

Influence of selenium and vitamin E supplementation in a fish oil-based diet on broiler performance, visceral organs, meat fatty acid composition, and antioxidant deposition

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ABSTRACT

This study aimed to evaluate the influence of selenium (Se) from selenomethionine and vitamin E (VE) from α -tocopherol acetate supplementation in a fish oil-based diet on broiler performance, visceral organs, fatty acids, VE, and Se in meat. A total of 200 one-day-old Cobb broiler chickens were reared for 35 days using a completely randomised design with 4 treatments and 5 replicates. The treatments were T0: control diet containing 3% fish oil; T1: T0 + Se (0.3 ppm) + VE (200 ppm); T2: T0 + Se (0.6 ppm) + VE (300 ppm); T3: T0 + Se (0.9 ppm) + VE (400 ppm). The treatments did not affect broiler performance ($P>0.05$). Compared to the T0, the T3 group improved Se and VE levels in meat by 105.4% and 83.2%, respectively, and had a lower colon length than the T0 and T1 groups ($P<0.05$). The T2 group increased total monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and omega-6 compared to the T0 and T1 groups ($P<0.05$). It was concluded that the inclusion of 400 ppm VE and 0.9 ppm Se in a diet rich in fatty acids effectively enhanced the deposition of Se, VE, and omega-3 in the meat as well as reduced colon length without adverse effect on the production performance of broiler chickens. The combination of 300 ppm VE and 0.6 ppm Se was the optimal dose for increasing the deposition of MUFAs, PUFAs, and omega-6 in the meat.

Keywords: *Body weight, Gastrointestinal tract, Meat quality, Sardinella Lemuru, Selenium, Vitamin E.*

INTRODUCTION

Increasing consumer awareness of the importance of nutritious and healthy food consumption has encouraged the livestock industry to focus not only on production efficiency but also on improving the nutritional quality of animal products, including broiler meat. Consumers are now more selective in choosing food products that not only have high nutritional value but

also contain bioactive components that are beneficial to health, such as essential fatty acids, natural antioxidants, and important minerals that support immune function and body metabolism (Maxim *et al.*, 2019; Petrescu *et al.*, 2019). One approach that has been widely studied is through diet manipulation, especially with the inclusion of unsaturated fatty acid sources and antioxidants such as Lemuru fish oil, selenium (Se), and vitamin E (VE).

Lemuru fish oil (*Sardinella lemuru*), which is obtained from the waste of the Lemuru fish canning process, is known to be rich in omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Lestari *et al.*, 2024). These fatty acids contribute to improving the body's immune system, reducing oxidative stress, and improving broiler growth and meat quality. Omega-3 is also beneficial for human health, especially in cardiovascular disease (Rodrigues *et al.*, 2024). Previous studies have suggested that the inclusion of 3% Lemuru fish oil in the diet could improve broiler productive performance in the starter period, improve carcass quality, and reduce fat content in meat (Sumiati *et al.*, 2022). However, the high level of unsaturated fatty acids make it susceptible to oxidation, which can reduce product quality (Pal *et al.*, 2024). To overcome this problem, it is necessary to include antioxidants such as Se and VE in the ration. Moreover, the VE and Se availability in broiler meat is also expected to be an antioxidant source for consumers.

VE prevents damage to cell membranes caused by oxidation, reduces inflammation, and supports the immune system (Khalifa *et al.*, 2021). Previously, dietary supplementation of 100 ppm VE with fish oil could increase omega-3 in meat and improve feed efficiency (Sumiati *et al.*, 2022), while the inclusion of 300 ppm (Taşdelen and Ceylan, 2017; Sadiq *et al.*, 2023) and 500 ppm (Albuquerque *et al.*, 2017) VE in the diet had strong potential to prevent lipid peroxidation without adversely affecting broiler performance. Meanwhile, Se acts as an antioxidant through the glutathione peroxidase enzyme (GPx), which decreases oxidative stress and improves the immunity and productivity of chickens (Darmawan *et al.*, 2024). It was reported that supplementation of 0.3 ppm Se in the diet had a favourable effect on the immunity and performance of broiler chickens (Khalifa *et al.*, 2021). Although the recommended level of Se supplementation is 0.35 ppm (Cobb-Vantress, 2022), higher supplementation levels have been studied to enhance antioxidant activity, particularly in diets rich in unsaturated fatty acids. For instance, supplementation with 0.6 ppm Se in a diet high in unsaturated fatty acids effectively enhanced antioxidant activity (Sumiati *et al.*, 2025). The addition of 0.8 ppm Se in the diet raised Se levels in meat and antioxidant capacity (Tang *et al.*,

2021). Also, dietary supplementation of 1 ppm Se and 250 ppm VE has been reported to improve productive performance and the immune system and reduce oxidative stress (Calik *et al.*, 2022). However, the evidence of combined supplementation with Se and VE levels in a diet rich in unsaturated fatty acids and its impact on meat nutritional quality and visceral organ development is still limited. We hypothesised that a supranutritional combination of Se and VE would synergistically improve antioxidant status and the deposition of unsaturated fatty acids, Se, and VE in broiler chicken meat without impairing visceral organ development and broiler performance. This study proposed to evaluate the impact of Se and VE inclusion in diets rich in unsaturated fatty acids on performance, visceral organs, fatty acids, Se, and VE in broiler chicken meat.

