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RESEARCH ARTICLE

The Effects of Vitamin E and Nano-Selenium Supplementation on Growth Performance, Intestinal Health, Antioxidant Status and Meat Quality Traits of Broilers

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ABSTRACT

This study investigated the effects of dietary supplementation with vitamin E (VE) and nano-selenium (Nano-Se) on growth performance, meat quality traits, intestinal morphology, antioxidant biomarkers, and gut microbiota in broiler chickens. A total of 250 one-day-old Ross 308 broiler chicks were randomly allocated to five dietary treatments viz a viz: (1) control (basal diet without additives), (2) basal diet supplemented with 150mg/kg VE, (3) 150mg/kg VE+2mg/kg Nano-Se, (4) 150 mg/kg VE+4mg/kg Nano-Se and (5) 150mg/kg VE+6 mg/kg Nano-Se. Growth performance parameters, including body weight, body weight gain, feed intake and feed conversion ratio, were not significantly affected ($P>0.05$) by dietary treatments. However, significant improvements ($P<0.05$) were observed in intestinal morphology, where supplementation with 2mg/kg Nano-Se increased duodenal villus height and width, while 4mg/kg Nano-Se enhanced crypt depth. Meat antioxidant status was markedly improved in supplemented groups, as evidenced by reduced lipid oxidation, particularly in birds receiving 6mg/kg Nano-Se. In addition, VE and Nano-Se supplementation significantly improved water-holding capacity, crude protein, ash, and fat contents of meat ($P<0.05$). Blood antioxidant biomarkers, including vitamin E, selenium, and glutathione concentrations, reached their highest levels in the group supplemented with 4mg/kg Nano-Se, whereas the highest Nano-Se level (6mg/kg) showed reduced antioxidant efficiency. Gut microbiota analysis revealed increased populations of Lactobacillus acidophilus and *Escherichia coli* in birds receiving 2mg/kg Nano-Se, while higher Nano-Se levels exerted inhibitory effects on microbial populations. Overall, the results indicate that the combined supplementation of vitamin E and Nano-Se at 2–4mg/kg improves intestinal health, meat quality and oxidative stability in broilers, whereas excessive Nano-Se supplementation may disrupt antioxidant balance and intestinal microbial homeostasis.

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INTRODUCTION

The poultry industry plays a pivotal role in global meat production and is essential for meeting the continuously increasing demand for animal protein (Chand *et al.,* 2018; Jabbar *et al.,* 2021). To maximize productivity, modern broiler production systems commonly employ intensive feeding strategies and high stocking densities. However, these practices often induce oxidative stress, which negatively affects bird health, immune function, growth performance, and overall production efficiency (Chand *et al.,* 2020; Hafeez *et al.,* 2020). Consequently, nutritional strategies aimed at minimizing oxidative damage have become increasingly important in sustainable poultry production (Alhidary *et al.,* 2017).

Dietary supplementation with antioxidants and trace minerals is widely used to alleviate the detrimental effects

of oxidative stress in broilers. Among these, selenium (Se), vitamin E (VE), and carotenoids are considered critical components of the antioxidant defense system in poultry (Khan *et al.,* 2012; Al-Khalaifah *et al.,* 2025). Proper diet formulation is therefore a key factor influencing broiler health, productivity, and metabolic efficiency (Hafeez *et al.,* 2020). Minerals are indispensable for numerous enzymatic reactions and physiological processes, thereby supporting growth, immunity, and nutrient metabolism in poultry. Selenium, in particular, plays an essential role in antioxidant defense, immune competence, reproductive performance, and intestinal health in broilers (Batool *et al.,* 2025; Haseeb *et al.,* 2025).

In recent years, nanotechnology has emerged as a promising approach in poultry nutrition due to the unique physicochemical properties of nanoparticles, including their high surface area, enhanced reactivity, targeted delivery, and reduced toxicity (Mahmood *et al.,* 2024). The growing concern regarding antimicrobial resistance and the reduced efficacy of antibiotics has further encouraged the exploration of nanoparticles as alternative feed additives to improve poultry health and productivity (Shami *et al.,* 2024). Among these, nano-selenium (Nano-Se) has gained substantial attention because of its superior bioavailability, lower toxicity, and greater biological activity compared with conventional selenium sources (Al-Khalaifah *et al.,* 2025). Nano-Se applications extend across medicine, biotechnology, and animal nutrition, offering innovative opportunities for nutrient delivery and disease management (Khan et al. 2025).

Nano-selenium, particularly in its elemental zero-valent form (Se^0), exhibits greater stability and bioactivity than other selenium forms (e.g., Se^{+}VI) (Al-Khalaifah *et al.,* 2025; Batool *et al.,* 2025; Haseeb *et al.,* 2025). Previous studies have demonstrated that Nano-Se enhances nutrient absorption, antioxidant status, immune responses, and resistance to intestinal degradation in broilers (Khan *et al.,* 2025). Moreover, supplementation of Nano-Se within recommended levels (0.3–0.5 ppm) has been shown to improve meat selenium deposition, intestinal morphology, gut microbial balance, and immune function while minimizing the risk of selenium toxicity (Al-Khalaifah *et al.,* 2025).

Vitamin E is another essential dietary antioxidant that plays a vital role in maintaining cellular integrity, reproductive performance, and immune responses in poultry (Rahman *et al.,* 2018). As a lipid-soluble antioxidant, VE protects biological membranes against lipid peroxidation and oxidative damage. Supplementation of VE has been associated with enhanced antioxidant enzyme activities, including total antioxidant capacity (TA), superoxide dismutase (SOD), and glutathione peroxidase (GPx), alongside reduced malondialdehyde (MDA) concentrations in broilers (Khan *et al.,* 2011). Importantly, vitamin E and selenium function synergistically within the antioxidant defense system. Selenium is an integral component of glutathione peroxidase, while vitamin E prevents oxidative deterioration of membrane lipids; together, they effectively reduce oxidative stress and tissue damage (Rahman *et al.,* 2018).

