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Vitamin E Supplementation in Feed Containing Lemuru Fish Oil to Improve IPB-D2 Chickens Performance and Eggs Rich in Vitamin E as a Potential Functional Food

Research Article

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ABSTRACT

This study aimed to evaluate the supplementation of vitamin E in diets containing Lemuru fish oil (LFO) on the performance, physical quality of egg, blood profile, malondialdehyde (MDA) level, yolk fatty acid, and functional egg production of IPB-D2 chicken. 120 IPB-D2 chickens were reared from 36 to 40 weeks. The treatment diets were consisted of: T0= control diet containing 2% LFO, T1= control diet containing 2% LFO + 100 ppm vitamin E, T2= control diet containing 2% LFO + 300 ppm vitamin E. Data were analyzed for significant differences using analysis of variance (ANOVA) and if the treatment significant effect followed by Tukey's significant difference test. The result showed that vitamin E supplementation to diet containing LFO had no effect on the performance, physical quality of eggs, and MDA level of IPB-D2 chicken. Supplementation of 100 ppm vitamin E significantly ($P<0.05$) increased red blood cell of IPB-D2 chicken. The supplementation of vitamin E as much as 100 ppm and 300 ppm in the diet containing LFO significantly ($P<0.01$) increased the content of vitamin E in the yolk by 262,22% to 1354,81% and has the potential to form of functional eggs rich in antioxidants. Based on calculations, women and men aged more than 14 years who consume 2 functional eggs high in vitamin E per day can satisfy the daily requirement of vitamin E by 39.33% of the daily requirement of vitamin E.

KEY WORDS functional egg, IPB-D2 chicken, Lemuru fish oil, performance, vitamin E.

INTRODUCTION

The development of local chickens as commercial livestock has potential because they can play an important role as protein source. Local chickens are able to adapt to the environment and climate in Indonesia (Nataamijaya, 2010). Local chicken tends to have no specific characteristics, resistant to several diseases, and has slow growth because there is not much genetic development (Lukmanudin *et al.* 2018). In Indonesia, along the time, there has been a lot of genetic development in local chickens. One of them is IPB-D2 chicken which is a candidate for local chicken lines developed by IPB University, Indonesia. IPB-D2 chicken is a selection of IPB-D1 chickens which has high body resistance to disease and allows it to grow faster than other local chickens. IPB-D2 chickens were selected based on a total IgY concentration that exceeded 9.55 mg/ml and an ND antibody titer of more than 3 log 2 HI units.

This chicken also produces eggs with white and slightly brown shells. In response to the continuous demand for eggs, higher efficiency is required in the production aspects of laying hens, one is by utilizing feed additives that can improve egg production and health over the laying periods (Adli *et al.* 2023). Eggs have high biological value because

they are rich in protein. Eggs contain essential unsaturated fatty acids such as linoleic acid, oleic acid, iron, phosphates, minerals, and fat-soluble vitamins (Kusum *et al.* 2018). Santoso and Fenita (2016) stated that eggs contain 16.60% protein, 31.90% fat, 147.08 mg/L calcium, 586.04 mg/L phosphorus, 7.25 mg/L iron, 3.03 mg/100 mg cholesterol, and 632 IU of vitamin A. Egg yolks also have an omega-6 to omega-3 ratio of 11.4 (Zotte *et al.* 2019). The nutritional quality of eggs can be affected by dietary factors (Zduńczyk *et al.* 2013), nutrient content of eggs can be modified by feed modification. One type of feed ingredient that is often used is oil. Oil can reduce dustiness in feed and increase palatability. Fish oil contains long chain fatty acids which can increase the unsaturated fat content in the egg.

Lemuru fish (*Sardinella lemuru*) oil in laying hen diet can be used as a source of omega-3 fatty acid, improves egg quality and egg production. Omega-3 as an unsaturated fatty acid contained in Lemuru fish oil (LFO) is needed as a hormone synthesis material for follicular development in laying hens (Indi *et al.* 2014). Omega-3 fatty acids are susceptible to oxidation which can have a negative impact on the nutritional value, color and texture of food products (Avila-Ramos *et al.* 2013). According to Tarigan *et al.* (2016), polyunsaturated fatty acids are easily oxidized and cause exogenous free radicals to easily enter cells and trigger oxidative cells. One effort that can be done to prevent fat oxidation, improving the immune system is by using antioxidant as functional feed supplementation (Adli *et al.* 2023). Vitamin E is the main antioxidant component that, play an important role in metabolic processes, protect cell structures, and maintain membrane stability (Rayani *et al.* 2017).

