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The impact of selenium yeast and vitamin E in blood profile and egg production of laying hens at the end of egg production period

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ABSTRACT

This study investigated the effects of selenium yeast and vitamin E supplementation on hematological parameters, egg production, and quality in laying hens aged beyond 94 weeks. The experiment adhered to ethical regulations and employed a Completely Randomized Design Dietary treatments included a basal diet (T0), or a basal diet supplemented with 0.450 mg selenium yeast and 100 mg vitamin E (T1), 0.675 mg selenium yeast and 100 mg vitamin E (T2), 0.450 mg selenium yeast and 200 mg vitamin E (T3), 0.675 mg selenium yeast and 200 mg vitamin E per kg diet (T4). There were 125 hens divided in five treatments and five replicates that contained five hens per replicate. Hematological parameters, egg production, and egg quality were determined. The T4 treatment reduced red blood cell counts ($P<0.05$), potentially impacting erythropoiesis. The T3 treatment increased lymphocyte content and decreased heterophil/lymphocyte ratio ($P<0.05$), which concurs with an increase in egg weight, egg mass, and Haugh Unit, and decreased feed conversion ratio ($P<0.05$). Thus, this study demonstrated that incorporating these supplements with the specified dosage (0.450 mg selenium yeast and 200 mg vitamin E per kg diet) in aged laying hens can enhance productivity and egg quality at the end of the production cycle.

Keywords: *Aged laying hens, Egg quality, Hematological parameters, Selenium yeast, Vitamin E*

INTRODUCTION

The laying hen industry is an important contributor to the sustainable production of high-quality protein through egg production. Evaluating the efficiency and profitability of laying hen farming relies significantly on factors such as egg production and quality. Maintaining productivity and egg quality in older laying hens for longer laying cycles is becoming important. Commercial laying hens typically last peak productivity at around 60 weeks of age, at which point the laying rate and egg quality start to decrease (Thiruvenkadan *et al*., 2010). The internal and shell quality of eggs deteriorates with aging laying hens, increasing the risk of breakage during collection and transportation. Therefore, in the later stages of the laying cycle, it is crucial to overcome the detrimental effects of aging on egg production and quality (Liu *et al.*, 2023). Furthermore, beyond egg quality, the blood profile of laying hens is a crucial indicator of overall health and reproductive status. Studies have shown that reproductive status influences blood parameters, including hemoglobin levels, highlighting the need to monitor blood profiles during egg production (Schumann *et al.*, 2014).

Various factors, including dietary supplements, such as selenium and vitamin E, are pivotal in influencing laying performance. Selenium is a crucial trace mineral, while vitamin E is a potent antioxidant. Selenium and vitamin E can contribute to laying hens' health status and productivity (Hu *et al.*, 2020). Selenium is one of the essential micronutrients for both human and farm animals, including poultry, which plays a vital role in antioxidant defense, redox state regulation, reproduction, and a wide variety of specific metabolic pathways (Roman *et al.*, 2014). Furthermore, selenium functions through integration into proteins as a component of selenocysteine, which is employed in producing selenoproteins. Most selenoproteins exhibit antioxidant activities (Chantiratikul *et al.*, 2017). A previous study revealed that selenium supplementation has improved productive traits in native Aseel chickens, emphasizing the importance of blood selenium status in relation to egg production (Zia *et al.*, 2016). Selenium yeast is a form of supplemental selenium, also referred to as organic selenium (Schrauzer, 2006). Because it is biologically safe and readily absorbed, selenium yeast is commonly utilized as a feed additive. Including selenium yeast in the diet has improved blood profile, egg production, and egg quality (Attia *et al.*, 2010; Liu *et al.*, 2023).

Meanwhile, vitamin E is an essential fat-soluble vitamin for domestic animals because they cannot synthesize it (Zhao *et al.*, 2021). Vitamin E is a lipid-soluble antioxidant that scavenges lipid peroxyl radicals and inhibits further oxidation to lipid molecules, which contributes significantly to oxidative stress (Surai *et al.*, 2016). It has been suggested that dietary vitamin E supplementation increases antioxidant capability and anti-inflammatory properties in poultry (Yang *et al.*, 2021). Vitamin E supplementation has demonstrated positive effects on plasma calcium levels, vitellogenin, and very low-density lipoprotein; thus, it is essential for egg production and quality (Leeson, 2007). A previous study demonstrated that vitamin E supplementation improved egg production (Çimrin, 2019). The combination of selenium yeast and vitamin E can positively influence laying hens' performance, egg quality, and selenium concentration in eggs, along with affecting serum biochemical indices during the production period (Mohiti-Asli *et al.*, 2008).