MATERIALS AND METHODS

Animals and Rearing Management

All animal procedures in this study were approved by the Animal Ethics Committee of IPB University. A total of 200-day-old Cobb broilers were reared for 35 days in a 1 m x 1 m x 0.5 m partitioned cage equipped with 1 tube feeder and a drinking water nipple to provide water ad libitum. The house temperature was set at 33°C for 3 days, and subsequently, due to the open house, the temperature was recorded between 26°C and 30°C, and the relative humidity was 70-78% during the grower period. Lighting was set at 24 hours on the first day, then 23 hours of light and 1 hour of darkness until harvest.

Diet and Treatment

The crumbled diet was prepared based on Cobb's standard broiler nutrition requirements. Crude protein was arranged at 21%-22% and metabolizable energy at 2,975 kcal /kg (starter phase), while crude protein was 19%-20% and metabolizable energy was 3,025 kcal/ kg (grower phase). The feedstuffs used were meat and bone meal, corn, rice bran, soybean meal, DL-methionine, L-lysine, salt, CaCO₃, premix, Sel-nomethionine, VE (α -tocopherol acetate), and Lemuru fish oil (Table 1). A completely randomised design with 4 treatments and 5 replicates (10 chickens per replicate) was applied in this study. The treatments were T0: a control diet containing 3% fish oil; T1: T0 + 0.3 ppm Se + 200 ppm VE;

Table 1. Composition and Nutrient Content of Basal Diets (% as fed)

| Ingredients (%) | Starter (1-14d) | Grower (15-35d) |
|--------------------------------|-----------------|-----------------|
| Yellow corn | 58.00 | 60.50 |
| Rice bran | 3.00 | 4.00 |
| Soybean meal | 28.00 | 25.50 |
| Meat bone meal | 6.50 | 5.00 |
| Lemuru fish oil | 3.00 | 3.00 |
| CaCO ₃ | 0.20 | 0.70 |
| NaCl | 0.20 | 0.20 |
| Premix* | 0.50 | 0.50 |
| DL-Methionine | 0.30 | 0.30 |
| L-Lysine | 0.30 | 0.30 |
| Total | 100.00 | 100.00 |
| Nutrient content** (%) | | |
| Dry matter | 90.50 | 90.36 |
| Metabolizable energy (kcal/kg) | 2995 | 2950 |
| Crude protein | 21.92 | 20.28 |
| Crude fat | 3.81 | 2.73 |
| Crude fiber | 2.72 | 2.81 |
| Calcium | 0.80 | 0.83 |
| Available phosphorus | 0.52 | 0.45 |
| Lysine | 1.32 | 1.21 |
| Methionine | 0.57 | 0.55 |
| Cysteine | 0.28 | 0.25 |
| Na | 0.17 | 0.16 |
| Cl | 0.20 | 0.20 |

*The premix composition per kg: 1,200,000 IU Vitamin A, 200,000 IU Vitamin D₃, 800 IU Vitamin E, 200 mg Vitamin K₃, 500 mg Vitamin B₂, 50 mg Vitamin B₆, 1,200 µg Vitamin B₁₂, 2,500 mg Vitamin C, 600 mg Calcium-D-pantothenate, 400 mg Niacin, 1,000 mg Choline chloride, 3,000 mg Methionine, 3,000 mg Lysine, 1,200 mg Manganese, 2,000 mg Iron, 20 mg Iodine, 10,000 mg Zinc, 20 mg Cobalt, and 400 mg Copper

** Calculated values.

T2: T0 + 0.6 ppm Se + 300 ppm VE; T3: T0 + 0.9 ppm Se + 400 ppm VE.

Performance Production Measurement

Broilers were weighed at the beginning of rearing to obtain initial body weight, and then body weight (BW) was measured weekly and expressed in g/bird. Body weight gain (BWG) was obtained as the difference in body weight between consecutive phases, expressed in g/bird. Feed intake (FI) was measured from the difference between feed provision and remaining feed expressed in g/bird. The feed conversion ratio (FCR) was obtained from FI divided by BWG.