Vitamin E is one of the vitamins contained in egg yolks, as stated by Cimrin (2019) that vitamin E supplementation in feed could increase the content of vitamin E in egg yolks and improve egg quality. Eggs high in vitamin E can be categorized as a functional food. Functional eggs are eggs that are rich in nutrients that are already in the egg and can have a positive effect on consumers.

Vitamin E supplementation in the feed containing LFO is expected to protect LFO in the diet from oxidation and play a role in improving the performance of IPB-D2 chicken, improving blood profiles, lowering blood malondialdehyde (MDA) levels, and forming functional eggs high in vitamin E. The aim of this study was to investigate the supplementation of vitamin E in diets containing LFO on the performance, physical quality of egg, blood profile, MDA level, yolk fatty acid, and functional egg production of IPB-D2 chicken.

MATERIALS AND METHODS

Experimental design

A total of 120 IPB-D2 chicken in layer phase, were reared from 36 to 40 weeks and received the treatment diets for 4 weeks. Before feeding the treatment diets, chickens were fed adaptation feed which was basal feed that cointaned corn, rice bran, soybean meal, meat bone meal, dicalcium phosphate, calcium carbonate ($CaCO_3$), NaCl, premix, and DL-methionine for two weeks and SINTA GT-1 commercial feed for the next two weeks. All chickens were housed in 60 battery cages, each cage containing 2 chickens. The feed ingredients as control diet used in the research included LFO, corn, rice bran, soybean meal, meat bone meal, dicalcium phosphate, calcium carbonate ($CaCO_3$), NaCl, premix, and DL-methionine. Vitamin E supplementation was given to the feed treatment T1 and T2 as much as 100 ppm and 300 ppm, respectively. Treatment were as follows: T0= control diet containing 2% LFO without vitamin E; T1= control diet containing 2% LFO + 100 ppm vitamin E; T2= control diet containing 2% LFO + 300 ppm vitamin E. Feed ingredients obtained from PT. Nuansa Baru and PT. Indofeed. Vitamin E was obtained from PT. Nutricell. The composition of formulated feed showed in the Table 1.

Sample collection

Blood was collected on the last day of chicken rearing as much as one chicken per replication. According to Martoenus and Djatmokowati (2015), blood in poultry is taken from the pectoralis vein which is the blood vessel at the bottom of the bird's wing. Sampling of yolks for vitamin E analysis was taken by taking 30 g of egg yolk from each replication.

Performance measurement of IPB-D2 chicken

Feed consumption (g/bird) was calculated by calculating the difference between the amount of diet given (g) and the remaining diet (g) which is done once a week (Syafwan and Noferdiman, 2020).

Hen day egg production (HDP) was obtained from the percentage of the total eggs produced by a number of chickens in a certain period of time, egg mass (g/bird) was obtained by multiplying daily egg production by egg weight, feed conversion ratio (FCR) was calculated by calculating the cumulative amount of feed consumed divided by the total weight of the eggs produced (Milenia *et al.* 2022), and mortality (bird) was obtained by the number of chickens that died during rearing.

Table 1 Feed composition and nutrient content of basal diet

Feed ingredients (%)	Composition
Corn	53.50
Rice bran	3.50
Soybean meal	22.00
Meat bone meal	10.00
Lemuru fish oil	2.00
Dicalcium phosphate	1.50
Calcium carbonate ($CaCO_3$)	6.50
NaCl	0.30
Premix	0.50
DL-methionine	0.20
Nutrient contents	
Dry matter (%)*	88.32
Ash (%)*	14.26
Metabolizable energy (kcal/kg)**	2803
Crude protein (%)*	20.93
Crude fat (%)*	4.46
Crude fiber (%)*	2.46
Lysine (%)**	1.10
Methionine (%)**	0.55
Calcium (%)**	4.06
Sodium (%)**	0.17
Chloride (%)**	0.25

Formulated using the trial and error method based on the needs of layer phase on Lohmann (2020).
* Results of analysis by the PAU IPB Laboratory (2022).
** Based on the results of the trial and error method.

Egg physical quality measurement of IPB-D2 chicken

Egg weight (g) was calculated by weighing whole eggs, egg index was calculated by dividing egg diameter by egg length multiplied by 100, percentage of albumen was calculated by dividing weight of the albumen by weight of the egg multiplied by 100%, percentage of yolk was calculated by dividing weight of the yolk by weight of the egg multiplied by 100%, eggshell percentage was calculated by dividing eggshell weight by egg weight multiplied by 100%, thickness of the eggshell was measured using a digital micrometer, albumen index was calculated by dividing height of albumen by diameter of the albumen, yolk index was calculated by dividing height of yolk by diameter of yolk, color of yolk was measured by matching the color of yolk with a Roche yolk color fan (Stadelman and Cotterill, 1995). Haugh Unit (HU) was calculated by the formula: $100\log (H+7.73–1.7\ W^{0.37})$. H is albumen height (mm) and W is egg weight (g) (Haugh, 1937).