Many researchers have extensively focused on the impact of selenium yeast and vitamin E during the peak production period, emphasizing their effects on shell weight and thickness, albumen and yolk index, and other essential parameters (Baylan *et al.*, 2010; Çimrin, 2019). The doses of 0.450-0.675 mg/kg selenium yeast and 100-200 mg/kg vitamin E were selected based on previous research indicating these levels provide benefits for egg production and quality in aged laying hens, while avoiding potential toxicity. There is a lack of information on the impact of combining selenium yeast and vitamin E in aged laying hens, particularly beyond 94 weeks of age, a period that has received limited attention in existing literature. Therefore, this study aimed to fill the gap in existing research by assessing the effects of selenium yeast and vitamin E supplementation on blood profile and egg production during the end of the egg production period in laying hens, specifically after 94 weeks. This study offers valuable insights for optimizing laying hen nutrition and management practices during the later stages of egg production.

MATERIALS AND METHODS

Ethical Regulation

The research procedure adhered to the regulations established by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, with ethical clearance number 027/EC-FKH/Eks. /2023.

Experimental Design and Diets

This study used 125 laying hens of the Hyline Brown strain at 94 weeks of age. The birds were allotted to five treatments with five replicates per treatment based on a completely randomized design. Each treatment contained five birds. The dietary treatments included a basal diet (T0) or a basal diet supplemented with 0.450 mg selenium yeast and 100 mg vitamin E (T1), 0.675 mg selenium yeast and 100 mg vitamin E (T2), 0.450 mg selenium yeast and 200 mg vitamin E (T3), 0.675 mg selenium yeast and 200 mg vitamin E per kg diet (T4). The selenium yeast contains organic selenium yeast based at 4500 ppm. A commercial laying diet L83-1A for the later stages of production (produced by New Hope, Tangerang, Indonesia) was used as the basal diet, and the nutrient content was presented in Table 1. The dietary treatments lasted eight weeks.

Determination of Egg Production and Quality

Laying hens at 94 weeks of age were housed individually in battery cages measuring 56×35×35 cm equipped with feed containers and nipple drinkers. The birds were fed daily with 120 grams per bird, and water was provided *ad libitum*. The birds were adapted for the feed for one week prior to dietary treatments. A similar management practice was applied to all the experimental groups. The egg number and egg weight were measured daily during the experimental period. Egg production was presented as a percentage of hen day production. Egg mass was calculated by multiplying hen day production with the average egg weight. Eggshell strength and Haugh Unit measurements were conducted in the 4th, 5th, and 6th weeks. Determination of eggshell strength followed the procedures Izquierdo (2018) outlined with an accuracy of 0.01N at a constant velocity of the measuring head shift set at 50 mm/min. The Haugh Unit was calculated using the formula: Haugh Unit = 100 log (H + 7.57 – 1.7W0.37), where H represents the albumen height, and W represents the egg weight. This formula was introduced by Raymond Haugh in 1937 (Khaleel, 2019).

Hematology Parameters

The blood samples were collected in the 7th week from brachial vein of chicken using a sterilized syringe and needles then transferred into vacutainer tubes with anticoagulant ethylenediaminetetraacetic acid. Blood analysis was performed using the Sysmex XP-100 Hematology Analyzer (Sysmec Corporation, Japan), following the methodology outlined by Seo *et al.* (2014). Total plasma protein was quantified using UV spectrophotometry at 260-280nm wavelengths, with the highest peak representing the maximum wavelength.