Despite the recognized antioxidant roles of selenium and vitamin E, limited information is available regarding the combined use of Nano-Se and VE in broiler diets and their interactive effects on growth performance, meat quality, antioxidant status, and intestinal health. Therefore, the present study was designed to evaluate the effects of dietary supplementation with Nano-Se (2–6mg/kg) in combination with vitamin E (150mg/kg) on broiler performance, meat quality characteristics, antioxidant capacity, and intestinal morphology, with the aim of developing an effective nutritional strategy for improving poultry health and sustainable broiler production.

MATERIALS AND METHODS

Experimental design: The present experiment was conducted at the Poultry Research Center, University of Baghdad. A total of 250 one-day-old Ross 308 broiler chicks were used in the study. The chicks were randomly allocated to five experimental groups, each consisting of five replicates with 10 birds per replicate. The experimental treatments were as follows: 1) control group (C; without additional vitamin E or nano-selenium supplementation), 2) control+150 mg/kg vitamin E supplementation (VE150), 3) VE150+2mg/kg nano-selenium supplementation (Se2), 4) VE150+4mg/kg nano-selenium supplementation (Se4) and 5) VE150+6 mg/kg nano-selenium supplementation (Se6).

The chicks were reared on floor pens divided by wire partitions (1m×2 m) under controlled environmental conditions. The ambient temperature was maintained at 35°C during the first three days and subsequently reduced by 2–3°C weekly throughout the experimental period. Wood shavings with a depth of approximately 8 cm were used as bedding material. Birds were provided with three phase-feeding diets, including starter (days 1–10), grower (days 11–24), and finisher (days 25–35) rations. The corn–soybean meal-based diets were formulated and manufactured at a commercial feed factory in Baghdad. The ingredient composition and nutrient contents of the experimental diets are presented in Table 1.

Table 1: Composition and nutritional values of experimental diets

Ingredient	Starter %	Grower %	Finisher%
Yellow Corn	48.1	48.1	48.1
Wheat	9.7	12.8	15.6
Soybean meal (48% protein)	33.0	29.1	25.3
Protein concentrate	5.0	5.0	5.0
Vegetable oil	2.0	3.1	4.3
Dicalcium phosphate	0.7	0.5	0.4
Sodium chloride	0.3	0.3	0.3
Limestone	1.2	1.1	1.0
Total	100	100	100
Chemical composition			
Crude Protein (CP, %)	23.04	21.50	20.02
Metabolizable Energy (ME, kcal/kg)	3004.54	3129	3204
Calcium (Ca, %)	0.95	0.87	0.80
Available phosphorus (AP, %)	0.39	0.35	0.32
Methionine (Meth, %)	0.53	0.51	0.49
Lysine (Lys, %)	1.40	1.28	1.17

The Protein Concentrate was from Al Wafi company, Netherland, contains: 40% crude protein, 2107 kcal, metabolizable energy/kg, 3.85% lysine, 3.7% methionine, 4.12% methionine + cysteine, 5% fat, 5% calcium, 4.68% phosphorus, 2.4% sodium, 2.26% fiber, 200,000 IU vitamin A, 60000 IU vitamin D3, 600mg vitamin E, 50mg vitamin K3, 60mg vitamin B1, 140mg vitamin B2, 80mg vitamin B6, 20mg folic acid, 100mcg biotin, 1 mg iron, 200mg copper, 1.6mg manganese, 1.2mg zinc, 20mg iodine, 5mg selenium, 900mg antioxidants (BHT).

At the end of the 35-day experimental period, two birds from each replicate (one male and one female) were randomly selected and humanely slaughtered by cervical dislocation for sample collection. Following slaughter, the intestinal tract was manually eviscerated, and duodenal and ileal segments were aseptically collected for morphological evaluation. In addition, thigh muscle samples were obtained for meat quality assessment. All collected samples were processed within 30 min after collection and were either analyzed immediately or stored at −80°C for subsequent analyses.

Productive parameters: Body weight (BW) was measured weekly for each replicate utilizing a 1gm sensitive balance. The difference between consecutive weight measurements was taken as body weight gain (BWG). Residual feed was subtracted from weekly feed supply to get feed intake (FI). Feed intake was divided by body weight gain to get feed conversion ratio (FCR).

Carcass parameters: At 35 days of age, the internal organs, including the heart, liver, gizzard, bursa of Fabricius, spleen, and abdominal fat, were collected after slaughter and carefully cleaned. The carcasses were subsequently dressed and individually weighed to determine dressing percentage excluding internal organs. The relative weights of the organs were calculated as a percentage of live body weight.

Meat quality parameters: The proximate composition and meat quality characteristics were determined using standard analytical procedures. Lipid content was quantified by Soxhlet extraction using hexane according to the method of Reynolds (1989), in which 10gm samples were extracted for 5h followed by solvent evaporation at 80°C. Crude protein content was determined using the Kjeldahl method as described by Douglas *et al.,* (2000), involving acid digestion and ammonia distillation, and the obtained nitrogen values were converted to protein using a conversion factor of 6.25. Ash content was analyzed by incinerating samples in a muffle furnace at 550°C for 5 h according to Association of Official Analytical Collaboration procedures (AOAC, 2013).

Drip loss was measured after 24h of storage at 5°C following the method of Alvarado and Sams (2002). Thawing loss was determined after 24h at −4°C according to Pesenti *et al.* (1981), while cooking loss was evaluated after cooking the samples at 70°C for 90 min following the procedure of Cyril *et al.,* (1996). Water-holding capacity (WHC) was assessed using the centrifugation method described by Honikel and Hamm (1994), in which homogenized samples mixed with distilled water were centrifuged at 5,000rpm for 10min. All gravimetric measurements were expressed as percentage weight changes relative to the initial sample weights.

