E, dan penambahan vitamin E harus dilakukan bersama-sama dengan peningkatan kadar lemak ransum.

*Kata kunci: Ayam Kedu pembibit, vitamin E, ransum perbaikan, deposisi vitamin E telur*

### ABSTRACT

The research aimed to evaluate dietary vitamin E deposited into the egg of Kedu breeder hen reared *in situ* and fed an improved diet supplemented with vitamin E. A total of 75 breeder hens and 15 cockerel of Kedu chicken aged  $\pm$  12 months, with average body weight of  $1667 \pm 237$  and  $2295 \pm 250$  g/bird, respectively, were distributed into 15 units of semi intensive housing system. A completely randomized designed (CRD) was arranged in the present research with 3 dietary treatments, namely farmer formulated ration (T0), improved ration (T1), and T1 + 6.28 mg/kg vitamin E (T2). Parameters observed were feed consumption, consumption and retention of vitamin E, egg's vitamin E, egg's vitamin E deposition efficiency, hen day production (HDP) and egg weight. All data gathered were subjected to analysis of variance and Duncan's multiple range test to differentiate between treatments. Variables, except feed consumption, HDP and egg weight, were significantly ( $P < 0.05$ ) influenced by dietary treatment. Vitamin E consumption and retention, and deposition increased due to feeding effect of improved diet and supplementation of vitamin E. Dietary vitamin E of farmer's ration deposited into the egg indicated the highest value (229.23%) followed by improved ration (51.85%), and improved

ration + vitamin E was the lowest (5.38%). In conclusion, Kedu hen is likely have a limitation on vitamin E deposited into the egg when the improved ration supplemented with vitamin E is fed, and vitamin E supplementation should be implemented together with the increase of fat content in the ration.

*Keywords: Kedu breeder hen, vitamin E, improved ration, egg's vitamin E deposition*

## INTRODUCTION

Kedu chicken is one of an Indonesian indigenous poultry originated from the village of Kedu Temanggung regency, Central Java. Kedu chickens reared either for meat producing purpose or for breeding flock. However, the farmers who maintained Kedu chicken as breeder hens have not been paying any attention on feed and feeding quality, especially the nutritional requirements of the birds for maximum production performance. Similar feed formulas were provided to the chickens without differentiate their physiological status. Feed or ration provided was mostly composed of protein concentrate, yellow corn, rice bran and vitamin-mineral premix with the portion of 15, 30, 50, and 0.5, respectively. In order to ensure the chickens are able to grow as good breeder flock, the feed should be sufficient in nutrients supply, especially in relation to egg fertility. As reported by Wahyuni *et al.* (2011) that addition of vitamin E up to 6 IU to Kedu hens showed a linear pattern. Kedu hens receiving vitamin E have improved their fertility and hatchability by 7 to 10% and 2 to 8%, respectively. Thus, dietary vitamin E is absolutely required to improve the function of poultry reproduction, especially for egg fertility and hatchability. Some previous studies have been done concerning dietary addition of vitamin E, in Indonesian local chicken (Nataamijaya, *et al.*, 2006; Iriyanti *et al.*, 2007; Wahyuni, *et al.*, 2011); Taiwan local chicken (Lin *et al.*, 2004); broiler breeder (Andi *et al.*, 2006); Indian native laying hen (Biswas *et al.*, 2010), and turkey (Adebisi *et al.*, 2014) to increase egg fertility and hatchability. On the other hand, Zhang *et al.* (2011) studied on the inclusion of commercial multi-vitamin preparation in laying hen ration produced egg containing higher level of most vitamin with least impact of dirty and cracked egg.