Data Analysis

Data analysis, including hematological parameters, blood proteins, egg production, and egg quality, was conducted using analysis of variance by employing the Systat Program ver.13. If

Table 1. Nutrient Content of the Basal Diet L83-1A

Nutrient	Content
Crude protein (%)	16.50
Metabolizable energy (kcal/kg)	2,750
Ether extract (%)	3.00
Crude fiber (%)	7.00
Crude ash (%)	14.00
Calcium (%)	3.25
Phosphorus (%)	0.60
Lysine (%)	0.80
Methionine (%)	0.40
Metionin+cystine (%)	0.67
Tryptophan (%)	0.18
Threonine (%)	0.55

the analysis of variance indicated significant effects on the measured parameters, differences among treatment groups were determined using the Duncan's multiple range test. Statistical significance was accepted when the P-value was less than 0.05.

RESULTS AND DISCUSSION

Hematological Parameters and Blood Proteins

Hematological parameters revealed that the red blood cell (RBC) counts of T4 treatment was lower than those of other treatments (P<0.05; Table 2), suggesting a potential impact of selenium yeast and vitamin E at the level of 675 mg and 200 mg per kg diet, respectively, on erythropoiesis in the later stages of egg production. This result corresponds to the findings investigated in laying hens during peak production fed selenite of selenium-enriched yeast (Petrovič *et al.*, 2006). In this study, the level of supplements is related to the observed impact on RBC counts. According to Surai (2000), the nutritional status of laying hens determines the efficiency of the antioxidant system, supporting the notion that dietary supplementation, such as yeast selenium and vitamin E, influences physiological parameters, including RBC counts at later stages of egg production.

Moreover, a trend of increase was found in mean corpuscular volume (MCV; P=0.063) and mean corpuscular hemoglobin (MCH; P=0.079), where the diets supplemented with selenium yeast and vitamin E had higher levels of MCV and MCH. This finding suggests a potential alteration in the average volume and hemoglobin content. On the other hand, dietary treatments did not affect packed cell volume (PCV) and hemoglobin (Hb) levels, indicating a consistent level of red cell mass and minimal influence on oxygen-carrying capacity in the later stages of egg production. Similarly, mean corpuscular hemoglobin concentration (MCHC), total plasma protein (TPP), and fibrinogen remained stable and were unaffected by the selenium yeast and vitamin E supplementation.

The findings of this study are consistent with previous observations on the effects of selenium and vitamin E supplementation in laying hens during various stages of production. Chantiratikul *et al.* (2017) demonstrated that selenium from selenium yeast and selenium-enriched kale sprouts were more efficient than sodium selenite. Additionally, Panda and Cherian (2014), who studied the effect of various concentrations of selenium in maternal laying hen diet, observed that higher concentrations of selenium increased the concentration of antioxidant enzymes and decreased susceptibility of lipid peroxidation in day-old chicks. Furthermore, organic selenium and vitamin E supplementation increased antioxidant enzymes' activities and decreased malonaldehyde concentrations in the serum and eggs of laying hens (Timur and Utlu, 2020). These studies collectively support that selenium and vitamin E supplementation have specific effects on laying hen health and blood profile, particularly in the later stages of egg production.

Previous observations showed alteration in hematological parameters of laying hens during different stages of production but not in the later stages. For example, Zhang *et al.* (2020) demonstrated that dietary supplementation with sodium humate can enhance egg quality and immune function in laying hens. Çetin *et al.* (2010) showed that supplementing diets with varying concentrations of propolis can impact hematological and immunological variables in laying hens. Sahin *et al.* (2002) explored the influence of vitamin C and E on lipid peroxidation, blood serum metabolites, and mineral concentrations in laying hens reared in high ambient temperatures, revealing the potential impact of dietary factors on hematological parameters.

Immunomodulatory and Blood Profile

Supplementation of 450 mg selenium yeast and 200 mg vitamin E per kg diet (T3) enhanced lymphocyte percentages and heterophil/ lymphocyte (H/L) ratio (P<0.05; Table 3). This treatment also tended to decrease the heterophil percentage (P=0.076). However, selenium yeast and vitamin E supplementation did not affect the white blood cell (WBC) count, heterophil, eosin-

Table 2. The Effect of Selenium Yeast and Vitamin E Supplementation on Hematological Parameters and Blood Proteins of Aged Laying Hens