Blood profiles measurement

Calculation of the number of erythrocytes and leukocytes were counted using hemacytometer, hematocrit was done by filling the hematocrit tube with blood and anticoagulant. The hematocrit value was determined by measuring the percentage of red blood cell volume using a microcapillary hematocrit reader, hemoglobin can be seen in the "gram %" column printed on the hemoglobin tube, the amount of hemoglobin in grams per 100 mL of blood, and differentiation of leukocytes were observed and counted under a microscope (Sastradipraja and Hartini, 1989).

Measurement of MDA (malonaldehyde) levels and yolk vitamin E content

Analysis of the MDA content in blood plasma was carried out using the thiobarbituric acid reactive substances (TBARS) which modified the amount of material used to follow the number of samples (Rice-Evans and Anthony, 1991). Measurement of vitamin E in egg yolk was carried out by injecting the sample solution into the HPLC (AOAC, 2005).

Measurement of egg fatty acid content

The first analytical procedure performed was fat extraction. Fat was extracted by the Soxhlet method to obtain fatty acids. Next, the sample of fat in the form of oil is weighed. The second analytical procedure is the formation of methyl esters (methylation). The third procedure is the identification of fatty acids by gas chromatography (AOAC, 2005).

Statistical analysis

A statistical analysis was conducted using analysis of variance using completely randomized design using SPSS software. At the end, if the treatment significant effect followed by Tukey's significant difference test. The following model was used:

$$Y_{ij} = \mu + r_i + \varepsilon_{ij}$$

Where:
Y_{ij}: parameters observed.
μ: overall mean.
r_i: effect different the effect level vitamin E.
e_{ij}: amount of error number.

Which, T0= control diet containing 2% lemuru fish oil without vitamin E; T1= control diet containing 2% lemuru fish oil + 100 ppm vitamin E; T2= control diet containing 2% lemuru fish oil + 300 ppm vitamin E.

RESULTS AND DISCUSSION

Table 2 shows that there was no significant difference between treatments on the performance of IPB-D2 chicken. Consumption can be affected by the energy content in the diet (Heldini, 2015). The treatment diets had the same energy content, the only difference was the level of vitamin E in diet. The vitamin E content in the diets did not affect the energy content (Lubis *et al.* 2015). This is likely to cause no significant difference in consumption.

Table 2 Performance of IPB-D2 chicken fed diet containing different level of vitamin E

Variables[2]	Treatments			P-value
	T0	T1	T2	
Feed consumption (×10^2 g/bird)	26.27±0.62	27.02±0.45	26.87±0.86	0.213
HDP (%)	47.90±8.20	48.00±11.31	49.29±7.68	0.965
Egg weight (g)	40.64±1.37	39.78±2.37	39.97±0.97	0.701
Egg mass (×10 g/bird)	66.87±12.81	67.63±20.59	67.01±9.86	0.996
FCR	4.06±0.86	4.26±1.13	4.08±0.06	0.926
Mortality (bird)	1	0	1	

T0: control diet containing 2% Lemuru fish oil (LFO) without vitamin E; T1: control diet containing 2% LFO + 100 ppm vitamin E; T2: control diet containing 2% LFO + 300 ppm vitamin E.
HDP: hen day production and FCR: feed conversion ratio.

Feed consumption can be influenced by palatability, environmental conditions, health status of chicken (Utomo, 2017). Rofii *et al.* (2018) stated that local chickens in Indonesia consume 90-97 g diet per bir per day. IPB-D2 chickens in this study produced egg production with range 47.90%-49.29%. Feed added to vitamin E which has antioxidant activity can increase chicken resistance to disease and maintain the chicken's body condition for maximum production. Hen day production in this study was similar to the study of Habiburahman *et al.* (2020) in IPB-D1 chickens which had an average egg production of 49.22%. Age, breed, health condition, feed, maintenance management, and environmental temperature can be factors that influence the production of laying hens. IPB-D2 chickens has purebred chicken genetics so they produce higher chicken eggs than some other local chickens.

