Effect of Vitamin E Supplementation and Storage Duration on Egg Physical Quality of IPB-D2 Candidate Chicken Strain

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Abstract. Eggs are one of the animal foods that can be easily damaged but have a long shelf life. The objective of the research was to analyze physical quality of IPB-D2 candidate chicken strain eggs which had been supplemented with vitamin E in feed and storaged for 1 and 14 days at room temperature. This research used 30 IPB-D2 candidate chicken strain eggs, from chickens that fed treatment diets during 30 days. This study used a factorial completely randomized design with 3 vitamin E supplementation levels (0 ppm, 100 ppm, 300 ppm), 2 duration of storage treatments (1 and 14 days), and 5 replications. The data were analyzed using ANOVA and if there was a significant difference, it was further analyzed using Duncan Multiple Range Test. The results showed that supplementation 300 ppm vitamin E significantly increased yolk score ($p<0.05$). Storage treatments affects the percentage of albumen, the percentage of yolk, albumen index, yolk index, and haugh unit ($p<0.05$). However, there was no interaction between vitamin E supplementation levels and storage treatments. The conclusion of this study that supplementation of vitamin E generally did not affect physical quality of egg stored at the different time, except yolk score. Supplementation of 100 ppm vitamin E increased yolk score. The storage of egg for 14 days can reduce egg quality.

1. Introduction

Sardinella lemuru fish oil is one of the sources of unsaturated fatty acids and naturally rich in omega-3 fatty acids. However, the weakness of omega-3 fatty acids is susceptible to lipid oxidation which has a negative impact on the quality of animal products such as eggs. The use of antioxidants was essensial to reduce the oxidation of lipids. One of the materials that could be used was vitamin E.

Increasing nutrition through comsumption of animal protein is a people demand for health. One of the efforts to sufficient the protein needs of livestock origin is to develop the potential of local livestock, such as native chickens [1]. Native chickens have good adaptations to the environment and weather changes in Indonesia [2]. Native chickens have been raised as produces of meat, eggs, or entertainment. One of the native chicken lines is the local chicken IPB-D2. IPB-D2 local chicken is a candidate chicken strain developed by the IPB University. IPB-D2 candidate chicken strain has a fast growth rate and more resistant to disease. IPB-D2 candidate chicken strain have an IgY indicator of at least 10 mg/mL of blood and this is the result of selection from IPB-D1 chickens [3]. This chicken produces eggs with white shells and some are slightly brownish.

Eggs is the one of the finest food but have a long shelf life, offering humans an almost complete balance of essential nutrients with proteins, vitamins, mineral, and fatty acids [4]. The external and internal qualities of eggs are very important for consumer health and from a marketing perspective [5]. The physical quality of eggs consists of egg weight, shell weight, shell thickness, egg shell color, egg yolk index, egg white index, and Haugh unit [6]. Physical eggs can be said to be of good quality if they have a clean, smooth, slippery, not cracked shell, normal shape and form. The physical quality of eggs is affected by feed and storage time. Oils are the most commonly applied sources of energy in feed diets for laying hens and exert multiple effects, such as improving palatability, feed intake, animal immunity, and reducing morbidity [7]. Lemuru fish oil is a source of unsaturated fatty acid.

The use of lemuru fish oil in laying hen rations can serve as a source of omega-3 fatty acids, improving egg quality and production.

However, too much addition of fish oil to the diet may affect the performance and can decrease the egg yolk weight. The addition of 8% fish oil to the diet significantly reduced the feed intake, egg production, FCR, egg weight, yolk color, yolk weight, and eggshell color without affecting the eggshell thickness [8]. Beside feed, storage time factors and storage temperature can also affect egg quality. The longer eggs are stored, the quality and freshness of eggs decreases. The content of unsaturated fatty acid in lemuru fish oil is easily oxidized during storage and feed containing these fatty acids need to be added with antioxidants [9]. Vitamin E is one of the fat-soluble vitamins, classified as a micronutrient, and can act as an antioxidant. The addition of vitamin E in feed containing oil can provide stability fot oxidized unsaturated fatty acids which can have a negative impact on livestock. Studies have shown that vitamin E supplementation increased quality of eggs [10]. Supplementation 200 mg/kg vitamin E increased the yolk percentage and decrease the albumen percentage [11]. Therefore, the addition of vitamin E in feed containing lemuru fish oil is expected to maintain egg quality during storage. The objective of the research was to analyze physical quality of IPB-D2 candidate chicken strain eggs which had been supplemented with vitamin E in feed and storaged for 1 and 14 days at room temperature.