Treatments	RBC (mil/ul)	PCV (%)	Hb (g/dL)	MCV (fl)	MCH (pg)	MCHC (%)	TPP (g/dL)	Fibrinogen (g/dL)
T0	3.12±0.53[a]	27.00±2.82	7.56±0.73	87.85±11.10	24.64±3.51	28.08±2.16	6..28±1.06	0.48±0.23
T1	2.99±0.21[a]	28.00±1.58	7.96±0.64	94.32±8.32	26.65±1.29	28.48±2.40	6.80±0.71	0.52±0.27
T2	2.72±0.18[a]	27.40±2.30	8.08±0.61	100.67±3.55	29.79±2.89	29.70±3.97	7.68±1.68	0.24±0.09
T3	2.99±0.29[a]	27.40±2.41	8.08±0.70	91.84±5.70	27.27±4.09	29.79±4.72	7.08±1.32	0.25±0.10
T4	2.54±0.12[b]	25.00±1.22	7.42±0.39	98.38±2.79	29.26±2.31	29.73±2.05	6.47±1.05	0.24±0.09
P value	0.041	0.258	0.332	0.063	0.079	0.865	0.410	0.511

[a,b] Means within a column with different superscripts indicated significant differences ($P<0.05$). T0: basal diet, T1: basal diet supplemented with 0.450 mg/kg selenium yeast and 100 mg/kg vitamin E, T2: basal diet supplemented with 0.675 mg/kg selenium yeast and 100 mg/kg vitamin E, T3: basal diet supplemented with 0.450 mg/kg selenium yeast and 200 mg/kg vitamin E, T4: basal diet supplemented with 0.675 mg/kg selenium yeast and 200 mg/kg vitamin E.

Table 3. The Effect of Selenium Yeast and Vitamin E Supplementation on Immunology and Blood Profile of Aged Laying Hens

Treatments	WBC (10^3/ul)	Heterophil (%)	Eosinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)	Heterophil/ Lymphocyte
T0	28.85±11.41	34.20±3.63	9.40±1.82	0.80±0.45	28.31±3.92[b]	7.00±3.32	1.22±0.15[a]
T1	24.65±2.01	32.00±3.94	8.40±2.70	0.60±0.89	28.53±0.76[b]	4.40±2.30	1.12±0.15[a]
T2	29.08±10.88	31.40±6.27	9.20±4.21	1.20±0.30	29.80±3.43[b]	4.00±3.00	1.08±0.31[a]
T3	31.57±4.58	25.40±3.13	9.60±1.34	1.00±1.22	35.86±1.70[a]	4.60±2.30	0.71±0.12[b]
T4	30.66±4.54	30.70±5.36	7.65±1.65	0.85±0.86	30.77±2,05[b]	5.75±1.60	1.01±0,24[a]
P value	0.666	0.076	0.736	0.903	0.001	0.373	0.010

[a,b] Means within a column with different superscripts indicated significant differences ($P<0.05$). T0: basal diet, T1: basal diet supplemented with 0.450 mg/kg selenium yeast and 100 mg/kg vitamin E, T2: basal diet supplemented with 0.675 mg/kg selenium yeast and 100 mg/kg vitamin E, T3: basal diet supplemented with 0.450 mg/kg selenium yeast and 200 mg/kg vitamin E, T4: basal diet supplemented with 0.675 mg/kg selenium yeast and 200 mg/kg vitamin E.

Table 4. The Effect of Selenium Yeast and Vitamin E Supplementation on Egg Production and Quality of Aged Laying Hens

Treatments	Egg Production (%)	Egg Weight (g)	Egg Mass (g)	Feed Conversion Ratio	Eggshell Strength (N)	Haugh Unit
T0	73.29±5.94	62.79±1.64[b]	46.04±4.22[b]	2.61±0.05[a]	0.23±0.06	86.76±2.40[b]
T1	79.18±4.14	63.99±2.84[b]	50.58±0.61[a]	2.37±0.08[a]	0.31±0.06	88.04±1.79[a]
T2	76.74±10.26	63.18±2.42[b]	48.36±5.58[a]	2.48±0.04[a]	0.30±0.03	87.05±2.57[b]
T3	80.79±2.33	67.00±1.30[a]	54.11±0.99[a]	2.21±0.04[b]	0.32±0.04	90.71±1.70[a]
T4	69.69±9.17	63.56±1.86[b]	44.37±6.66[b]	2.70±0.06[a]	0.30±0.05	84.67±2.45[b]
P value	0.127	0.034	0.017	0.037	0.299	0.006