Vitamin E supplementation did not affect egg weight in this study. This is because the consumption between treatments did not differ. Egg weight in this study had a ranged from 39.78-40.64 g. The weight of the eggs in this research was greater than that of the research of Ardika *et al.* (2017) which stated that native chicken eggs with commercial feed produced a weight of 34.66 g. Egg weight can be affected by environmental factors, egg laying period, laying hen age, egg composition, and feed nutrients (Okatama *et al.* 2018).

Older chickens will produce larger eggs. The treatment of vitamin E did not make a difference to the egg mass. This is in accordance with the statement of Mohiti-Asli *et al.* (2008) that vitamin E supplementation in feed did not make a significant difference to egg mass. Egg mass is related to feed consumption, egg weight, and number of eggs. High consumption of feed will be in line with high egg production and the growth of mature follicles which will increase egg mass (Tumiran *et al.* 2017).

Feed conversion ratio is used to measure efficiency in the use of feeds (Subekti *et al.* 2006). Feed conversion ratio of IPB-D2 chicken is currently not available yet. This research resulted in feed conversion ratio of 4.06-4.26. Vitamin E supplementation did not affect feed conversion ratio. Previous research on IPB-D2 chickens in the layer phase resulted in a feed conversion ratio of 6.63 (Falah, 2023).

The different may be caused by vitamin E supplementation and environmental conditions (Sumiati *et al.* 2022). Feed conversion ratio can be influenced by egg production, metabolic energy content in diet, body size of chicken, environmental temperature, and animal health status (Afandi *et al.* 2018). Vitamin E supplementation can increase the efficiency of feed use. Utomo (2017) stated that brown laying hens produce a ration conversion of 2.18-2.33. Vitamin E supplementation can increase the immune system of livestock because it reduces the negative impact of unsaturated fatty acid oxidation (Kusharto *et al.* 2015). Salsabila *et al.* (2022) stated that supplementation of 200 ppm of vitamin E could increase the immunity of chickens exposed to heat stress during rearing.

The results of the effect of adding vitamin E to the diet on the physical quality of IPB-D2 chicken eggs are presented in Table 3. The results showed that Vitamin E supplementation did not affect the physical quality of IPB-D2 chicken eggs. Vitamin E supplementation in feed did not significantly affect egg weight, this is in accordance with the statement of Beyzi *et al.* (2020) that the supplementation of vitamins in the feed did not affect egg weight. The nutrient content of the diet can affect egg weight. This research contained the same nutrient diets, except that T1 and T2 were added with vitamin E. The egg weight in this research produced eggs that were heavier than the parent IPB-D2 chicken, namely IPB-D1 chickens, which had an average egg weight of 40.54 g (Habiburahman *et al.* 2020). Yılmaz *et al.* (2011), stated that egg weight has a negative correlation with egg index. There was no significant effect of vitamin E supplementation for egg index. Research by Marlya *et al.* (2021) stated that native chicken eggs produced an index of 74.34. The rounder the egg shape, the greater the egg index number (Shoimah *et al.* 2019). Feed with a high amount of metabolic energy can increase the egg index (Andri *et al.* 2015). Vitamin E supplementation can maximize the use of LFO which contains high energy and reduces fatty acid oxidation.

Vitamin E supplementation in this research did not affect the percentage of egg yolk and albumen. This is in accordance with the statement of Nemati *et al.* (2020).

Table 3 Physical quality of IPB-D2 chicken eggs fed diet containing different level of vitamin E

Variables	Treatments[1]			P-value
	T0	T1	T2	
Egg weight (g)	43.91±2.16	42.35±3.39	42.32±2.15	0.564
Egg index	75.50±2.90	77.54±0.95	76.65±5.15	0.657
Yolk percentage (%)	38.61±4.51	34.71±1.23	35.91±1.54	0.272
Albumen percentage (%)	50.57±4.26	54.14±2.02	52.76±1.93	0.561
Albumen index	0.10±0.01	0.10±0.01	0.10±0.02	0.482
Yolk index	0.43±0.03	0.43±0.01	0.44±0.02	0.893
Shell percentage (%)	10.82±0.53	11.15±1.18	11.33±0.71	0.903
Shell thickness (mm)	0.31±0.01	0.32±0.06	0.31±0.02	0.251
Haugh unit	88.69±3.70	87.74±4.37	87.75±5.86	0.936
Yolk score	5.45±0.65	5.50±1.00	5.85±0.55	0.670

T0: control diet containing 2% Lemuru fish oil (LFO) without vitamin E; T1: control diet containing 2% LFO + 100 ppm vitamin E; T2: control diet containing 2% LFO + 300 ppm vitamin E.

Yolk contain high fat, therefore the use of fat from LFO is maximized by adding vitamin E and forming more yolks. Jiang *et al.* (2013) stated that the supplementation of 200 ppm of vitamin E increased the percentage of egg yolks compared to feed without vitamin E, but decreased the percentage of albumens.