2. Methods

Sample of 30 eggs were randomly selected from 120 candidate chicken strains of IPB-D2 which has been maintained for 30 days. The feed ingredients used in the study were lemuru fish oil, yellow corn, rice bran, soybean meal, meat bone meal, DCP, CaCO3, NaCl, premix, and DL-methionine (Table 1). The addition of vitamin E was given to the feed treatment T1 and T2 as much as 100 ppm and 300 ppm. Feeding system was based on the needs of layer-phase chickens. The treatment diets were:

T0 = Basal diet
T1 = Basal diet + 100 ppm vitamin E
T2 = Basal diet + 300 ppm vitamin E

Table 1. Feed composition and nutrient content of basal diet

Feed material	Unit	Composition
Yellow corn	%	53.5
Rice bran	%	3.5
Soybean meal	%	22.0
Meat bone meal	%	10.0
Lemuru fish oil	%	2.0
DCP	%	1.5
$CaCO_3$	%	6.5
NaCl	%	0.3
Premix	%	0.5
DL-methionine	%	0.2
Total	%	100
Nutrient content		
Dry matter	%	88.32
Metabolic energy	kkal kg^{-1}	2803
Protein	%	18.5
Fat	%	5.00
Crude fiber	%	1.5
Lysin	%	1.1
Methionine	%	0.55
Methionine + cystine	%	0.84
Available P	%	0.46
Calsium	%	4.06
Sodium	%	0.17
Chloride	%	0.25

The selected sample was divided into two equal groups. One grup was stored for 1 day while the other was stored for 14 days. The eggs stored at room temperature. Physical quality parameters observed were: egg weight, egg index, percentage of yolk, percentage of albumen, albumen index, yolk index, percentage of egg shell, haugh unit, shell thickness, and yolk score.

1. **Egg weight.** Egg weight calculation (g) was carried out by weighing whole eggs which included the weight of the shell, yolk, and albumen with an analytical scale.
2. **Egg index.** Egg index is done by measuring the length and diameter of the egg using a digital caliper. Egg index was calculated as a ratio of the egg length to its diameter as follows:

$$\frac{egg\ diameter}{egg\ length}$$

3. **Albumen percentage.** The eggs were carefully cracked and placed on a glass table. The percentage of albumen was calculated by weighing the albumen using an analytical scale. Albumen percentage was calculated by dividing the albumen by the egg weight multiplied by 100%
4. **Yolk percentage.** The eggs were carefully cracked and placed on a glass table. The percentage of yolk was calculated by weighing the yolk using an analytical scale. Yolk percentage was calculated by dividing the yolkby the egg weight multiplied by 100%
5. **Albumen index.** The diameter and height of the thick albumen were measured using a digital caliper. The albumen index calculated by dividing the albumen height (mm) by the albumen diameter (mm)
6. **Yolk index.** The diameter and height of the thick yolk were measured using a digital caliper. The yolk index calculated by dividing the yolk height (mm) by the yolk diameter (mm)
7. **Egg shell percentag.** The eggs were carefully cracked and placed on a glass table. The percentage of egg shell was calculated by weighing the egg shell using an analytical scale. Egg shell percentage was calculatedby dividing the egg shell by the egg weight multiplied by 100%
8. **Haugh unit.** After performing the albumen height measurement, the haugh unit (HU) was determined by following equation proposed by Haugh (1937):

$$Haugh\ unit = 100 \log(H + 7{,}73 - 1{,}7\ W^{0{,}37})$$

9. Where: H=height of dense albumen (mm), and W: egg weight (g)
10. **Shell thickness.** The shell thickness was measured using a digital micrometer. The measurements were taken, thesemeasurements being made at the two poles and in the middle of the egg. Calculation results in millimeters (mm)
11. **Yolk score.** Yolk score was measured by matching the yolk color with a *Roche yolk color fan*.

This study used a factorial completely randomized design with 3 vitamin E supplementation levels (0 ppm, 100 ppm, 300 ppm), 2 duration of storage treatments (1 and 14 days), and 5 replications. The data were analyzed using ANOVA and if there was a significant difference, it was further analyzed using Duncan Multiple Range Test.

3. Results and Discussion

Results of the effects of supplementation vitamin E and storage period are presented in Table 2. The results showed that supplementation of 100 ppm vitamin E significantly increased yolk score($p<0.05$). Storage treatments affected the percentage of albumen, the percentage of yolk, albumen index,yolk index, and haugh unit ($p<0.05$).