[a,b] Means within a column with different superscripts indicated significant differences ($P<0.05$). T0: basal diet, T1: basal diet supplemented with 0.450 mg/kg selenium yeast and 100 mg/kg vitamin E, T2: basal diet supplemented with 0.675 mg/kg selenium yeast and 100 mg/kg vitamin E, T3: basal diet supplemented with 0.450 mg/kg selenium yeast and 200 mg/kg vitamin E, T4: basal diet supplemented with 0.675 mg/kg selenium yeast and 200 mg/kg vitamin E.

ophil, basophil, and monocyte percentages of laying hens at the later stages of production. Selenium yeast and vitamin E are known for their immunomodulatory properties. The T3 treatment, with a higher lymphocyte percentage, suggests a potential enhancement of cellular immune responses. Lymphocyte plays a crucial role in the adaptive immune system, and variations in their percentages may reflect the effectiveness of dietary supplements in bolstering immune function. Moreover, as a potential antioxidant, vitamin E could enhance the antioxidant status, which may influence lymphocyte viability and function, affecting their relative abundance in the blood (Pavlik *et al.*, 2007).

The H/L ratio is recognized as an indicator of stress in poultry; thus, the lower H/L ratio in T3 treatment suggests a potentially lower stress level by inducing an immune response. Selenium yeast and vitamin E are known to mitigate oxidative stress, and their inclusion in the diet may have a calming effect on poultry, reflected in the reduced H/L ratio (Haryuni *et al.*, 2021). The finding of this study is consistent with previous observations that dietary selenium and vitamin E improved immune response and blood biological parameters in broilers (Habibian *et al.*, 2013) and laying hens (Invernizzi *et al.*, 2013; Lu *et al.*, 2019). Similarly, an enriched diet with organic selenium and selenium yeast-methionine emphasizes the immunomodulatory effects in laying hens (Wei-Xian *et al.*, 2021). Selenium positively impacts physiology, tissue functions, and immune response regulation. Feeding organic selenium also improved antioxidant status and reduced possible selenium deficiency-related diseases (Invernizzi *et al.*, 2013). Specific selenium sources might play a crucial role in maintaining immune homeostasis and potentially mitigating age-related stress responses. Selenium is essential for the activity of selenoproteins involved in antioxidant defense and other vital physiological functions (Skřivan *et al.*, 2013). Moreover, the synergistic effect of selenium yeast and vitamin E supplementation improved the reproductive performance of laying hens (Haryuni *et al.*, 2021).

However, it is important to note that the literature also encompasses studies on various factors that influence the performance and health of laying hens, such as essential oils and oligosaccharide supplementation, and housing systems (Ding *et al.*, 2017; Meng *et al.*, 2010; Pavlik *et al.*, 2007). These studies demonstrated the potential effects of different dietary components and environmental factors on laying hen physiology and egg production. Additionally, research has explored using organic selenium and vitamin E to enhance antioxidant enzyme activities in laying hens, indicating potential benefits for overall health and oxidative stress management (Timur and Utlu, 2020).

It is also important to note that previous studies focused on the effect of selenium yeast and vitamin E in laying hens during peak production or growing periods. For example, Surai (2000) reported that inclusion of organic selenium and vitamin E in the maternal diet of the laying hens determines the efficiency of the antioxidant system throughout the offspring's embryonic and early post-hatch development. Furthermore, selenium yeast is more effective in maintaining laying hens' antioxidant and selenium status than selenite (Petrovič *et al.*, 2006; Han *et al.*, 2017; Kralik *et al.*, 2018), which is reflected in the improvement of laying performance, egg quality, antioxidant capacity, and selenium contents in tissues and eggs. Moreover, the positive effects of selenium and vitamin E supplementation on the reproduction performance of breeding stock have been observed (Haryuni *et al.*, 2022). Considering the age-related decline in physiological functions, including the potential impact on egg quality and immune response, this study offers the positive effects of dietary supplements in the later stages of egg production.