Blood profile: Blood samples were collected from the wing veins of 40 euthanized birds (two birds per replicate) using sterile disposable needles. The collected samples were divided into EDTA-coated tubes for hematological analysis and gel separator tubes for serum biochemical assays. After storage at 4°C for 12 h, the samples were centrifuged at 3,000 rpm to separate the serum, which was subsequently stored at −18°C until analysis.

Serum biochemical parameters, including glucose, cholesterol, total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were determined using a Mindray BS-1200 automated blood chemistry analyzer with Agape diagnostic kits.

For antioxidant biomarker analysis, vitamin E was extracted from 500µL of serum using an ethanol–hexane mixture (1:2). The extracts were analyzed using a high-performance liquid chromatography (HPLC) system (SYKAM) equipped with a C18-octadecylsilane (ODS) column maintained at 35°C. The mobile phase consisted of methanol, acetonitrile, and water (60:35:5) at a flow rate of 1.5mL/min, and detection was performed at 292 nm.

Selenium concentration was determined following wet acid digestion using an HNO_3–H_2O_2 mixture (2:1) at 70°C, followed by analysis using atomic absorption spectroscopy (Shimadzu AA-7000). Glutathione levels were measured after trichloroacetic acid (TCA) precipitation and ortho-phthalaldehyde (OPA) derivatization using HPLC with fluorescence detection. Separation was carried out on a C18-amino (NH_2) column using a mobile phase composed of acetonitrile, buffer, and water (60:10:30) at a flow rate of 1mL/min, according to the method described by Pearce *et al.,* (1992).

Histological analysis of intestinal morphology: Duodenal and ileal segments measuring approximately 1cM were aseptically collected and fixed in 10% buffered formalin for 72h. The tissue samples were subsequently processed through a graded ethanol dehydration series (70–100%) at 2h intervals, followed by xylene clearing and paraffin embedding at 56–58°C for 4h with two paraffin changes. Tissue sections of 5µM thickness were prepared using a rotary microtome according to the method described by Drury (1983). Morphometric measurements, including villus height, villus width, and crypt depth, were determined using ImageJ software.

Antioxidant indicators: Three complementary analyses were performed to evaluate lipid oxidation characteristics. The peroxide value (PV), which reflects the formation of primary oxidation products, was determined according to the method of Egan *et al.* (1981). Briefly, 2gm of fat extract was reacted with an acetic acid–chloroform mixture (3:2) and potassium iodide, followed by titration with 0.01 N sodium thiosulfate using starch as an indicator. The acceptable PV range was considered ≤10 mEq/kg.

Secondary lipid oxidation products were assessed using the thiobarbituric acid reactive substances (TBARS) assay according to Witte *et al.,* (1970). Samples homogenized in 20% trichloroacetic acid (TCA) and 2M phosphoric acid were reacted with 0.005M thiobarbituric acid (TBA) reagent and incubated

in darkness for 16h prior to spectrophotometric measurement at 530nM. Malondialdehyde (MDA) concentration was calculated using the formula: MDA = $A_{530} \times 5.2$, with acceptable values considered ≤0.09mg MDA/kg.

Free fatty acid (FFA) concentration, an indicator of hydrolytic rancidity, was determined according to Egan *et al.,* (1981). Briefly, 10g of fat was dissolved in an ether: ethanol mixture (1:1) and titrated with 0.1N NaOH using phenolphthalein as the endpoint indicator. The results were expressed as the percentage of oleic acid equivalent.

Intestinal microbial population: Gut microbiota composition was evaluated using quantitative culture-based techniques targeting both beneficial and pathogenic bacterial populations. Enumeration of *Escherichia coli* was performed using serial decimal dilutions of intestinal samples, which were plated on MacConkey agar and aerobically incubated at 37°C for 48h. Lactobacillus populations were quantified on de Man, Rogosa, and Sharpe (MRS) agar under anaerobic conditions at 37°C for 48h. Only plates containing 30–300 colonies were considered suitable for bacterial enumeration.

Statistical analysis: The experiment was conducted using a completely randomized design (CRD) with five dietary treatments and five replicates per treatment. Prior to statistical analysis, data normality was evaluated using the Shapiro–Wilk test. Because the experimental design consisted of five independent dietary treatments rather than a factorial arrangement, treatment effects were analyzed using one-way analysis of variance (ANOVA) in SAS software. The obtained data were analyzed by one-way analysis of variance (ANOVA) using SAS software. When significant treatment effects were detected, mean differences among treatments were compared using Tukey's post hoc test. Differences were considered statistically significant at $P<0.05$. Effect sizes and an a priori power analysis were not performed and are acknowledged as limitations of the study.

RESULTS

The effects of dietary vitamin E (VE) and nano-selenium (Nano-Se) supplementation on growth performance parameters are presented in Table 2. Experimental treatments did not significantly affect ($P>0.05$) body weight (BW), body weight gain (BWG), feed intake (FI), or feed conversion ratio (FCR). Although minor numerical variations were observed among treatments, none reached statistical significance.

The effects of experimental treatments on intestinal morphology are shown in Table 3. Significant differences ($P\leq0.05$) were observed in villus height, villus width, and crypt depth of the duodenum and ileum. The VE150+Se2 treatment produced the greatest duodenal villus height (613.15±18.28μM) and villus width (86.71±4.13μM), whereas the greatest duodenal crypt depth (138.91±5.68μM) was recorded in the VE150+Se4 group. In the ileum, the VE150 treatment resulted in the highest villus height (789.12±24.63μM), villus width (86.71±3.52μM) and crypt depth (120.46±5.27μM). In general, the control group exhibited the lowest intestinal morphometric values.

The effects of VE and Nano-Se supplementation on peroxide value (PV), thiobarbituric acid reactive substances (TBA), and free fatty acids (FFA) before and after freezing are summarized in Table 4. Significant treatment effects ($P\leq0.05$) were observed for all lipid oxidation parameters. The control treatment showed the highest PV, TBA, and FFA values both before and after freezing. Conversely, the VE150+Se6 treatment produced the lowest values for all oxidation indices, indicating improved oxidative stability of broiler meat.