Visceral Organs Weight and Intestine Length Measurement

The weight of visceral organs and the length of intestinal measurements were measured on 2 male chickens per replicate at 32 days of age. Chickens with body weights close to the average per replication were fasted for 12 hours,

and the fasted body weights were weighed immediately before slaughter and recorded as the slaughter weight for calculating relative organ weight. After slaughtering, the internal organs and digestive tract were immediately removed, and the digesta content was carefully emptied without washing to be weighed separately. The organ's weight was determined using a digital scale with an accuracy of 0.01 grams, and the length of the digestive tract was determined with a measuring tape with an accuracy of 0.1 cm. The weight of visceral organs was expressed as a percentage, while the length of the digestive tract was in cm/100 g.

The calculation was as follows:

Percentage of visceral organ weight (%) = (visceral organ weight (g)) / (slaughter weight (g)) × 100%

Relative length of intestine (cm/100g) = (length of intestine (cm)) / (slaughter weight (g)) × 100

Meat Fatty Acid Analysis

On day 35, 2 chickens from each replicate were slaughtered, and thigh meat was collected. The profile of fatty acids was analyzed based on the method described by Zaki *et al.* (2018). The fatty acids were first methylated using boron trifluoride in methanol, then extracted with heptane, and then analysed via gas chromatography with the flame ionisation detector. The carrier gas used was helium with a pressure of about 25 psi, an airflow of 450 ml/minute, hydrogen of 45 ml/minute, and a split ratio of 100 ml/minute. Lipid extraction and methylation procedures were performed based on the method of Folch *et al.* (1957).

VE and Se Analysis

On day 35, 2 chickens from each replicate were slaughtered, and then the breast meat was collected. VE concentration was measured using HPLC (Hitachi L-7000). 0.5 g of meat was homogenised in a mixture of pyrogallol, ethanol, and BHT, extracted with hexane, and then redissolved in a methanol solution as described by Rupérez *et al.* (1998).

Se concentration in breast meat was measured using the method of Tang *et al.* (2021). A total of 1.0 g of meat was mineralised using microwaves with a mixture of nitric acid (HNO₃) and chloric acid (HClO₄), then kept overnight. The mixture was then heated (340°C) until clear, cooled, had HCl (6.0 mol/L) added, and reheated until white smoke formed. After cooling, the solution was transferred into a 50 mL volumetric flask, and the solvent was added until it reached the final volume. Se levels (µg/100 g) were determined in an atomic absorption spectrometer (Solaar M-6 Atomic Spectrometer).

Data Analysis

The data normality test was conducted using the Shapiro-Wilk method. Furthermore, the data were analyzed using IBM SPSS Statistics 22 with the one-way ANOVA method. Differences that were considered significant were determined at P<0.05. For further analysis of differences between treatments, the Duncan test was used. All data analysis results are presented in the form of mean values accompanied by standard deviations.

RESULTS AND DISCUSSION

Broiler Productive Performances

In our study, BW, BWG, FI, and FCR were not influenced by treatments (P>0.05) (Table 2). In line with Pečjak *et al.* (2022), who found that the inclusion combination of 200 IU VE and 0.20 ppm Se in the diet did not affect BWG, FCR, or FI. In line with Albuquerque *et al.* (2017), the addition of Se (0.3 ppm) and VE (500 ppm) did not affect the growth or production efficiency index of broiler chickens. Zhao *et al.* (2023) also showed that 0.3 ppm organic Se in the diet had no effect on FI and BWG in broilers. However, some studies have shown different results. For instance, Ekunseitan *et al.* (2021) reported that dietary supplementation of 400 ppm VE and 0.2 ppm Se increased FI and BW of broilers. The inclusion of 100 ppm VE and 0.22 ppm Se in the diet improved the BW and BWG of broilers (Salahuddin *et al.*, 2017). Furthermore, the inclusion of 100 ppm VE or 0.3 ppm Se not only improved production parameters but also supported the regulation of growth genes such as insulin-like growth factor 1 (IGF1) and growth hormone receptor (GHR), without affecting the health status of broilers (Khalifa *et al.*, 2021).