Egg Production and Quality

Supplementation of selenium yeast and vitamin E in specific doses of T1, T2, or T3 enhanced the egg weight, egg mass, or Haugh Unit, and decreased the feed conversion ratio ($P<0.05$; Table 4). The T3 treatments (0.450 mg selenium yeast and 200 mg vitamin E) resulted in the highest improvements, where these supplements enhanced the egg weight, egg mass, Haugh Unit, and decreased the feed conversion ratio ($P<0.05$). This indicates the positive effects of these supplements at the specified doses in boosting productivity and egg quality in the later production stages of aged laying hens. The selenium yeast provides a bioavailable source of selenium, an essential micronutrient, while vitamin E is a potent lipid-soluble antioxidant. Supplementation at the optimal levels likely enhanced antioxidant status and supported immune function, as well as promoting nutrient absorption and utilization. The increases in egg output and mass signify that the hens directed more nutrients towards egg production. Meanwhile, improved feed conversion highlights better feed efficiency. The increase in egg weight and Haugh Unit further suggest that the supplements promoted albumen quality. One contributing factor that improved performance and egg quality in this study was related to increase ntioxidant capacity and reducing oxidative damage, which indirectly boosted health status and laying performance (Chantiratikul *et al.*, 2023). However, the higher dose of these supplements with 0.675 mg selenium yeast and 200 vitamin E (T4) did not affect the productive performance and egg quality, probably due to adverse effects when these supplements were combined (Schrauzer, 2006; Kim and Kil, 2020; Rahbari, 2020). In addition, these supplements did not affect egg production and eggshell strength. The T3 treatment showed numerically higher egg production, although not statistically significant, it probably due to a high standard deviation in this variable. In agreement with this finding, a previous study showed inconsistent egg productivity in response to Se dosage (Chantiratikul *et al.*, 2023).

The improvements in egg weight and quality in this study correspond with previous observations on the effects of selenium and vitamin E supplementation on laying hens. Supplementation of several selenium sources enhanced egg quality parameters, including egg yolk selenium content (Hu *et al.*, 2020). Selenium-enriched yeast has been observed to improve the egg's physical parameters in laying hens (Skřivan *et al.*, 2006). Similarly, dietary supplementation with selenium-enriched yeast in laying hens' diet enhanced egg production and eggshell quality (Invernizzi *et al.*, 2013). Furthermore, using selenium conjugated to animal protein in laying hens' diets increased productive performance, egg quality, antioxidant capacity, and biochemical indices (Qiu *et al.*, 2021). These findings demonstrated that selenium supplementation can positively affect the egg quality parameters.

Moreover, previous studies revealed the positive influence of vitamin E on egg quality. Skřivan *et al.* (2013) observed that vitamin E supplementation increased laying performance and egg quality of laying hens. In addition, dietary vitamin E supplementation has been linked to improve eggshell strength and color intensity in laying hens (Kim *et al.*, 2022). However, in the current study, supplementation with vitamin E and selenium yeast did not significantly impact eggshell strength. This may be because the aged laying hens already had compromised shell quality due to their stage of the production cycle. Similarly, no differences were found in Haugh unit between treatment groups, likely because of high variability in albumen quality of eggs from older flocks. The supplements may have had a greater effect if provided earlier in the laying period before age-related declines in egg parameters. These findings corroborate the observed impact of vitamin E supplementation on egg weight and quality parameters in this study, even though this study used aged laying hens.

On the contrary, several researchers did not observe any effects of selenium sources on egg quality parameters (Eldin, 2015). This discrepancy in the effects of selenium might be due to variations in experimental conditions, such as the specific selenium source used or the dosage administered. Additionally, the interaction effect of selenium and vitamin E supplementation and metabolizable energy levels on reproductive performance in breeders highlights the possibility of dietary interventions and their potential interactions with other factors (Haryuni *et al.*, 2021).

CONCLUSION

This study showed that selenium yeast and vitamin E supplementation modulated hematological parameters, immune response, and egg quality in laying hens beyond 94 weeks of age. The most improvements were observed when 0.450 mg selenium yeast and 200 mg vitamin E were supplemented, indicating the positive effects of these supplements in the specific dose in the later production stages of laying hens. Therefore, supplementation with 0.450 mg selenium yeast and 200 mg vitamin E can be recommended for laying hens in the later stages of production.

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