The effects of dietary treatments on meat quality characteristics are presented in Table 4. Significant improvements ($P\leq0.05$) were observed in crude protein, crude ash, fat content, water-holding capacity (WHC), and dry matter (DM) in birds receiving VE150+Se6 supplementation. The same treatment also resulted in the lowest thawing loss, cooking loss, and drip loss values. In contrast, the control group exhibited the poorest meat quality characteristics, including lower WHC, crude ash, fat, and DM values, along with higher thawing, cooking and drip losses.

Table 2: Effects of experimental treatments of growth performance parameters

Item	Control	VE150	VE150+Nano-Se2	VE150+Nano-Se4	VE150+Nano-Se6	P
BW						
1-10 d	38.75± 0.47	39.5± 0.28	39.25± 0.75	39.75± 0.63	38.75± 0.25	0.573
10-24 d	274.25± 11.01	247.75± 11.05	246± 8.25	257.75± 16.53	246.5± 10.42	0.415
24-35 d	1247± 20.10	1167.5± 55.53	1159.25± 13.92	1161.5± 27.63	1174± 18.31	0.275
1-35 d	2173± 75.80	2090.5± 109.92	1974.8± 111.46	2058.3± 191.46	2041± 95.75	0.843
BWG						
1-10 d	235.5±10.90	208.25±11.29	206.75±8.98	218±16.25	207.75±10.28	0.407
10-24 d	972.75±29.94	919.75±66.57	913.25±8.43	903.75±13.53	927.5±9.26	0.646
24-35 d	926±91.66	923±68.07	815.5±108.74	896.75±175.89	867±93.09	0.954
1-35 d	2134.25±75.72	2051±109.75	1935.5±111.79	2018.5±190.93	2002.25±95.68	0.841
FI						
1-10 d	252±8.73	219.25±16.84	223.75±12.47	223.25±11.48	233±21.92	0.563
10-24 d	1075.25±47.59	1077.75±45.71	993.5±20.43	981±16.31	1000.75±37.76	0.201
24-35 d	1700±0	1688.75±11.25	1692.5±7.5	1700±0.00	1700±0.00	0.562
1-35 d	3027.25±42.36	2985.75±39.87	2909.75±24.67	2904.25±24.96	2933.75±46.31	0.136
FCR						
1-10 d	1.072±0.01	1.05±0.05	1.07±0.07	1.037±0.03	1.12±0.09	0.814
10-24 d	1.11±0.05	1.19±0.10	1.09±0.02	1.09±0.03	1.08±0.03	0.589
24-35 d	1.88±0.16	1.86±0.13	2.20±0.32	2.15±0.44	2.04±0.24	0.873
1-35 d	1.42±0.06	1.47±0.09	1.52±0.09	1.48±0.15	1.48±0.08	0.974

Control (Basal diet, no additives), VE150: Vitamin E (150mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg) + nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150mg/kg) + nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150mg/kg) + Nano-selenium (6 mg/kg diet), BW: Body Weight, BWG: Body Weight Gain, FI: Feed Intake, FCR: Feed Conversion Ratio, P: probability.

Table 3: Effects of experimental treatments on villus morphometry and crypt depth in the duodenum and ileum

Item	Control	VE150	VE150+Se2	VE150+Se4	VE150+Se6	P
Duodenum						
Villus height (µM)	476.56±15.72^{c}	549.12±19.88^{b}	613.15±18.28^{a}	487.74±13.95^{c}	394.61±6.31^{d}	0.0001
Villus width (µM)	67.26±2.57^{cb}	75.84±4.30^{ab}	86.71±4.13^{a}	58.23±3.80^{cd}	53.56±3.74^{d}	0.0001
Crypt depth (µM)	88.54±2.63^{b}	77.58±3.67^{b}	90.70±4.67^{b}	138.91±5.68^{a}	131.55±4.36^{a}	0.0001
Ileum						
Villus height (µM)	376.69±24.72^{c}	789.12±24.63^{a}	428.86±15.15^{c}	555.88±14.36^{b}	377.76±10.18^{c}	0.0001
Villus width (µM)	60.99±1.63^{c}	86.71±3.52^{a}	71.11±2.51^{cb}	62.26±2.58^{c}	78.84±5.10^{ab}	0.0001
Crypt depth (µM)	85.72±1.29^{bc}	120.46±5.27^{a}	80.65±6.65^{c}	95.48±2.80^{b}	110.74±4.47^{a}	0.0001

Control (Basal diet, no additives), VE150: Vitamin E (150mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg) + nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150 mg/kg)+nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150mg/kg) + Nano-selenium (6mg/kg diet), P: probability. Different superscript letters within a row indicate significant differences (P≤0.05).

Table 4: Effects of experimental treatments on meat quality and lipid oxidation parameters in broiler meat before and after freezing