The yolk index and albumen index in this research were not affected by vitamin E supplementation. The mean yolk index and albumen index in this research had values of 0.43 and 0.10, respectively. Yolk index can be affected by the age of the chicken, the older the chicken, the larger the size of the eggs produced, and the greater the yolk. Yolk index is also affected by the condition of the vitelline membrane (Nys and Guyot, 2011), where a decreased state of the vitelline membrane can cause a shift of water from the albumen to the yolk, this can cause the yolk to become runny and have a low index value. Albumen index can be affected by storage. The longer the storage of eggs, the thinner the albumen will be due to the evaporation of H_2O and CO_2.

Vitamin E treatment did not give a significant difference on shell percentage and shell thickness. IPB-D2 chickens in this research had a shell percentage 10.82% to 11.35%. The percentage of IPB-D1 chicken shells was 11.30% (Habiburahman *et al.* 2020). Another research stated that native chicken eggs had a shell percentage of 11.35% (Marlya *et al.* 2021). The thickness of the shell can be affected by the age of the chicken, the genetics of the laying hens, the health of the chickens, the environment, and the nutrient of feed, especially vitamin D, Ca, and P. The shell thickness in this research had a ranged of 0.31-0.32 mm, this value was still the same as that of IPB-D1 chickens, which was 0.3 mm.

Haugh unit (HU) is also one of the variables that can determine the quality of an egg. HU in this research was not affected by vitamin E supplementation, this is in accordance with the statement of Jiang *et al.* (2013) that the level of vitamin E in feed does not significantly affect HU.

The higher the HU value, the better the egg quality (De Almeida *et al.* 2021). The HU in this research resulted in 87.74-88.69, with the highest value in the control treatment. Habiburahman *et al.* (2020) stated that IPB-D1 chickens produced HU 87.45, thus the egg quality of IPB-D2 chicken and their parents can be said to be of good quality.

The egg yolk score in this research with vitamin E supplementation to the feed was not significantly different from the control feed, this is in accordance with the statement of Narimanirad *et al.* (2012). The average egg yolk score was still below the IPB-D1 chicken egg yolk score in the research by Habiburahman *et al.* (2020), which is 7.16. The color of the yolk can be affected by the carotenoid content in the feed and make a difference in egg yolk pigmentation (Suresh *et al.* 2015), the higher the carotenoid content in the feed, the greater the yolk score.

Blood functions as a means of transportation and defense of the body which is formed in the bone marrow. The blood profile of IPB-D2 chickens with vitamin E supplementation in the feed is presented in Table 4. Supplementation 100 ppm vitamin E had a significant effect ($P<0.05$) on red blood cells, but did not make a difference on white blood cells, hemoglobin, hematocrit, and leukocyte differentiation compared to control feed.

The results of the statistical analysis showed that the supplementation of 100 ppm of feed had a significant effect ($P<0.05$) on increasing the number of red blood cells. This is in accordance with the statement of Attia *et al.* (2017) that vitamin E supplementation in diet increases red blood cells. The increase in red blood cells caused by inhibition of lipid peroxidation in the erythrocyte membrane due to antioxidant effect of vitamin E. Vitamin E did not significantly affect hemoglobin and hematocrit. Hemoglobin levels that have the same value indicate the ability of chickens to bind oxygen to function properly (Mahmud *et al.* 2017). This research produced hemoglobin values with an average of 8.50-9.34 G %, this is still within the normal range.

Hematocrit or packed cell volume is a percentage based on the volume of red blood cells, where the increase and decrease depend on the volume of blood cells compared to the total blood volume (Alfian *et al.* 2017). The hematocrit value in this research was not significantly different. The range of hematocrit in this research was 24.40% to 26.70%. This research resulted in a lower hematocrit value of 15.2%-22.5% compared to the normal range. This can be caused because livestock in the tropics tend to have high water consumption. Increased consumption of drinking water, according to Ulupi and Ihwantoro (2014), can increase fluid in the livestock's body and cause a decrease in hematocrit values.

White blood cells are distributed to body organs that need defense (Ulupi and Ihwantoro, 2014). Vitamin E supplementation did not significantly affect white blood cells. There are several factors that can affect the number of white blood cells, such as environmental conditions, bird age, bird physiological status, and nutrient content in feed (Addass *et al.* 2012). Nutrient factors such as protein can play an important role in the process of forming white blood cells. The immune system of local chickens tends to be better than other chicken breeds because they have been able to adapt to the environment in Indonesia.