The lack of efficacy of Se and VE on BW, BWG, FI, and FCR broilers is probably due to several factors. One reason is that the effectiveness of antioxidant compounds on chicken growth performance can be strongly influenced by the type, nutrient content, and feed ingredients (Pompeu *et al.*, 2018). More specifically, Se and VE in the diet may have been sufficient to meet the nutritional requirements of the chickens; hence, supplementation did not have a significant effect (Pitargue *et al.*, 2019). Moreover, with the isocaloric and isonitrogenous composition in all diet treatments, VE probably did not contribute additionally to improving protein or energy utilisation efficiency (Taşdelen and Ceylan, 2017). Furthermore, oxidative stress in broilers did not occur significantly in a proper environment, allowing the body's antioxidant system to work normally without the need for additional supplements (Calik *et al.*, 2022). It should be emphasised that the main role of VE and Se is as antioxidants that protect cells from oxidative damage, rather than directly stimulating the growth of the chicken body. When in extreme environmental conditions exceeding the thermoneutral zone, the body will activate the antioxidant de-

Table 2. Productive Performance of Broiler Chickens Fed Diets Rich in Unsaturated Fatty Acids and Supplemented with VE and Se at 35 Days of Age

| Parameters | Period (day) | Treatments | | | |
|---------------------------|--------------|------------------|------------------|------------------|------------------|
| | | T0 | T1 | T2 | T3 |
| Feed intake (g /bird) | 1-14 | 428.59 ± 25.78 | 398.48 ± 20.08 | 413.22 ± 17.13 | 411.02 ± 19.95 |
| | 15-35 | 2270.50 ± 107.67 | 2244.02 ± 82.86 | 2331.36 ± 135.27 | 2297.97 ± 200.00 |
| | 1-35 | 2699.09 ± 127.98 | 2642.50 ± 102.83 | 2744.58 ± 145.44 | 2708.99 ± 208.98 |
| Body weight (g/bird) | 1 | 41.90±0.67 | 42.20±0.27 | 42.20±0.27 | 42.90±0.82 |
| | 14 | 396.20 ± 29.64 | 387.80 ± 12.39 | 395.74 ± 18.21 | 385.40 ± 14.35 |
| | 35 | 1770.31 ± 103.07 | 1788.28 ± 96.58 | 1812.14 ± 101.96 | 1852.57 ± 111.80 |
| Body weight gain (g/bird) | 1-14 | 354.30 ± 29.48 | 345.60 ± 12.39 | 353.54 ± 18.06 | 342.50 ± 13.71 |
| | 15-35 | 1374.10 ± 75.60 | 1400.48 ± 90.63 | 1416.40 ± 90.21 | 1467.17 ± 105.00 |
| | 1-35 | 1728.41 ± 102.43 | 1746.08 ± 96.37 | 1769.94 ± 101.81 | 1809.67 ± 111.45 |
| Feed conversion ratio | 1-14 | 1.21 ± 0.04 | 1.15 ± 0.03 | 1.17 ± 0.04 | 1.20 ± 0.01 |
| | 15-35 | 1.66 ± 0.05 | 1.61 ± 0.08 | 1.65 ± 0.08 | 1.57 ± 0.09 |
| | 1-35 | 1.56 ± 0.04 | 1.52 ± 0.06 | 1.55 ± 0.05 | 1.50 ± 0.06 |

T0: Control diet containing 3% Lemuru fish oil; T1: T0+0.3 ppm Se + 200 ppm VE; T2: T0+0.6 ppm Se + 300 ppm VE; T3: T0+0.9 ppm Se + 400 ppm VE.

fence system to neutralise excess free radicals. This activation involves the Nrf2 signalling pathway, which stimulates the various antioxidant genes and increases the antioxidant enzyme activity (Miao *et al.*, 2020). However, in this study, after the initial brooding period (33°C for 3 days), the ambient temperature was recorded at 26–30°C, which might not be extreme enough to trigger significant oxidative stress. Thus, the endogenous antioxidant system might have been functioning normally without additional supplements, and the antioxidant response contributing to improved performance was not optimally activated.

Visceral Organs Weight and Intestine Length

The treatment did not influence ($P>0.05$) the weight percentage of the liver, gallbladder, heart, pancreas, lymph, thymus, bursa of Fabricius, small intestine, and cecum. Dietary supplementation of 400 ppm VE and 0.9 ppm Se significantly lowered the relative length of the colon ($P<0.05$) compared to the control and supplementation of 0.3 Se and 200 ppm VE groups (Table 3 and 4). It indicated that VE and Se supplementation in diets rich in unsaturated fatty acids could maintain the physiological stability of the digestive and immune organs of broiler chickens without causing toxic effects. These two antioxidant compounds function not only in counteracting free radicals and preventing cell damage, but also in increasing the nutrient ab-

sorption efficiency in the intestine and maintaining the health of the digestive tract (Hassanpour *et al.*, 2016; Chen *et al.*, 2024). At the appropriate levels, VE and Se act synergistically as antioxidants, with Se acting as a cofactor for glutathione peroxidase (GSH-Px) and VE inhibiting lipid peroxidation in cell membranes, thereby maintaining cellular integrity and metabolic efficiency (Sadiq *et al.*, 2023; Oke *et al.*, 2025). The mechanism of VE is by donating a hydrogen atom to neutralise peroxy free radicals into hydroperoxides (Shastak *et al.*, 2023).