Table 2. Egg physical qualities of IPB-D2 candidate chicken strain

Physical Attributes	Vitamin E Levels	Storage treatments (days)		Mean	Vitamin E × Storage
		1	14		
Egg weight (g)	T0	41.82 ± 2.46	38.26 ± 3.05	40.04 ± 2.76	NS
	T1	40.88 ± 7.26	41.80 ± 8.85	41.34 ± 8.06	
	T2	43.50 ± 1.65	43.20 ± 4.89	43.35 ± 3.27	
		42.07 ± 3.79	41.09 ± 5.60		
Egg index	T0	0.77 ± 0.03	0.76 ± 0.03	0.77 ± 0.03	NS
	T1	0.79 ± 0.04	0.75 ± 0.02	0.77 ± 0.03	
	T2	0.76 ± 0.04	0.80 ± 0.05	0.78 ± 0.05	
		0.77 ± 0.04	0.77 ± 0.03		
Percentage of yolk (%)	T0	34.71 ± 0.99	43.25 ± 1.80	38.98 ± 1.40	NS
	T1	35.78 ± 6.85	41.26 ± 4.95	38.52 ± 5.90	
	T2	34.82 ± 1.84	40.21 ± 1.09	37.52 ± 1.47	
		35.10 ± 3.23a	41.57 ± 2.61b		
Percentage of albumin (%)	T0	55.41 ± 1.70	46.05 ± 3.35	50.73 ± 2.53	NS
	T1	54.48 ± 7.55	49.39 ± 3.48	51.94 ± 5.52	
	T2	54.91 ± 2.31	47.86 ± 1.74	51.39 ± 2.03	
		54.93 ± 3.85b	47.77 ± 2.86a		
Albumin index	T0	0.10 ± 0.02	0.02 ± 0.004	0.06 ± 0.01	NS
	T1	0.11 ± 0.03	0.03 ± 0.01	0.07 ± 0.02	
	T2	0.12 ± 0.06	0.01 ± 0.005	0.07 ± 0.03	
		0.11 ± 0.04b	0.02 ± 0.01a		
Yolk index	T0	0.42 ± 0.02	0.17 ± 0.08	0.30 ± 0.05	NS
	T1	0.46 ± 0.05	0.24 ± 0.12	0.35 ± 0.09	
	T2	0.44 ± 0.04	0.22 ± 0.02	0.33 ± 0.03	
		0.44 ± 0.04b	0.21 ± 0.07a		
Percentage of egg shell (%)	T0	10.42 ± 1.40	10.70 ± 1.86	10.56 ± 1.63	NS
	T1	9.74 ± 1.78	9.35 ± 1.73	9.55 ± 1.76	
	T2	10.27 ± 1.18	11.93 ± 0.95	11.10 ± 1.07	
		10.14 ± 1.45	10.66 ± 1.51		
Haugh unit	T0	88.09 ± 5.90	49.43 ± 2.68	68.76 ± 4.29	NS
	T1	90.26 ± 4.98	55.19 ± 14.75	72.73 ± 9.87	
	T2	88.67 ± 4.91	34.18 ± 19.02	61.43 ± 11.97	
		89.01 ± 5.26b	46.27 ± 12.15a		
Shell thickness (mm)	T0	0.35 ± 0.04	0.30 ± 0.05	0.33 ± 0.05	NS
	T1	0.31 ± 0.07 0.31	0.27 ± 0.09	0.29 ± 0.08	
	T2	± 0.05	0.35 ± 0.02	0.33 ± 0.04	
		0.32 ± 0.05	0.31 ± 0.05		
Yolk score	T0	4.80 ± 0.84	4.20 ± 0.45	4.50 ± 0.65a	NS
	T1	5.60 ± 0.55	5.80 ± 1.64	5.70 ± 1.10 b	
	T2	5.00 ± 0.71	4.80 ± 0.84	4.90 ± 0.78 ab	
		5.13 ± 0.70	4.93 ± 0.98		

a-b Different superscript at the same row or colomn indicate significant differences ($p<0.05$)

NS: No significant interaction between vitamin E supplementation and storage time. T0 = without vitamin E supplementation (control); T1 = supplementation of 100 ppm vitamin E; T2 = supplementation of 300 ppm vitamin E

3.1 Egg Weight. There was no interaction between vitamin E supplementation in feed and storage time, and also there was no significant effect of each treatment on egg weight (Table 2).