Vitamin E supplementation during the laying period could be beneficial for reproductive performance improvement of breeder pullets. Supplemental vitamin E at 80 mg/kg feed increased egg fertility and hatchability by 7.7 and 13.4%, respectively (Lin *et al.*, 2004), but the

amount of 40 IU/kg could be the requirement for persisting hatchability and for maximizing the progeny immune responses (Andi *et al.*, 2006) with the higher yolk tocopherol content (Loetscher *et al.*, 2014). Chick embryo development and their viability in the early posthatch life was significantly affected by nutrients density of the maternal diet. Increasing vitamin E supplementation in maternal diet increased vitamin E (carotenoids) concentration in developing embryonic tissues and also significantly decreased susceptibility to lipid peroxidation (Surai *et al.*, 2015). Rengaraj and Yeong (2015) stated that moderate amount of dietary vitamin E supplementation (75–100 mg/kg diet) protected the qualities of sperm in male or egg in female birds by decreasing lipid peroxidation. Lipid peroxidation of egg yolk efficiently reduced with vitamin E supplementation from 20 to 100 mg/kg in laying hens fed diet containing fish oil (An *et al.*, 2010).

Feeding different level of dietary vitamin E (Mori *et al.*, 2003) or combined with others vitamin (Zang *et al.*, 2011) or combined with selenium (Scheideler *et al.*, 2010) in laying hens had increased its deposition into the egg (egg yolk). Vieira (2007) indicated that metabolic lipid peroxidation increased, and vitamin E concentration in the egg yolk decreased when the laying hens exposed to heat stress. Dietary vitamin E supplementation is considered to be an important nutrient to overcome or minimize stress condition. The natural vitamin E deposited into the egg was slower than vitamin A (Leeson, 2007), and affected the embryonic susceptibility against lipid peroxidation. The awareness was also focused on the vitamin E as an antioxidant deposited into the egg yolk of Kedu hens since the birds possibly suffered continuous heat stress due to the open house rearing model. Therefore, it is important to evaluate the extent of dietary vitamin E that can actually be deposited into the egg of Kedu breeder hen fed improved ration with vitamin E supplementation.

## MATERIALS AND METHODS

Research was conducted *in situ* on the farm

owned by Kedu chicken farmer at Kedu village, Kedu District, Temanggung Regency. The Kedu breeder hens of each mating group was raised in a cage equipped with exercise area, nesting facilities and perches. Modifications were done for feeder placement in order to monitor feed consumption of the hens and cockerels separately. Ration given by the farmer usually consisted of rice bran, yellow corn, protein concentrate for layer and premix with the portion of 5, 3, 1.5, and 0.5, respectively. Composition and nutrient content of dietary treatment are presented in Table 1.

A total of 75 birds of 12 months Kedu breeder hens with an average body weight of  $1.667 \pm 0.24$  kg were used in this research. Completely randomized design was applied with 3 treatments and 5 replicates. Each replicate consisted of 5 hens. Dietary treatments were as follows T0: farmer's ration formula; T1: improved ration and T2: improved ration plus vitamin E. Synthetic vitamin E in the form of  $\alpha$ -tocopherol acetate with the concentration of 400 mg was added into T2 improved ration.

Adaptation to the dietary treatments was

Table 1. Composition and Nutrient Content of Dietary Treatments

Ingredients (%)	Farmer's Formulation	Improved Ration	Improved Ration + Vitamin E
	----- % -----		
Concentrate layer mash <sup>1</sup>	15.00	–	–
Yellow corn	30.00	50.00	50.00
Rice bran	50.00	19.60	19.60
Soybean meal	–	18.20	18.20
Fish meal	–	5.00	5.00
Premix <sup>2</sup>	5.00	–	–
CaCO <sub>3</sub>	–	3.00	3.00
Oyster shell	–	4.20	4.20
Synthetic vitamin E <sup>3</sup> (IU)	–	–	10.00
Calculated nutrient composition <sup>4</sup> (%)	100.00	100.00	100.00
Crude protein	12.50	16.70	16.70
Crude fiber	16.87	12.07	12.07
Ether extract	5.01	3.66	3.66
Calcium	1.51	2.81	2.81
Phosphor	0.71	0.66	0.66
Vitamin E (mg/kg)	0.09	1.01	6.28
Metabolizable energy <sup>5</sup> (kcal/kg)	2332.04	2578.25	2578.25

<sup>1</sup> Composed of soybean meal, corn gluten meal, palm olein, premix, vitamin, essential amino acid and mineral, and the proximate analysis based on commercial label as follows water 11%, crude protein 32.5%, ether extract 2%, crude fiber 8% and ash 35%.