Item	Control	VE150	VE150+Se2	VE150+Se4	VE150+Se6	P
Lipid oxidation before freezing						
P.V (meq/kg)	2.53±0.01^{a}	2.37±0.01^{b}	2.17±0.01^{d}	2.22±0.01^{c}	2.13±0.01^{e}	0.0001
T.B.A (mg MDA / kg)	0.05±0.00^{a}	0.04±0.00^{b}	0.03±0.00^{d}	0.04±0.00^{c}	0.03±0.00^{e}	0.0001
FFA %	0.49±0.01^{a}	0.43±0.00^{b}	0.37±0.00^{d}	0.40±0.00^{c}	0.33±0.01^{e}	0.0001
Lipid oxidation after freezing						
P.V (meq/kg)	7.16±0.05^{a}	5.15±0.03^{b}	4.84±0.04^{d}	5.03±0.03^{c}	4.72±0.02^{e}	0.0001
T.B.A (mg MDA / kg)	0.08±0.00^{a}	0.05±0.00^{b}	0.04±0.00^{c}	0.05±0.00^{b}	0.04±0.00^{d}	0.0001
FFA %	0.79±0.01^{a}	0.58±0.01^{b}	0.51±0.01^{d}	0.54±0.01^{c}	0.47±0.01^{e}	0.0001
Meat Quality						
Crud Protein %	17.10±0.01^{e}	18.46±0.02^{d}	19.13±0.01^{b}	18.94±0.02^{c}	19.5±0.01^{a}	0.0001
Crud Ash %	0.84±0.02^{e}	0.96±0.02^{d}	1.2±0.00^{b}	1.12±0.01^{c}	1.28±0.00^{a}	0.0001
WHC %	29.16±0.05^{e}	30.21±0.03^{d}	32.16±0.02^{b}	31.10±0.03^{c}	33.14±0.03^{a}	0.0001
FAT%	8.13±0.03^{e}	9.05±0.03^{d}	10.035±0.02^{b}	9.73±0.01^{c}	10.2±0.01^{a}	0.0001
Thawing loss%	3.16±0.02^{a}	2.98±0.01^{b}	2.62±0.01^{d}	2.83±0.01^{c}	2.42±0.01^{e}	0.0001
Cooking loss%	32.20±0.08^{a}	30.21±0.04^{b}	25.94±0.02^{d}	28.17±0.03^{c}	22.9±0.01^{e}	0.0001
Drip loss %	3.74±0.02^{a}	2.115±0.02^{b}	1.63±0.01^{d}	1.83±0.03^{c}	1.42±0.01^{e}	0.0001
Dry Matter (DM), %	30.75±0.2^{e}	33.35±0.32^{d}	36.45±0.20^{b}	35.10±0.00^{c}	38.05±0.09^{a}	0.0001

Control (Basal diet, no additives), VE150: Vitamin E (150mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg)+nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150 mg/kg) + nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150mg/kg) + Nano-selenium (6mg/kg diet), P.V: Peroxide Value, T.B.A: Thiobarbituric Acid, MDA: Malondialdehyde, FFA: Free Fatty Acids, WHC: Water Holding Capacity DM: Dry Matter, P: probability. Different superscript letters within a row indicate significant differences (P≤0.05).

The effects of dietary VE and Nano-Se supplementation on carcass traits are shown in Table 5. No significant differences (P>0.05) were observed among treatments for carcass yield, heart weight, liver weight, gizzard weight, bursa of Fabricius weight, spleen weight, or abdominal fat percentage. However, slight numerical variations were observed between groups.

Table 5: Effects of dietary vitamin E and nano-selenium treatments on carcass parameters

Item	Control	VE150	VE150+Se2	VE150+Se4	VE150+Se6	P
Carcass%	72.92±2.04	71.25±0.50	73.25±2.00	76.25±1.25	71.25±0.75	0.145
Heart weight, %	0.745±0.08	0.87±0.06	0.76±0.05	0.77±0.04	0.71±0.05	0.429
Liver weight, %	3.66±0.29	3.53±0.11	3.33±0.18	3.68±0.23	3.69±0.57	0.918
Gizzard weight, %	2.1±0.13	2.18±0.09	2.08±0.06	2.24±0.18	2.30±0.08	0.645
B. fabricus, %	0.28±0.07	0.3±0.03	0.23±0.02	0.34±0.15	0.30±0.05	0.888
Spleen, %	0.17±0.03	0.18±0.04	0.11±0.01	0.16±0.01	0.14±0.03	0.278
Crude fat, %	1.39±0.17	1.36±0.13	1.0825±0.06	1.12±0.12	1.185±0.08	0.309

Control (Basal diet, no additives), VE150: Vitamin E (150 mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg) +nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150 mg/kg) +nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150 mg/kg) +Nano-selenium (6mg/kg diet), P: probability.

The effects of experimental treatments on blood biochemical parameters are presented in Table 6. Dietary supplementation with VE and Nano-Se did not significantly influence (P>0.05) serum glucose, cholesterol, total protein, AST, or ALT concentrations. All treatment groups showed comparable values for these parameters.

Table 6: Effects of dietary vitamin E and nano-selenium treatments on blood biochemical parameters

Item	Control	VE150	VE150+Se2	VE150+Se4	VE150+Se6	P
Glucose (mg/dl)	147.03±15.84	130.51±14.66	168.59±5.43	158.05±14.94	151.23±9.87	0.345
Cholesterol (mg/dl)	100.10±6.68	106.24±6.22	108.25±3.37	106.44±11.11	112.58±6.04	0.808
Protein (g/dl)	3.39±0.21	3.30±0.37	3.29±0.25	3.50±0.18	3.48±0.23	0.963
AST/GOT (U/L)	15.53±4.82	26.32±12.04	18.14±3.99	19.92±9.70	16.47±8.56	0.899
ALT/GPT (U/L)	23.28±15.13	17.45±5.38	12.96±5.94	7.71±2.73	14.79±4.36	0.726

Control (Basal diet, no additives), VE150: Vitamin E (150 mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg) +nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150mg/kg) +nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150mg/kg) +Nano-selenium (6mg/kg diet), AST/GOT: Aspartate Aminotransferase /Glutamic-Oxaloacetic Transaminase, ALT/GPT: Alanine Aminotransferase /Glutamic-Pyruvic Transaminase, P: probability

The effects of treatments on serum vitamin E, selenium, and glutathione (GSH) concentrations are shown in Table 7. Significant differences (P<0.05) were detected among treatments. The VE150+Se4 treatment resulted in the highest serum VE (12±0.03ppm), selenium (106.25±0.49 ppb), and glutathione concentrations, whereas lower values were observed in the control group and VE150+Se6 treatment.

The effects of dietary treatments on intestinal microbial populations are presented in Table 8. Significant differences (P≤0.01) were observed for both *Escherichia coli* and *Lactobacillus acidophilus* populations. The VE150+Se2 treatment showed the

highest counts of both microorganisms (7.58±0.23 and 9.17±0.42 log CFU/g, respectively), while higher Nano-Se levels tended to reduce bacterial populations.