Supplementation of vitamin E as much as 300 ppm had a higher weight than the 0 ppm and 100 ppm, but did not differ significantly. This is in accordance with Mohiti-Asli (2008) [12] that addition of vitamin E in the ration has no effect on egg weight.. At 14 days of storage (42.07 g), eggs decreased in weight compared to 1 day of storage (41.09 g). The loss of egg weight is mostly due to evaporation of water in the albumen, and a small part is due to the evaporation of gases such as CO2, NH3, N2, and H2S due to the degradation of egg protein components [13]. The longer the storage time, the greater the egg weight loss will be. Research states that eggs stored for 2 weeks have a shrinkage value of 3.63% ± 1.66% [14]. Evaporation of water and gases such as CO2, NH3, and H2S as a result of the degradation of egg organic matter occurs since the egg comes out of the chicken's body and evaporates through the pores in the egg shell and lasts continuously, causing a decrease in egg weight. The decrease in egg weight is also influenced by storage temperature, relative humidity, and eggshell porosity [15].

3.2 Egg Index. There was no interaction effect between vitamin E supplementation in the ration and storage time on egg index. Egg index of vitamin E supplementation of 0 ppm, 100 ppm, and 300 ppm, were not significant different. Storage time also has no effect on the egg index.

Egg index is a parameter used to assess egg shape. In this study, the index of eggs stored for 1 and 14 days had a same value. This is not much different from the index value of eggs stored at a emperature of 18-30℃ which has a value of 0.78-0.79 [16]. Egg shape and weight are not affected by storage temperature and storage time, but are influenced by heredity, brood age, season of the year, and feed quality. Amount of metabolizable energy in feed possibly increase egg shape index [17]. Some studies reported that high energy level in feed significantly increased egg shape index of laying hen as compared to low energy level in feed [18]. In this study, energy in the feed with the addition of vitamin E to maximize the use of lemuru fish oil and reduce the oxidation of fatty acids contained in it.

3.3 Percentage of Yolk and Albumen. There was no interaction between vitamin *E* supplementation and storage time on the percentage of yolk and albumen percentage. Storage for 14 days increased the percentage of yolk and decreased the percentage of egg whites ($p<0.05$). Supplementation of vitamin E had no effect on the percentage of yolk and albumen.

This is in accordance with previous studies, which stated that the administration of vitamin E did not affect the percentage of egg yolks [12]. The research reported that the addition of vitamin E (100 or n200 ppm) in laying hens diets significantly decreased albumen weight with increased yolk weight [19], which resulted in albumen and yolk percentages which were not much different from this study, namely 51% and 38% respectively. Other studies showed that the hens fed diets containing 200 mg/kg of vitamin E increased yolk percentage and reduced albumen percentage than hens fed 0 mg/kg of vitamin E [11].

During the storage period, egg yolk increased from 35.10% on 1 day storage to 41.57% on 14 days storage, egg white decreased accordingly from 54.93% to 47.77%. The loss of albumen percentage or quality during storage time is mainly attributed to the original albumen quality and the movement of water from albumen to yolk [20]. Measuring components as proportions of the whole egg removed any inconsistencies, and longer periods of storage in greater percentages of shell and yolk, but a lesser percentage of albumen. It was reported that decreased yolk cholesterol levels could decrease yolk weight [21].

3.4 Albumen Index and Yolk Index. Vitamin E supplementation had no effect on albumen index and yolk index. This is the same as previous studies which stated that the administration of vitamin E in laying hens did not affect the albumen index and yolk index and resulted 0.06-0.07 of albumen index and 0.34-0.36 of yolk index [22]. There was no interaction between vitamin E supplementation and storage period on variables observed.

Storage for 14 days significantly decreased albumen index compared to storage for 1 day ($p<0.05$). This is in accordance with previous research that storage had a significant effect on

decreasing albumen index [23]. The longer the storage time, the higher the evaporation of CO_2 and H_2O so that the albumen decreases in thickness. Dilution of albumen occurs due to changes in gel structure, due to physico-chemical damage to ovomucin fibers. Ovomucin is a glycoprotein in the form of fibers and can bind water to form a gel structure [24]. The thicker the egg white, the higher the egg white index value to maintain egg white quality during storage [25]. The albumen index value decreased rapidly after 14 days of storage at room temperature. In addition to storage time, other factors that affect the egg white index value include storage temperature, feed nutrition, and chicken age.

The index value of yolk stored for 1 and 14 days in this study was significantly decreased ($p<0.05$). This is in accordance with previous research that storage had a significant effect on decreasing the yolk index [23]. Factors that affect the yolk index are storage, temperature, vitelline membrane quality, and feed nutrition. The quality of the vitelline membrane and feed with fat content and vitamin E supplementation had a major influence on the egg yolk index. The state of the convex and firm yolk is determined by the strength and condition of the vitelline membrane. A decrease in the binding strength and a decrease in the state of the vitelline membrane can cause water to easily move from the albumen to the yolk. This displacement causes the yolk to become watery and relatively flat, so the index value will be low [25]. Supplementation of vitamin E in feed containing fish oil or fat can prevent oxidation and maintain egg yolk quality, thereby maintaining the index value.