Our findings are supported by Salman *et al.* (2007), who found that Se and VE can help the liver combat free radicals without causing organ enlargement. In this study, the unchanged heart weight indicates the absence of toxicity or excessive contraction due to antinutrients. Similarly, our results showed that the liver and bile size remained unchanged, probably because Se and VE only acting as antioxidants, without directly affecting bile production or fat metabolism. The liver has an important function in fat metabolism, including producing bile to emulsify fats such as fish oil for absorption in the gut (Alamri, 2018). Similarly, the pancreas, which plays a role in the secretion of amylolytic, lipolytic, and proteolytic enzymes (Vertiprakhov *et al.*, 2024), did not show significant weight changes. This suggests that the digestive enzymatic system also remained stable and was not overburdened by feed components, particularly fat from Lemuru oil.

Table 3. Visceral Organ Weights of Broiler Chickens Fed Diets Rich in Unsaturated Fatty Acids Supplemented with VE and Se at 35 Days of Age

| Parameters (%) | Treatments | | | |
|--------------------|-------------|-------------|-------------|-------------|
| | T0 | T1 | T2 | T3 |
| Liver | 2.24 ± 0.32 | 2.32 ± 0.23 | 2.37 ± 0.20 | 2.08 ± 0.14 |
| Heart | 0.53 ± 0.07 | 0.49 ± 0.07 | 0.52 ± 0.13 | 0.51 ± 0.05 |
| Bile | 0.14 ± 0.06 | 0.17 ± 0.04 | 0.18 ± 0.04 | 0.15 ± 0.03 |
| Pancreas | 0.29 ± 0.07 | 0.28 ± 0.04 | 0.25 ± 0.04 | 0.27 ± 0.03 |
| Thymus | 0.22 ± 0.08 | 0.15 ± 0.08 | 0.11 ± 0.07 | 0.17 ± 0.05 |
| Bursa of Fabricius | 0.14 ± 0.07 | 0.13 ± 0.05 | 0.08 ± 0.03 | 0.12 ± 0.05 |
| Lymph | 0.10 ± 0.03 | 0.10 ± 0.02 | 0.11 ± 0.08 | 0.07 ± 0.02 |

T0: Control diet containing 3% Lemuru fish oil; T1: T0+0.3 ppm Se + 200 ppm VE; T2: T0+0.6 ppm Se + 300 ppm VE; T3: T0+0.9 ppm Se + 400 ppm VE.

Table 4. Weights and Lengths of The Digestive Tract of Broiler Chickens Fed Diets Rich in Unsaturated Fatty Acids and Supplemented with VE and Se at 35 Days of Age

| Parameters (%) | Treatments | | | |
|----------------|----------------------------|--------------------------|---------------------------|--------------------------|
| | T0 | T1 | T2 | T3 |
| | Relative weight (%) | | | |
| Proventriculus | 0.60 ± 0.23 | 0.55 ± 0.10 | 0.45 ± 0.07 | 0.48 ± 0.05 |
| Ventriculus | 1.58 ± 0.20 | 1.42 ± 0.20 | 1.50 ± 0.20 | 1.40 ± 0.11 |
| Duodenum | 0.74 ± 0.13 | 0.69 ± 0.15 | 0.72 ± 0.14 | 0.73 ± 0.10 |
| Jejunum | 1.45 ± 0.29 | 1.44 ± 0.39 | 1.18 ± 0.08 | 1.21 ± 0.14 |
| Ileum | 1.13 ± 0.18 | 0.95 ± 0.40 | 1.03 ± 0.08 | 1.07 ± 0.23 |
| Caeca | 0.36 ± 0.04 | 0.35 ± 0.08 | 0.36 ± 0.04 | 0.37 ± 0.03 |
| Colon | 0.14 ± 0.05 | 0.13 ± 0.04 | 0.12 ± 0.02 | 0.10 ± 0.02 |
| | Relative length (cm /100g) | | | |
| Duodenum | 2.03 ± 0.27 | 1.98 ± 0.36 | 1.86 ± 0.19 | 2.01 ± 0.31 |
| Jejunum | 4.96 ± 0.74 | 5.78 ± 2.28 | 4.55 ± 0.30 | 4.77 ± 0.73 |
| Ileum | 4.90 ± 0.56 | 4.52 ± 0.94 | 4.61 ± 0.26 | 5.02 ± 0.77 |
| Caeca | 2.34 ± 0.28 | 2.21 ± 0.33 | 2.34 ± 0.15 | 2.11 ± 0.19 |
| Colon | 0.64 ± 0.16 ^b | 0.69 ± 0.27 ^b | 0.51 ± 0.03 ^{ab} | 0.45 ± 0.07 ^a |

T0: Control diet containing 3% Lemuru fish oil; T1: T0+0.3 ppm Se + 200 ppm VE; T2: T0+0.6 ppm Se + 300 ppm VE; T3: T0+0.9 ppm Se + 400 ppm VE. Means with different superscripts on the same row represent significant differences (P<0.05).