<sup>2</sup> Vitamin and mineral contents per kg are vitamin A 1200000 IU, vitamin D<sub>3</sub> 200000 IU, vitamin E 800 IU, vitamin K<sub>2</sub> 200 mg, vitamin B<sub>1</sub> 200 mg, vitamin B<sub>2</sub> 500 mg, vitamin B<sub>6</sub> 50 mg, vitamin B<sub>12</sub> 1200 µg, vitamin C 2500 mg, Ca D-pantothenate 600 mg, Niacin 4000 mg, Choline chloride 1000 mg, Methionine 3000 mg, Lysine 3000 mg, Manganese 12000 mg, Iron 2000 mg, Iodine 20 mg, Zinc 10000 mg, Cobalt 20 mg, Copper 400 mg, Santoquin (antioxidant 1000 mg, Zinc bacitracin 2100 mg, carrier material up to 1 kg.

<sup>3</sup> In the form of  $\alpha$ -tocopherol acetate contained 400 mg vitamin E;

<sup>4</sup> Proximate analysis value; <sup>5</sup> Calculated value based on formula of Bolton (1967)

done for 3 weeks, except for chickens fed ration of farmer's formula. The first week was the adaptation period prior to feeding dietary treatment, at the second and third weeks were the monitoring period for feed consumption until constant which it was used as the basic references of providing the amount of daily feed during 4 weeks treatment period. All the treatment rations served in wet form (mixed with warm water with the ratio of 5 and 1, w/v) and fed 60% in the morning and 40% in the afternoon. Drinking water was provided *ad libitum*.

Variables observed were vitamin E consumption and retention, egg's vitamin E, egg's vitamin E deposition efficiency, feed consumption, hen day production (HDP), and egg weight. Vitamin E retention was measured using a combination method of total collection and indicator to avoid fasting period that would affect egg production as described by Indreswari *et al.* (2009). Vitamin E of the egg and excreta were analyzed using high performance liquid chromatography (HPLC) according to the modified procedure of Renzi *et al.* (2005). The egg's vitamin E deposition efficiency was calculated using the following formula: Egg's vitamin E (mg/egg) was divided by the retained vitamin E (mg/bird/day) and multiplied by 100%. Data were subjected to analysis of variance, and followed by Duncan's multiple range test to determine the differences between treatments.

## RESULTS AND DISCUSSION

### Egg's Vitamin E Deposition

Improved diet with higher nutrients density and supplemented with vitamin E significantly ( $P < 0.05$ ) influenced vitamin E consumption and retention, and percentage of deposition into the egg of Kedu breeder chicken (Table 2). Since dietary vitamin E treatment increased while feed consumption was similar, therefore the vitamin E consumption and retention increased. The increased vitamin E would be a cause of the increased vitamin E deposition into the egg, and closely related to the reproductive efficiency. It has been reported by Vieira (2007) that the concentration of vitamin E in the egg yolks was a direct function of its level in the feed. It was also found that vitamin E levels in the embryo were similar to those found in the yolk at the early stages of incubation. In this study, additional vitamin E was found to have low effect on its deposition in the egg yolk. However, the previous

findings indicated that the number of white ovarian follicles increased (Zaghari *et al.*, 2013), and lead to the increase in egg mass (Sedaqat *et al.*, 2011) due to additional vitamin E at the level of 400 mg/kg. Therefore, it should be better to pay attention for the future study focusing on the development of follicles, besides yolk vitamin E deposition.