The results of the analysis showed vitamin E supplementation did not have a significant effect on leukocyte differentiation, but produced higher heterophils and eosinophils than the normal range according to Swenson and William (1993). The heterophile percentage in this research indicated that IPB-D2 chicken had great potential to produce non-specific immunity against bacterial or viral infections because heterophils acted as phagocytic cells. Vitamin E supplementation can increase the immune system and endurance (Lubis *et al.* 2015). Eosinophils function to detect allergies and parasitic infections that may occur in livestock (Asmara *et al.* 2019). High eosinophils indicate that chickens are more reactive when there are infection and stress.

High phagocytic activity in eosinophils and heterophils can reduce the antibodies produced by lymphocytes. This research produced an average lymphocyte count of 51.33% to 52.59%. Lymphocytes are a specific immune system consisting of specific humoral (B) cells and cellular (T) cells (Khairunnisa *et al.* 2021). Chickens that are exposed to disease agents will stimulate lymphocyte cells to proliferate. High environment temperature can increase corticosteroid hormones and causes the formation of lymphocytes to be inhibited (Davis *et al.* 2008). Monocytes migrate to inflamed tissues and turn into large macrophages. Basophils have a very small concentration in white blood cell differentiation but have an important role in inhibiting the blood clotting process because of the heparin they contain (Ulupi and Ihwantoro, 2014).

Vitamin E supplementation in the diet did not make a difference on the blood MDA levels of IPB-D2 chicken (Table 5). The oxidized unsaturated fatty acids can cause free radicals. Malondialdehyde is a product produced from lipid peroxidation and is formed due to the presence of free radicals in unsaturated fatty acids in cell membranes (Wang *et al.* 2011). Vitamin E supplementation does not produce significant blood MDA levels because in the blood and cells there are other antioxidants besides vitamin E, making it possible for vitamin E supplementation not significantly different. Antioxidants can be formed in the body, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase which are produced to scavenge exogenous and endogenous free radicals (Maharani *et al.* 2021). Feed containing oil added with antioxidants such as vitamin E can protect polyunsaturated fatty acid bonds from oxidation, maintain the amount of free fatty acids, and maintain the peroxide value below standard (Kusharto *et al.* 2015). Vitamin E acts as an antioxidant and anti-inflammatory which can reduce MDA levels which indicate cellular damage due to free radicals (Zaetun *et al.* 2017).

Vitamin E supplementation of 100 ppm and 300 ppm in feed containing LFO was very significant ($P<0.01$) increased the content of vitamin E in egg yolks. Vitamin E supplementation at a dose of 100 ppm increases vitamin E content 262.22% greater than rations without vitamin E and vitamin E supplementation of 300 ppm increases vitamin E content 1354.81% greater than rations without vitamin E. Based on research, vitamin E supplementation in feed as much as 100 ppm and 300 ppm has the potential to form functional eggs rich in vitamin E. This is in accordance with the statement of Müller *et al.* (2012) that vitamin E in feed can affect vitamin E levels in eggs after going through a physiological process in egg formation.

Vitamin E has various benefits for the human body, preventing diseases by increasing immune activity, such as cardiovascular disease, cancer, diseases that attack immunity, and cataracts (Rizvi *et al.* 2014). The human need for vitamin E and RDA percentage is presented in Table 6. Recommended dietary allowances (RDA) percentage is a value that indicates the average need for a nutrient that must be met every day by all people with certain characteristics, such as age, gender, level of physical activity, and physiological conditions, this statement is contained in the regulation of Menteri Kesehatan Republic Indonesia (Gurnida *et al.* 2020). Through the RDA percentage, it can be seen how much the need for certain nutrients has been fulfilled from a food product. Table 5 shows that one IPB-D2 chicken egg in the T2 treatment contains 2.95 mg of vitamin E, therefore by consuming one IPB-D2 chicken functional eggs can supply 19.67% of daily vitamin E needs.