Ventriculus organ weights that did not differ indicated normal functioning in response to feed fiber below 5% (Darmawan and Ozturk, 2025).

The relative length of the colon decreased significantly in this study, which may be due to the synergistic activity of Se and VE as antioxidants in strengthening the integrity of the small intestinal tissue and reducing inflammation, thereby improving nutrient absorption (Surai and Kochish, 2019; Hassanpour *et al.*, 2021). Although nutrient absorption was not measured directly in this study, the trend of increased body weight and feed efficiency with higher levels of Se and VE may indicate an improvement in nutrient utilisation. This condition may reduce the flow of undigested material to the colon and decrease its functional load, which may contribute to the observed reduction in colon length. This condition also indicated that supplementation up to 400 ppm VE and 0.9 ppm Se in a diet high in

fatty acids was safe and did not show any signs of toxicity. However, excessive levels of VE and Se can have an adverse impact on intestinal histomorphology, such as a reduction in villus height and surface area due to inhibition of intestinal epithelial cells' proliferation in the crypts, which contribute to the renewal of the intestinal villi (Chen *et al.*, 2019; Li *et al.*, 2025).

The treatments also had no impact on the immune organs' weight. The inclusion of VE and Se is necessary to neutralise oxidants from fatty acid oxidation and maintain the stability of immune cell membranes. In addition, omega-3 in fish oil, which is immunomodulatory, may be able to suppress excess inflammatory responses (Rodrigues *et al.*, 2024); hence, this combination supports optimal immune function without causing immune organ hyperplasia, including thymus, bursa Fabricius, and lymph.

VE and Se in Broiler Meat

Dietary supplementation of 0.9 ppm Se and 400 ppm VE had the highest Se concentration and a higher VE level ($P < 0.05$) than the control group and Se (0.3 ppm) and VE (200 ppm) groups (Table 5). A previous study found that selenomethionine inclusion at 0.3 ppm significantly increased Se deposits in broiler breast muscle (Vieira *et al.*, 2020). Similarly, Tang *et al.* (2021) reported that the usage of 0.8 ppm selenomethionine in the diet effectively increased Se accumulation in breast muscle with a linear dose-response. Also, the combination of the three antioxidants (Se, vitamin C, and E) was evidenced to have a synergistic effect in increasing Se accumulation in muscle tissue (Pečjak *et al.*, 2022). Meanwhile, the utilisation of VE in the diet exhibited a linear pattern of α -tocopherol retention in thigh and breast muscle (Mazur-Kušnerek *et al.*, 2019).

In general, the application of Se in the diet has been proven to improve tissue Se levels, and organic Se sources show higher absorption efficiency than inorganic Se. This difference is closely related to the metabolic pathways of each form of Se, where organic Se tends to be more efficient in tissue storage (Kim and Kil, 2020). The absorption of organic Se, especially selenomethionine, is reported to reach an efficiency of more than 90%, while inorganic forms such as sodium selenite are only about 50% (Fairweather-Tait *et al.*, 2010). This efficiency is associated with the absorption mechanism whereby inorganic Se is absorbed passively through simple diffusion in the intestine, while selenomethionine utilises an active transport pathway through an amino acid transport mechanism. Furthermore, the similarity in chemical structure between selenomethionine and methionine allows selenomethionine to be used as a substitute in the protein synthesis process, as tRNA is unable to distinguish between the two. This condition causes selenomethionine to be stored in tissues such as muscle, kidney, liver, pancreas, and digestive

tract mucosa (Wang *et al.*, 2011). Moreover, Se acts as a necessary component of the GPx enzyme, which functions in the detoxification of lipid peroxides, thus reducing the need for VE as an antioxidant. Se protective function against pancreatic integrity also contributes to optimising fat digestion and VE absorption, which indirectly increases VE retention in plasma and body tissues (Skřivan *et al.*, 2008). Meanwhile, VE is absorbed with fat and carried by lipoproteins to muscle tissue. α - and γ -tocopherol are absorbed in the intestine along with dietary fat and are packed in chylomicrons. Part of the VE in chylomicrons is transported to peripheral tissues through lipoprotein lipase activity, while the remaining is carried to the liver. From the liver, VE is then circulated to body tissues via HDL and LDL (Jiang, 2022).