Table 7: Effects of dietary vitamin E and Nano-Selenium Supplementation on blood Vitamin E, selenium, and Glutathione peroxidase (GSHPx) concentrations in broiler.

Item	Control	VE150	VE150+Se2	VE150+Se4	VE150+Se6	P
VE (ppm)	9.38±0.17^{d}	10.24±0.03^{c}	11.00±0.03^{b}	12±0.03^{a}	6.28±0.07^{e}	0.0001
Se (ppb)	26.03±0.38^{d}	34.68±0.27^{c}	41.65±0.76^{b}	44.75±0.40^{a}	18.65±0.41^{e}	0.0001
GSH (ppb)	74.63±1.28^{d}	84.08±0.31^{c}	95.45±0.95^{b}	106.25±0.49^{a}	40.53±0.57^{e}	0.0001

Control (Basal diet, no additives), VE150: Vitamin E (150mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg) + nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150mg/kg) +nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150mg/kg) +Nano-selenium (6mg/kg diet), VE: Vitamin E, Se: Selenium, GSH: Glutathione, P: probability. Different superscript letters within a row indicate significant differences (P≤0.05).

Table 8: Effects of dietary vitamin E and Nano-Selenium Supplementation on *Escherichia coli* and *Lactobacillus acidophilus* populations in broiler

Item	Control	VE150	VE150+Se2	VE150+Se4	VE150+Se6	P
Escherichia coli	6.86 ±0.17^{b}	7.35 ±0,12^{ab}	7.58 ±0.23^{a}	4.94 ±0.17^{d}	6.18 ±0.14^{c}	0.0001
Lactobacillus acidophilus	7.55 ±0.25^{bc}	7.96 ±0.26^{b}	9.17 ±0.42^{a}	6.52 ±0.44^{cd}	6.33 ±0.39^{d}	0.0004

Control (Basal diet, no additives), VE150: Vitamin E (150mg/kg diet), VE150+Nano-Se2: Vitamin E (150mg/kg) +nano-selenium (2mg/kg diet), VE150+Nano-Se4: Vitamin E (150mg/kg) +nano-selenium (4mg/kg diet), VE150+Nano-Se6: Vitamin E (150mg/kg) +Nano-selenium (6mg/kg diet), P: probability. Different superscript letters within a row indicate significant differences (P≤0.01).

DISCUSSION

The absence of significant effects of VE and Nano-Se supplementation on growth performance parameters suggests that the basal diet may have already provided sufficient antioxidant and nutrient levels to support normal growth. These findings are consistent with studies reporting that antioxidants primarily improve oxidative balance rather than growth efficiency in nutritionally adequate broiler diets (Flachowsky, 2003; Surai, 2020). However, the present findings disagree with reports demonstrating enhanced weight gain following Nano-Se supplementation (Abd Allah *et al.,* 2020). Such discrepancies may be related to differences in selenium dosage, bird strain, environmental conditions, or the antioxidant status of the basal diet (Surai and Kochish, 2019). The lack of growth response in the present study may also indicate that higher selenium inclusion levels could exert mild pro-oxidant effects, thereby limiting productive performance. The lack of significant effects on carcass traits suggests that VE and Nano-Se supplementation did not markedly influence carcass development under the current experimental conditions. Similar findings have been reported previously, indicating that antioxidant supplementation may improve physiological and oxidative status without necessarily altering carcass yield or organ weights (Pečjak *et al.,* 2022).

The improvements observed in villus morphology and crypt depth indicate that VE and Nano-Se positively influenced intestinal health and absorptive capacity. Increased villus height and width are generally associated with enhanced nutrient digestion and absorption, whereas increased crypt depth reflects active intestinal tissue renewal (Chen *et al.,* 2019). The beneficial effects of VE on intestinal morphology agree with previous reports emphasizing its role in maintaining intestinal integrity and reducing oxidative damage (Zia ur Rehman *et al.,* 2018). Nano-Se may have enhanced intestinal development through its involvement in selenoprotein synthesis and antioxidant defense mechanisms (Chen *et al.,* 2024). However, the reduced duodenal villus dimensions observed at the highest Nano-Se level suggest that excessive supplementation may negatively affect intestinal tissue structure. These findings indicate that moderate Nano-Se supplementation levels may be more beneficial than excessive inclusion. Mechanistically, improved villus increases the absorptive surface area available for nutrient transport, while deeper crypts reflect enhanced epithelial cell proliferation and turnover. Because selenium is incorporated into selenoproteins involved in redox regulation and vitamin E protects membrane phospholipids from oxidative injury, their combined action likely preserves epithelial integrity and accelerates intestinal renewal, thereby improving nutrient utilization.

The reduction in PV, TBA, and FFA values in supplemented groups demonstrates the effectiveness of VE and Nano-Se in preventing lipid oxidation and improving meat oxidative stability. Vitamin E acts as a lipid-soluble antioxidant that protects cellular membranes against peroxidation, while selenium enhances the activity of glutathione peroxidase, an important antioxidant enzyme (Chen *et al.,* 2019). The synergistic interaction between selenium and VE likely contributed to the improved oxidative stability observed in the present study. This response is biologically plausible because vitamin E interrupts lipid peroxidation chain reactions within cellular membranes, whereas selenium functions through glutathione peroxidase and other selenoproteins to eliminate hydrogen peroxide and lipid hydroperoxides. Together, these complementary antioxidant pathways reduce oxidative damage to muscle lipids and proteins, thereby improving meat stability during storage. This response is biologically plausible because vitamin E interrupts lipid peroxidation chain reactions within cellular membranes, whereas selenium functions through glutathione peroxidase and other selenoproteins to eliminate hydrogen peroxide and lipid hydroperoxides. Together, these complementary antioxidant pathways reduce oxidative damage to muscle lipids and proteins, thereby improving meat stability during storage.The protective effects against oxidation after freezing further confirm the important role of dietary antioxidants in maintaining meat quality during storage (Pečjak *et al.,* 2022).