Table 4 Blood profiles of IPB-D2 chicken fed diet containing different level of vitamin E

Variables	Treatments			P-value	Normal range*
	T0	T1	T2		
RBC (10^6)	2.20±0.51[a]	3.02±0.51[b]	2.15±0.23[a]	0.014	2.50-3.20
Hemoglobin (G %)	8.76±0.62	8.50±0.39	9.34±0.83	0.147	6.50-9.00
PCV (%)	26.70±2.82	24.40±1.67	25.90±2.70	0.354	30.00-33.00
WBC (10^3)	25.23±4.38	25.01±5.93	21.68±4.66	0.480	20.00-30.00
Lymphocyte (%)	51.33±2.84	52.40±1.08	52.59±1.38	0.555	55.00-60.00
Heterophil (%)	32.11±2.62	32.10±2.35	31.95±2.23	0.993	25.00-30.00
Eosinophil (%)	11.79±1.25	10.83±1.44	10.60±1.19	0.342	3.00-8.00
Monocyte (%)	3.85±0.76	3.72±0.77	3.89±0.81	0.944	10.00
Basophil (%)	0.92±0.04	0.95±0.10	0.97±0.10	0.645	-

T0: control diet containing 2% Lemuru fish oil (LFO) without vitamin E; T1: control diet containing 2% LFO + 100 ppm vitamin E; T2: control diet containing 2% LFO + 300 ppm vitamin E.
RBC: red blood cell; WBC: white blood cell; PCV: packed-cell volume (hematocrit).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 Blood MDA levels and egg yolk vitamin E content of IPB-D2 chicken fed diet containing different level of vitamin E

Variables	Treatments			P-value
	T0	T1	T2	
Blood MDA (µL/L)	1.85 ± 0.86	1.20 ± 0.32	1.62 ± 0.83	0.377
Vitamin E (mg/100 g)	1.35 ± 0.19[a]	4.89 ± 0.50[b]	19.64 ± 2.89[c]	0.000
Vitamin E (mg/egg)	0.20	0.73	2.95	

T0: control diet containing 2% Lemuru fish oil (LFO) without vitamin E; T1: control diet containing 2% LFO + 100 ppm vitamin E; T2: control diet containing 2% LFO + 300 ppm vitamin E.
MDA: malondialdehyde.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Amount of vitamin E (α-tocopherol) recommended for humans and recommended daily allowance (RDA) percentage

Age	Vitamin E recommendations (mg)		RDA percentage (%)[1]	
	Male	Female	Male	Female
0-6 months	4	4	73.75	73.75
7-12 months	5	5	59.00	59.00
1-3 years	6	6	49.17	49.17
4-8 years	7	7	42.14	42.14
9-13 years	11	11	26.82	26.82
> 14 years	15	15	19.67	19.67
Pregnant		15		19.67
Breast-feed		19		15.53

It is assumed that 1 egg yolk weighs 15 g, so that the T2 treatment contains 2.95 mg of vitamin E per egg.

Adult men and women aged over 14 years, consuming 2 eggs per day can meet 39.33% of their vitamin E needs. The daily need for vitamin E if you consume non-functional eggs, 2 eggs can only fulfill 2. 66%.

The effect of vitamin E supplementation on the fatty acid profile of the egg yolk can be seen in Table 7. The fat content in the yolk can be affected by feed, environment, and age of the chicken. Yolks contain more fat than albumen.

IPB-D2 chicken eggs have lower saturated fatty acids compared to unsaturated fatty acids. The dominant saturated fatty acids in IPB-D2 chicken were palmitic acid and stearic acid (Table 7).

This is in accordance with the statement of Réhault-Godbert *et al.* (2019), that in general, palmitic and stearic fatty acids are the dominant saturated fatty acids in chicken egg yolks. IPB-D2 chicken unsaturated fatty acids ranged from 35.56%-41.99%.

IPB-D2 chicken eggs have dominant oleic and linoleic fatty acids compared to other fatty acids (Suci *et al.* 2020).

Unsaturated fatty acids are divided into two, namely monounsaturated fatty acids and polyunsaturated fatty acids. Cook and Briggs (1977) stated that eggs are a food source of unsaturated fatty acids, especially oleic fatty acids. Oleic fatty acid is an omega 9 fatty acid. Oleic fatty acid can function as a counterweight to the ratio of essential fatty acids such as linolenic, linoleic and oleic fatty acids for body health (Nugraheni *et al.* 2015). Oleic and linoleic fatty acids influence each other. The higher the linoleic fatty acid, the lower the oleic fatty acid. Linoleic fatty acid is part of the omega-6 fatty acids. Excessive consumption of linoleic acid will result in an unequal production of prostaglandins and linoleic acids. DHA (docosahexaenoic acid) is the dominant omega-3 fatty acid in IPB-D2 chicken eggs in this study.