The two important parameters, fertility and hatchability, in laying chickens could not be separated from the transfer mechanism of vitamin E from mother hen to offspring through the physiological steps during egg formation or egg laying. Muller *et al.* (2012) have found the phenomenon that the transfer of vitamin E may depend on the current environmental variation of the mother hen which further brought about the different results. This physiological phenomenon is assumed to have impact on the rate of vitamin E deposition into the eggs. A clear evidence concerning nutritional supply as an important environmental variation experienced by the mother hen was provided by Kenny and Kemp (2017), that a high ratio of protein to energy reduced egg hatchability and chick performance. It was recommended that the optimum dietary protein and energy levels is 15.18% and 2,750 kcal/kg feed, respectively, for breeder chickens. In this study, the ratio of protein to energy of farmer's ration was 1.0 vs. 186.56 and much lower than that of two other feeds (Table 1, 1.0 vs. 154.38), but it was similar to previous study which recommended to be 1.0 vs. 182.16 (Kenny and Kemp, 2017). It was predicted that high protein to energy ratio was the cause of vitamin E addition became ineffective in Kedu chickens fed either improved diet with or without additional vitamin E.

Lipid peroxidase of egg yolk reduced in laying hens fed diet containing fish oil with additional vitamin E, and promoted the development of liver of chick offspring (An *et al.*, 2010). This phenomenon indicated that retained vitamin E was stored in the liver and transported well later to the ovarium and finally deposited into the ovum/yolk. Therefore, such metabolic physiology gave an impact on the rate of egg vitamin E deposition from retained vitamin E. However, the present results indicated that the lowest vitamin E content of the farmer's ration formula (Table 1) resulted the highest retained vitamin E deposited into the egg (229%), while the improved ration without added vitamin E was much lower (51.85%), and the improved ration

with added vitamin E was the lowest (5.38%). The phenomenon of mechanism could be occurred because ration of farmer's formula had higher fat content as compared to the other two rations (Table 1), since fat was the carrier of the vitamin E metabolism. The biochemical processes of vitamin E metabolism involving the absorption of the common dietary lipids under the aid of bile salt to form fat-soluble vitamins. The hydrolysis product of dietary lipids provide a better milieu for the solubilization of vitamin E to form mixed-micelle. Micelle was further hydrolyzed by pancreatic enzyme before being absorbed by intestinal villi. Vitamin E in the form of chylomicron passed through the small intestine and entered blood circulation to be transported to the liver (Iqbal and Hussain, 2009). Absorption and transportation processes were similar to all type of vitamin E, but after reaching the liver, blood circulating vitamin E were mostly in the form of  $\alpha$ -tocopherol.

The present results provided an interesting illustration that utilization efficiency of vitamin E synthetic was lower compared to that of natural vitamin E from the feedstuffs of the ration. The utilization efficiency between natural sources and additional sythetic vitamin E was due to the difference of biological activity. Biological activity of natural vitamin E such as  $\alpha$ -tocopherol was much more effective compared to the synthetic form and thus increased muscular retention and muscular antioxidant activity in broiler (Cheng *et al.*, 2016). Similar results reported by Peisker *et al.* (2014) that single stereoisomer (natural  $\alpha$ -tocopherol) composition in egg yolk of newly hatched chicken were closely related to dietary vitamin E sources when

compared to mixture stereoisomer, a synthetic all-rac- $\alpha$ -tocopherol. Other findings (Andi *et al.*, 2006; and Zang *et al.*, 2011) indicated that utilization of synthetic vitamin E became more effective when served together with other vitamin and mineral in the form of vitamin-mineral premix. In case of the present research, the mineral content of the rations, especially Ca and P, were almost the same and other vitamin was not added. Therefore, it was suggested that vitamin E utilization efficiency of improved diet was very low (Table 2) even with added vitamin E of synthetic form. However, all parameters due to feeding improved diet without added vitamin E, except vitamin E of the egg, indicated the medium values, and vitamin E utilization for the egg was higher than that of diet with additional vitamin E. It could be assumed that there was no interfere with the biological activity of natural vitamin E derived from the feed since no addition of synthetic vitamin E. As it has been described previously that single stereoisomer of natural vitamin E had higher biological activity compared to synthetic addition either single source or in combination with natural form (Ognik and Wertelecki, 2012; Peisker *et al.*, 2014; and Cheng *et al.*, 2016).