The improvements in WHC, crude protein, fat content, and reduced cooking and drip losses indicate that combined VE and Nano-Se supplementation enhanced meat quality characteristics (Habibian *et al.,* 2016). Improved antioxidant status may preserve muscle cell membrane integrity, thereby reducing moisture loss and improving nutrient retention (Choi *et al.,* 2023). Previous studies have similarly reported that selenium and vitamin E improve protein stability, nutrient retention, and meat shelf life through enhanced antioxidant protection (Joksimovic-Todorovic *et al.,* 2012; Xiao *et al.,* 2021).

Although high selenium concentrations have been associated with toxicity and impaired meat quality in some studies, no adverse effects were observed in the present experiment.

The absence of significant changes in serum glucose, cholesterol, AST and ALT concentrations indicates that dietary VE and Nano-Se supplementation did not adversely affect liver function or metabolic status. These findings suggest that the supplementation levels used in this study were physiologically safe and did not disrupt normal metabolic processes. The results also imply that blood biochemical indices may be more strongly influenced by genetics, dietary energy level and management conditions than by antioxidant supplementation alone.

The increased serum concentrations of VE, selenium, and glutathione confirm the enhanced antioxidant status of birds receiving VE and Nano-Se supplementation. The superior response observed in the VE150+Se4 treatment suggests that moderate Nano-Se supplementation may optimize antioxidant defense mechanisms more effectively than higher inclusion levels. The decline observed in the VE150+Se6 treatment may indicate reduced efficiency or potential oxidative imbalance at excessive selenium concentrations. Although molecular markers were not evaluated, the coordinated improvements in intestinal morphology, antioxidant biomarkers, oxidative stability, and gut microbiota support the involvement of selenium-dependent antioxidant pathways and vitamin E-mediated membrane protection. Future studies incorporating selenoprotein expression, antioxidant enzyme activities, and inflammatory signaling pathways are warranted to confirm these underlying mechanisms.

The significant effects on intestinal microbial populations indicate that VE and Nano-Se influenced gut microbial ecology. The increased *Lactobacillus acidophilus* population in the VE150+Se2 treatment may be attributed to improved intestinal health and reduced oxidative stress, creating favorable conditions for beneficial bacteria (Zainudin *et al.,* 2023). The simultaneous increase in *E. coli* populations at lower Nano-Se levels suggests that low selenium concentrations may support general microbial proliferation, whereas higher concentrations exert antibacterial effects through membrane disruption and oxidative stress mechanisms. These findings highlight the dose-dependent antimicrobial properties of Nano-Se and emphasize the importance of optimizing supplementation levels for maintaining intestinal microbial balance. The changes in intestinal microbiota may reflect improved intestinal morphology and reduced oxidative stress, whereas the decline at the highest Nano-Se level suggests dose-dependent antimicrobial effects.

Conclusions: This study demonstrated that dietary supplementation with vitamin E (150 mg/kg) and nano-selenium (2–6 mg/kg) improved intestinal morphology, antioxidant status, meat quality, and gut microbial composition without affecting growth performance. Although 6 mg/kg nano-selenium produced the greatest improvements in lipid oxidation and several meat quality traits, antioxidant biomarkers (vitamin E, selenium, and glutathione) peaked at 4 mg/kg, while higher supplementation reduced beneficial microbial populations. These findings suggest that 4 mg/kg nano-selenium, combined with vitamin E, provides the best overall balance between antioxidant capacity, intestinal health, and physiological responses, whereas 6 mg/kg offers additional improvements in meat quality but with diminished biological advantages.

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REFERENCES

Abd Allah D, Tawfeek M, Rashwan A, *et al.,* 2020. Impact of dietary supplementation with nano and organic selenium without or with vitamin E on growth performance and selenium metabolism in growing rabbits. Journal of Production and Development Research 25:323-342.

Alhidary IA, Rehman ZU, Khan RU, *et al.,* 2017. Anti-aflatoxin activities of milk thistle (*Silybum marianum*) in broiler. Worlds Poultry Science Journal 73:559-566.

Al-Khalaifah H, Naz S, Al-Atiyat R, *et al.,* 2025. Eco-friendly selenium nanoparticles from Capsicum annuum: Impact on growth efficiency, blood biochemistry, immune response, intestinal morphology, and profitability in broiler chickens. Poultry Science 104:105915. doi:10.1016/j.psj.2025.105915.

Alvarado CZ and Sams AR, 2002. The role of carcass chilling rate in the development of pale, exudative turkey pectoralis. Poultry Science 81:1365-1370. doi:10.1093/ps/81.9.1365.

Batool S, Khan MA, Naz S, *et al.,* 2025. Anticoccidial effect of bitter apple (*Citrullus colocynthis*) seed powder and organic selenium nanoparticles in mitigating Eimeria tenella-induced challenge in quails. Journal of Applied Animal Research 53:2526393. doi:10.1080/09712119.2025.2526393.

Chand N, Naz S, Rehman ZU, *et al.,* 2018. Blood biochemical profile of four fast-growing broiler strains under high ambient temperature. Applied Biological Chemistry 61:273-279.

Chen J, Xing Y, Nie M, *et al.,* 2024. Comparative effects of various dietary selenium sources on growth performance, meat quality, essential trace elements content, and antioxidant capacity in broilers. Poultry Science 103:104057. doi:10.1016/j.psj.2024.104057.

Chen C, Wang Z, Li J, *et al.,* 2019. Dietary vitamin E affects small intestinal histomorphology, digestive enzyme activity, and the expression of nutrient transporters by inhibiting proliferation of intestinal epithelial cells within jejunum in weaned piglets. Journal of Animal Science 97:1212-1221.

Choi J, Kong B, Bowker BC, *et al.,* 2023. Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: A review. Animals 13:1386.

Douglas BW, MacLeod JA, Mellish TM, *et al.,* 2000. A method for measuring Prince Edward Island soil quality. Communications in Soil Science and Plant Analysis 31:1837-1845. doi:10.1080/00103620009370541.