3.5 Percentage of Eggshell. There was no interaction between vitamin E supplementation and storage time. Vitamin E supplementation did not significant different and storage time also did not significant effect. Storage for 14 days resulted in a higher percentage of egg shells compared to storage for 1 day. The percentage of eggshells in this study resulted in a higher average than the study where the average eggshell of chickens given vitamin E supplementation was 9.20% [26]. In contrast to other studies, that the percentage of local chicken egg shells was 11.35% [27]. The egg shell is the outermost part that encloses the contents of the egg and serves to reduce physical and biological damage, and has pores for gas exchange from the inside to the outside of the egg shell. It was further explained that the egg shell weight was strongly influenced by the feed consumed, egg weight, and the age of the chicken. In addition, the content of calcium and phosphorus in feed plays a role in eggshell quality, such as thickness, weight, and eggshell structure [28].

3.6 ***Haugh Unit.*** The result obtained in Table 2 shows that there's significant differences ($p<0.05$) between storage time for 1 day and the ones storage time for 14 days. There was no interaction between vitamin E supplementation and storage time.

Storage for 1 day resulted in HU of 89.01 while the storage of 14 days resulted in HU of 46.27. The HU is a measure for evaluating egg internal quality that relates albumen height with egg weight. Higher HU means better egg quality [29]. The decrease in HU value is related to albumin dilution. The value of HU decreases with increasing age of eggs, storage time will cause albumin quality to decrease due to physical and chemical changes so that albumin becomes watery and ovomucin content becomes low [30]. Determination of egg quality based on the Haugh Unit value is based on the standards of the United State Department of Agriculture (2000), namely the Haugh Unit value less than 31 is classified as C quality, the Haugh Unit value less than 31-60 is classified as B quality, the Haugh Unit value is less than 60-72 classified as A quality and a Haugh Unit score of more than 72 is classified as AA quality. So, in this study, IPB-D2 candidate chicken strain eggs at 1 day storage had AA quality and at 14 days storage had B quality.

3.7 ***Shell Thickness.*** The effect of vitamin E supplementation treatment and storage time on the interior quality of eggs such as shell thickness gave results that were not significantly different and there was no interaction between vitamin E supplementation and storage time.

The thickness of the shell in this study ranged from 0.29-0.33. It is appropriate that the average thickness of native chicken shells ranges from 0.34-0.40 [13]. Egg shell quality is influenced by the nutritional adequacy of livestock, livestock health, maintenance management, and the livestock environment. Improving the quality of the eggshell can be done by stabilizing the flow of calcium into the blood vessels, because the main source of CaCO3 for shell formation comes from bicarbonate ions

in the blood. Age can also affect the thickness of the shell, the older age of the chicken, the quality of shell will decrease, the eggshell will be thinner, the color of the shell will fade, and the weight of the egg will get bigger. Thin egg shells can be sourced from nutrition or due to infection. One way that can be done to prevent thin shells is by supplementing with vitamin E in the feed as an antioxidant. Vitamin E can stimulate immunological responses in increasing immunity [31].

3.8 ***Yolk Color Score.*** Supplementation of vitamin E significantly ($p<0.05$) increased yolk color score. There was no interaction between vitamin E supplementation and storage time. Storage time has no effect on the yolk color score.

Color is important quality trait of foods since it affects the consumers perception of quality and internsity of aroma and flavor [32]. Other research states that supplementation with 250 mg/kg vitamin E had no significant effect on egg quality [26]. In 1 day storage the yolk color score was 5.13 and at 14 days storage it was 4.93. The average egg yolk color score obtained is still below the score the yolk color score of IPB-D1 chickens in other research which got 7.16 [33]. Reduction in yolk color score as storage time have been reported previously [34]. Yolk color score is chiefly dependent upon the content of yolk carotenoids, which can be degraded by oxidative processes, varying the yolk pigmentation during storage [20]. Duffision of water from albumen during storage time accelarate oxidative process of yolk pigment. Feed that was supplemented with vitamin E had a better yellow color than egg yolks without vitamin E supplementation. These finding that probably vitamin E could reduce oxidative process and preserve yolk color during more time [35].

4. Conclusions

It can be concluded, that the supplementation of vitamin E generally did not affect physical quality of egg stored at the different time, except yolk score. Supplementation of 100 ppm vitamin E increased yolk score. The storage of egg for 14 days can reduce egg quality reflected by decreasing of albumen index, yolk index, and Haugh Unit.

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