Table 7 Fatty acid profile of egg yolk of chicken fed diet containing different level of vitamin E

Fatty acids	Treatments[1]		
	T0	T1	T2
Fat content	**7.02**	**8.91**	**5.37**
Saturated fatty acid			
Butyric acid, C4:0	0.30	0.33	0.34
Caprilic acid, C8:0	1.89	0.00	0.00
Myristic acid, C14:0	0.29	0.33	0.33
Pentadecanoic Acid, C15:0	0.04	0.00	0.04
Palmitic acid, C16:0	17.21	17.79	18.18
Heptadecanoic Acid, C17:0	0.10	0.12	0.10
Stearic acid, C18:0	4.76	4.67	5.22
Behenic acid, C22:0	0.02	0.00	0.00
Lignoceric acid, C24:0	0.11	0.00	0.00
SFA total	**24.72**	**23.24**	**24.21**
Monounsaturated fatty acid			
Myristoleic acid, C14:1	0.08	0.05	0.10
Palmitoleic acid, C16:1	2.80	3.01	3.02
Cis-10-heptadecanoic acid, C17:1	0.09	0.12	0.00
Cis-11-eicosenoic acid, C20:1	0.13	0.20	0.83
MUFA total	**3.10**	**3.38**	**3.95**
Polyunsaturated fatty acid			
Cis-11,14-eicosedienoic acid, C20:2	**0.08**	**0.08**	**0.00**
Omega 9			
Elaidic acid, C18:1n9t	0.14	0.12	0.11
Oleic acid, C18:1n9c	24.07	26.43	28.35
Linolelaidic acid, C18:2n9t	0.03	0.00	0.03
Omega 9 total	**24.24**	**26.55**	**28.49**
Omega 6			
Linoleic acid, C18:2n6c	5.55	6.37	5.34
γ-Linolenic acid, C18:3n6	0.05	3.24	1.60
Cis-8,11,14-eicosetrienoic acid, C20:3n6	0.11	0.11	0.00
Arachidonic acid, C20:4n6	1.00	0.89	0.93
Omega 6 total	**6.71**	**10.61**	**7.87**
Omega 3			
Linolenic acid, C18:3n3	0.10	0.17	0.00
Cis-5,8,11,14,17-eicosapentaenoic acid, C20:5n3	0.15	0.00	0.00
Cis-4,7,10,13,16,19-docosahexaenoic acid, C22:6n3	1.18	1.20	1.08
Omega 3 total	**1.43**	**1.37**	**1.08**
Omega 6:omega 3	**4.69**	**7.74**	**7.29**
Total fatty acid		**60.29**	**65.23**

T0: control diet containing 2% Lemuru fish oil (LFO) without vitamin E; T1: control diet containing 2% LFO + 100 ppm vitamin E; T2: control diet containing 2% LFO + 300 ppm vitamin E.

LFO contains high and dominant DHA fatty acids (Maulana *et al.* 2014), possibly causing the DHA content to be high in IPB-D2 chicken eggs. 2% LFO on diet in this study increased the omega 3 fatty acid content by 2.85%-36.19% compared to chickens that were not given lemuru fish oil. Riyanto (2006) research resulted in the omega 3 fatty acid content of chicken eggs not contain LFO of 1.05%. Vitamin E supplementation in feed containing LFO maintains omega-3 fatty acids, so that it is not easily oxidized, is more stable, and can be used optimally.

Balanced composition of omega-6 and omega-3 fatty acids needs to be considered to support the important functions of essential fatty acids.

Meliandasari *et al.* (2015) stated that a good balance of omega-6 and omega-3 is important for poultry because it functions in metabolic functions, physiological functions, and lipid membrane composition. Omega-6 that too high can be a pro-inflammatory, therefore omega-3 is needed to suppress omega-6 because omega-3 is anti-inflammatory (Nisa *et al.* 2017). Ratio of omega-6 and omega-3 fatty acids in this research ranged from 4.69 to 7.74. A good balance of omega-6 and omega-3 fatty acids for consumption is 10:1 to 15:1 (Kouba and Mourot, 2011). NRC (1989) suggested the ratio of omega-6 to omega-3 is 4:1 to 10:1. Based on this, IPB-D2 chicken eggs have a good ratio of omega-6 and omega-3 and eggs are healthy food product.

CONCLUSION

Based on the results of the research, supplementation vitamin E improved high performance that remains as high as without supplementation vitamin E in feed. Supplementation of 100 ppm vitamin E increases red blood cells and is indicated to improve health in IPB-D2 chickens. Supplementation of 100 ppm vitamin E increased the vitamin E content by 262.22% and supplementation of 300 ppm vitamin E in feed containing LFO increased the vitamin E content of egg yolk by 1354.81%. Vitamin E supplementation of 100 ppm and 300 ppm has the potential to form functional eggs. Consuming 2 eggs high in vitamin E, which is produced from vitamin E supplementation of 300 ppm, per day can supply the daily vitamin E requirement of 39.33% for women and men aged over 14 years.

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