Drury R, 1983. Theory and practice of histological techniques. Journal of Clinical Pathology 36:609-–609. doi:10.1136/jcp.36.5.609-d.

Egan H, Kirk RS and Sawyer R, 1981. Pearson's chemical analysis of foods. 8th ed.

Flachowsky G, 2003. Natural antioxidants in avian nutrition and reproduction. Animal Feed Science and Technology 103:187-188. doi:10.1016/S0377-8401(02)00260-2.

Habibian M, Ghazi S and Moeini MM, 2016. Effects of dietary selenium and vitamin E on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. Biological Trace Element Research 169:142-152. doi:10.1007/s12011-015-0404-6.

Hafeez A, Shah SAA, Khan RU, *et al.,* 2020. Effect of diet supplemented with phytogenics and protease enzyme on performance, serum biochemistry and muscle histomorphology in broilers. Journal of Applied Animal Research 48:326-330.

Hafeez A, Ullah Z, Khan RU, *et al.,* 2020. Effect of diet supplemented with essential coconut oil on performance and intestinal injury in broiler exposed to avian coccidiosis. Tropical Animal Health Production 52:2499-2504. doi:10.1007/s11250-020-02279-6.

Haseeb A, Naz S, Satti S, *et al*., 2025. Comparative impact of selenium sources and doses on sexual behaviour, productivity and gonadal bioaccumulation in *Coturnix coturnix* japonica. Journal of Applied Animal Research 53:2475919. doi:10.1080/09712119.2025.2475919.

Honikel KO and Hamm R, 1994. Measurement of water-holding capacity and juiciness. In: Quality attributes and their measurement in meat, poultry and fish products. p. 125–161. doi:10.1007/978-1-4615-2167-9_5.

Jabbar A, Tahir M, Alhidary MA, *et al*., 2021. Impact of microbial protease enzyme and dietary crude protein levels on growth and nutrients digestibility in broilers over 15 to 28 days. Animals 11:2499. doi:10.3390/ani11092499.

Joksimovic-Todorovic M, Davidovic V and Sretenovic L, 2012. The effect of diet selenium supplement on meat quality. Biotechnology of Animal Husbandry 28:553-561. doi:10.2298/BAH1203553J.

Khan RU, Al-Khalaifah H, Usama M, *et al*., 2025. Effect of dietary supplementation of biosynthesized nano-selenium particles on growth, blood indices, antioxidant status, immune response and histological features of intestine in broilers. Food and Agriculture Immunology 36:2529315. doi:10.1080/09540105.2025.2529315.

Khan RU, Naz S, Nikousefat Z, *et al*., 2011. Effect of vitamin E in heat-stressed poultry. Worlds Poultry Science Journal 67:469-478.

Khan RU, Rahman ZU, Javed I, *et al*., 2012. Effect of vitamins, probiotics and protein on semen traits in post-molt male broiler breeders. Animal Reproduction Science 135:85-90.

Mahmood Q, Shaheen S and Azeem M, 2024. Nanobiosensors: Application in healthcare, environmental monitoring and food safety. Asian Journal of Agricultural Biology 2024:2023157. doi:10.35495/ajab.2023.157.

Pearce BC, Parker RA, Deason ME, *et al*., 1992. Hypocholesterolemic activity of synthetic and natural tocotrienols. Journal of Medical Chemistry 35:3595-3606. doi:10.1021/jm00098a002.

Pečjak M, Leskovec J, Levart A, *et al*., 2022. Effects of dietary vitamin E, vitamin C, selenium and their combination on carcass characteristics, oxidative stability and breast meat quality of broiler chickens exposed to cyclic heat stress. Animals 12:1789.

Pesenti A, Pelizzola A, Mascheroni D, *et al*., 1981. Low frequency positive pressure ventilation with extracorporeal CO2 removal (LEPPV-ECCO2R) in acute respiratory failure (ARF): Technique. Transactions of American Society for Artificial Internal Organs 27:263-266.

Rehman ZU, Che L, Ren S, *et al*., 2018. Supplementation of vitamin E protects chickens from Newcastle disease virus-mediated exacerbation of intestinal oxidative stress and tissue damage. Cellular and Physiological Biochemistry 47:1655-1666. doi:10.1159/000490984.

Reynolds HL, 1989. Association of official analytical chemists: 1964–1988. Reviews in Environmental Contamination and Toxicology 109:109-136. doi:10.1007/978-1-4684-7086-4_3.

Shami A, Abdallah M, Alruways MW, *et al*., 2024. Comparative prevalence of virulence genes and antibiotic resistance in *Campylobacter jejuni* isolated from broilers, laying hens and farmers. Pakistan Veterinary Journal 44:200-204. doi:10.29261/pakvetj/2024.133.

Surai PF, 2020. Antioxidants in poultry nutrition and reproduction: An update. Antioxidants 9:105. doi:10.3390/antiox9020105.

Surai PF, Kochish II, 2019. Nutritional modulation of the antioxidant capacities in poultry: The case of selenium. Poultry Science 98:4231-4239.

Witte VC, Krause GF and Bailey ME, 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of Food Science 35:582-585. doi:10.1111/j.1365-2621.1970.tb04815.x.

Xiao J, Khan MZ, Ma Y, *et al*., 2021. The antioxidant properties of selenium and vitamin E; their role in periparturient dairy cattle health regulation. Antioxidants 10:1555. doi:10.3390/antiox10101555.

Zainudin NN, Hemly NIM, Muhammad AI, *et al*., 2023. Effects of supplementing organic- and inorganic-based selenium with vitamin E on intestinal histomorphology, caecal bacterial proliferation, and short-chain fatty acid profile in layer hens. Tropical Animal Health and Production 55:90.

Zia ur Rehman, Chand N, Khan RU, *et al*, 2018. Serum biochemical profile of two broiler strains supplemented with vitamin E, raw ginger (*Zingiber officinale*) and L-carnitine under high ambient temperatures. South African Journal of Animal Science 48:935-942.