The increasing percentage of vitamin E deposition into the egg of Kedu breeder chickens was calculated between those fed improved ration compared to those given farmer's formula. Deposition of vitamin E into the egg of chickens fed improved ration without and with added vitamin E increased by 1779 and 1980%, respectively (Table 2). However, the increasing deposition of vitamin E into the egg of chickens fed improved ration without added vitamin E

Table 2. Vitamin E Consumption, Retention and Deposition Efficiency of Kedu Breeder Chicken Fed Ration with Different Nutrients Density

Parameters	Dietary Treatments		
	Farmer's Formulation	Improved Ration	Improved Ration + Vitamin E
Vitamin E consumption (mg/bird/d)	0.0079 <sup>c</sup>	0.8900 <sup>a</sup>	6.0700 <sup>a</sup>
Vitamin E retention (mg/bird/d)	0.0065 <sup>c</sup>	0.5400 <sup>b</sup>	5.7600 <sup>a</sup>
Egg's vitamin E (mg/egg)	0.0149 <sup>b</sup>	0.2800 <sup>a</sup>	0.3100 <sup>a</sup>
Egg's vitamin E deposition efficiency (%)	229.2300 <sup>a</sup>	51.8500 <sup>b</sup>	5.3800 <sup>c</sup>

<sup>a-c</sup>Different superscript in each row show significantly different (P<0.05)

Table 3. Production Performance of Kedu Breeder Chicken Fed Ration with Different Nutrients Density

Parameters	Dietary treatments		
	Farmer's Formulation	Improved Ration	Improved Ration + Vitamin E
Feed consumption (g/bird/day)	93.67	90.23	90.41
Hen day production (%)	32.98	34.78	34.80
Egg weight (g)	42.38	40.81	42.07

compared to those given improved ration with additional vitamin E was only 10.71%. These results suggested that additional vitamin E to the diet was not really effective deposited into the egg, because only very small dietary vitamin E deposited into the egg. In addition to the increasing nutrients level, especially  $\alpha$ -tocopherol, in the egg yolk, the strength of vitelline membrane could also be an indicator of the efficiency vitamin E utilization in laying chicken. Yolk  $\alpha$ -tocopherol increased and vitelline membrane strength improved due to feeding dietary supplementation vitamin E at the levels of 100 and 150 mg/kg (Scheideler *et al.*, 2010).

### Production Performance

Feed quality improvement by increasing crude protein, energy metabolism and vitamin E, in one side, and decreasing crude fiber and fat, in other side (Table 1) did not influence production performance (Table 3) indicated by feed consumption, HDP and egg weight that were not significantly different. Different results were found in Hy-Line laying hens given dietary vitamin E at the level of 200 mg/kg improved HDP from 80.7 to 82.7% with the same amount of feed consumption that was 101 g/hen/day (Jiang *et al.*, 2013). Open house rearing model of the chickens in the hot climate region cause apprehension about the productive performance because the birds were assumed suffering continuous heat stress. These results were quite different when compared to the report of Mohiti-Asli *et al.* (2010) that the significant decrease in feed consumption (109.6 to 105.9 g/hen/day), HDP (72.9 to 68.4%), and egg weight (61.5 to 59.7 g) were observed in laying hens exposed to the high temperature. However, an interesting phenomenon shown in the present study that Kedu breeder chickens kept under hot climate

produced the same HDP although given diet with different nutritional density including vitamin E supplementation. Vitamin E functioned as an antioxidant which could managed stress so that those Kedu chicken fed improved ration supplemented with vitamin E should had improved their egg production, but the assumption was not true in the present research. This could be attributed to the low utilization of added synthetic vitamin E that was shown on its low deposition in the egg (Table 2). The phenomenon can be explained that the effectiveness of feeding vitamin E is dependent upon some factors such as strain, ration adaptability, raising model and environment.

### CONCLUSION

Kedu breeder chickens showed limited capability to deposit synthetic vitamin E into the egg when the improved ration supplemented with vitamin E was fed. It is, therefore, recommended that vitamin E supplementation should be implemented together with the increasing fat content of the ration.

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