Fatty Acid Content in Broiler Meat

Feeding chickens with unsaturated fatty acids from oils has been proven to produce meat with higher unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs) and lower saturated fatty acids (SFAs) (Kralik *et al.*, 2012; Sumiati *et al.*, 2022; Cruz *et al.*, 2023). However, this high PUFA content makes the meat more susceptible to lipid oxidation processes, which can degrade meat quality both during the life phase of the chicken, after slaughter, and during storage and meat product processing (Pečjak *et al.*, 2022). Our results proved that there were no significant differences ($P > 0.05$) in saturated fatty acid levels. Dietary supplementation of 0.6 ppm Se and 300 ppm VE increased ($P < 0.05$) total monounsaturated fatty acid, total polyunsaturated fatty acid, and omega-6 compared to the control and 0.3 ppm Se and 200 ppm VE groups. The highest omega-3 content was obtained in the treatment with Se (0.9 ppm) and VE (400 ppm) (Table 6). Our finding is consistent with Cruz *et al.* (2023), who showed that the inclusion of 0.5 ppm Se was able to prevent oxidation of lipid and increase the PUFA content in thigh and

Table 5. Se and VE Content of Broiler Meat Fed Diets Rich in Unsaturated Fatty Acids and Supplemented with VE and Se

| Parameters (%) | Treatments | | | |
|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | T0 | T1 | T2 | T3 |
| Se ($\mu\text{g}/100\text{ g}$) | 13.27 \pm 0.88 ^a | 16.69 \pm 1.26 ^b | 21.36 \pm 1.10 ^c | 27.25 \pm 1.58 ^d |
| VE ($\text{mg}/100\text{ g}$) | 1.29 \pm 0.02 ^a | 2.07 \pm 0.10 ^b | 2.39 \pm 0.14 ^c | 2.36 \pm 0.13 ^{bc} |

T0: Control diet containing 3% Lemuru fish oil; T1: T0+0.3 ppm Se + 200 ppm VE; T2: T0+0.6 ppm Se + 300 ppm VE; T3: T0+0.9 ppm Se + 400 ppm VE. Means with different superscripts on the same row represent significant differences ($P < 0.05$).

Table 6. Fatty Acid Profile of Broiler Meat Fed Diets Rich in Unsaturated Fatty Acids and Supplemented with VE and Se

| Parameters (%) | Treatments | | | |
|--|--------------------------|--------------------------|--------------------------|--------------------------|
| | T0 | T1 | T2 | T3 |
| Saturated fatty acids (SFAs) | | | | |
| C 14:0 (Myristic acid) | 0.068±0.004 | 0.064±0.004 | 0.168±0.010 | 0.196±0.011 |
| C 15:0 (Pentadecanoic acid) | 0.010±0.001 | 0.006±0.001 | 0.017±0.001 | 0.017±0.001 |
| C 16:0 (Palmitic acid) | 0.828±0.047 | 0.867±0.055 | 0.467±0.058 | 0.548±0.030 |
| C 17:0 (Heptadecanoic acid) | 0.012±0.001 | 0.013±0.001 | 0.036±0.002 | 0.039±0.002 |
| C 18:0 (Stearic acid) | 0.246±0.014 | 0.209±0.013 | 0.550±0.031 | 0.695±0.039 |
| C 20:0 (Arachidic acid) | 0.004±0.001 | 0.005±0.002 | 0.009±0.001 | 0.008±0.001 |
| Total SFAs | 1.169±0.066 | 1.164±0.073 | 1.246±0.024 | 1.181±0.090 |
| Monounsaturated fatty acids (MUFAs) | | | | |
| C 14:1 (Myristoleic acid) | 0.008±0.001 | 0.009±0.001 | 0.023±0.001 | 0.023±0.001 |
| C 16:1 (Palmitoleic acid) | 0.202±0.012 | 0.271±0.017 | 0.708±0.039 | 0.688±0.039 |
| C 17:1 (Heptadecanoic acid) | 0.007±0.001 | 0.008±0.001 | 0.023±0.001 | 0.024±0.001 |
| C 18:1 (Oleic acid) | 1.389±0.076 | 1.392±0.068 | 2.663±0.410 | 2.633±0.162 |
| C 20:1 (Eicosenoic acid) | 0.013±0.001 | 0.011±0.001 | 0.029±0.002 | 0.034±0.002 |
| C 22:1 (Erucic acid) | 0.013±0.006 | 0.004±0.001 | 0.011±0.001 | 0.010±0.001 |
| Total MUFAs | 1.631±0.097 ^a | 1.697±0.085 ^a | 3.429±0.366 ^b | 3.442±0.207 ^b |
| Polysaturated fatty acids (PUFAs) | | | | |
| C 18:2 n6 (Linoleic acid) | 0.562±0.030 | 0.593±0.037 | 1.612±0.090 | 1.784±0.097 |
| C 20:3 n6 (Eicosatrienoic acid) | 0.013±0.006 | 0.006±0.001 | 0.010±0.001 | 0.013±0.001 |
| C 20:4 n6 (Arachidonic acid) | 0.019±0.001 | 0.017±0.001 | 0.042±0.002 | 0.051±0.003 |
| C 18:3 n3 (Alpha-linolenic) | 0.023±0.001 | 0.026±0.001 | 0.073±0.004 | 0.082±0.005 |
| C 20:5 n3 (Eicosapentaenoic acid) | 0.061±0.003 | 0.053±0.003 | 0.121±0.009 | 0.171±0.007 |
| C 22:6 n3 (Docosahexanoic acid) | 0.080±0.005 | 0.044±0.003 | 0.087±0.007 | 0.131±0.005 |
| Total omega-6 | 0.595±0.037 ^a | 0.616±0.037 ^a | 1.665±0.093 ^b | 1.849±0.101 ^b |
| Total omega-3 | 0.164±0.010 ^b | 0.123±0.008 ^a | 0.281±0.021 ^c | 0.384±0.016 ^d |
| Total PUFAs | 2.389±0.144 ^a | 2.436±0.131 ^a | 5.375±0.328 ^b | 5.675±0.256 ^b |

T0: Control diet containing 3% Lemuru fish oil; T1: T0+0.3 ppm Se + 200 ppm VE; T2: T0+0.6 ppm Se + 300 ppm VE; T3: T0+0.9 ppm Se + 400 ppm VE. Means with different superscripts on the same row represent significant differences (P<0.05).

breast meat. Similarly, Kralik *et al.* (2012) suggested that the omega-3 in meat increased when chickens were fed with 0.3 ppm Se and a combination of 3% sunflower oil and 3% linseed oil. Furthermore, Shahid *et al.* (2019) noted that feeding VE and flaxseed not only enriched the PUFAs in meat but also lowered the n-6 to n-3 PUFAs ratio. On the other hand, high-dose supplementation (400 ppm VE and 0.9 ppm Se) was ineffective in increasing the levels of MUFAs and PUFAs in the meat. This phenomenon can be explained by the fact that antioxidant compounds can exhibit varying effects depending on their concentration. At optimal levels, Se and VE act as antioxidants; conversely, at excessive doses, these compounds produce pro-oxidant effects and promote lipid peroxidation (Wijayanti *et al.*, 2023; Barchielli *et al.*, 2022). Consequently, such behaviour may reduce the effectiveness of the protection of unsaturated fatty acids, thereby decreasing the efficiency of MUFA and PUFA deposition in tissues.

The same SFA levels among treatments in this study may be due to the structure of SFAs, which is more stable against oxidation due to the

absence of double bonds. Therefore, although Se and VE function as important antioxidants in suppressing lipid peroxidation, their protective effects are more pronounced against unsaturated fatty acids, especially PUFAs that have a high susceptibility to oxidative degradation. The synergistic interaction between VE and Se minerals, as strong antioxidants, is believed to be able to maintain the stability of MUFAs and PUFAs in muscle tissue, thus supporting the retention and deposition of healthy fatty acids (Ekunseitan *et al.*, 2021). The treatment with the highest dose (400 ppm VE and 0.9 ppm Se) resulted in the highest omega-3 levels, indicating that higher antioxidant protection was able to inhibit the oxidation of highly reactive omega-3 fatty acids (DHA and EPA). Previous studies have suggested that antioxidant protection may be associated with desaturase and elongase activity involved in long-chain PUFA biosynthesis (Konieczka *et al.*, 2015). In addition, VE supplementation can also reduce lipid oxidation, specifically linolenic acid (18:3n-3) (Taşdelen and Ceylan, 2017). This process may explain the improvement in the total deposition of unsaturated fatty acids in tissues

such as thighs and breasts.

CONCLUSION

The inclusion of 400 ppm VE and 0.9 ppm Se in a diet rich in unsaturated fatty acids effectively enhanced the deposition of Se, VE, and omega-3 in the meat as well as reduced colon length without adverse effect on the production performance of broiler chickens. The combination of 300 ppm VE and 0.6 ppm Se was the optimal dose for increasing the deposition of MUFAs, PUFAs, and omega-6 in the meat.

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