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Effects of Supplementing Vitamin E and Nanoparticle-Sized Vitamin E on Growth Performance, Blood Profile, and Meat Quality in Broilers

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ABSTRACT This study was conducted to investigate the effect of vitamin E and nanoparticle-sized vitamin E (NVE) in a broiler diet on growth performance, blood profiles, and meat quality. A total of 45 one-day-old Arbor Acres broilers (initial body weight of 37.00 ± 0.41 g) were used in this experiment for 28 days. All broilers were randomly allocated into three dietary treatments in a randomized complete block design. The dietary treatments were as follows: a basal diet (CON), a basal diet supplementing with 380 mg/kg of vitamin E (T1), and a basal diet supplementing with 380 mg/kg of NVE (T2). Each treatment had five replicates with three birds per cage. On days 0-7, the T1 and T2 groups significantly increased (P<0.05) body weight gain compared to the CON group. Also, the T1 and T2 groups significantly increased (P<0.05) vitamin E content in blood and breast meat compared to the CON group. In shearing force, the T2 group showed a lower tendency (P=0.070) than the CON group. The T1 group showed a higher tendency (P=0.086) in the b* (yellowness) value than the T2 group. On day 7 after the end of the experiment, the T2 group significantly decreased (P<0.05) TBA values compared to the CON group. In conclusion, supplementation with vitamin E or NVE can improve broiler growth performance in the starter period, reduce TBA value through the antioxidant action of vitamin E, prevent lipid oxidation, and improve shelf life.

(Key words: broiler, nanoparticle-sized vitamin E, vitamin E)

INTRODUCTION

Vitamin E can protect cell membranes and tissues from lipid peroxidation damage induced by free radicals and reduce lipid peroxidation (Traber and Stevens, 2011; Pompeu et al., 2018). In broilers, lipid oxidation is one of the main causes of meat quality degradation due to off-flavor production, reduction of polyunsaturated fatty acids, and formation of toxic compounds such as peroxides and aldehydes (Morrissey et al., 1994). Vitamin E can act as an antioxidant (Rey et al., 2015). Supplementing vitamin E in a broiler diet can inhibit lipid oxidation in broiler muscles and prevent chicken meat from rancidity by increasing α-tocopherol levels in the diet (Ruiz et al., 2001). According to Mazur-Kuśnirek et al. (2019), the addition of 200 mg/kg of vitamin E in broiler diets can result in a higher breast muscle content in the carcass, improve meat water holding capacity (WHC), and reduce drip loss (DL), thus improving meat quality. Vitamin E can also strengthen cellular and humoral immune functions with a positive effect on the immune system of broilers, which can improve the growth performance of broilers (Rizvi et al., 2014; Pompeu et al., 2018).

Nanoparticle-sized materials exhibit a large surface area and high catalytic activity, resulting in increased absorption in the body (Vijayakumar and Balakrishnan, 2014). Nanoparticle-sized vitamin E (NVE) has the advantage of maximizing the effectiveness of vitamin E by increasing its bioavailability.

However, there are not many studies to support that NVE can maximize the effect of vitamin E. Also, there are no studies comparing the effects of different vitamin E types in broilers. Therefore, the objective of this study was to investigate the effects of vitamin E and NVE in a broiler diet on growth performance, blood profiles, and meat quality.

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MATERIALS AND METHODS

1. Ethics Approval and Consent to Participate

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-2010-22-01).

2. Animals and Experimental Design

A total of 45 one-day-old Arbor Acres broilers (initial body weight of 37.00±0.41 g) were obtained from a local hatchery (Cherrybro Co., Eumseong, Korea) and used in this experiment for 28 days. All broilers were randomly allocated into three dietary treatments in a randomized complete block design. The dietary treatments were as follows: a basal diet (CON), a basal diet supplementing with 380 mg/kg of vitamin E (T1), and a basal diet supplementing with 380 mg/kg of NVE (T2). The vitamin E content in the basal diet was 37.5 mg/kg as the vitamin E content in the vitamin premix included in the diet. Each treatment had five replicates with three birds per cage. Each cage was 100 cm in width, 40 cm in depth, and 45 cm in height. The experiment initiation temperature was 33±1℃, and after that, the temperature was gradually lowered to maintain 25±1℃. All diets were formulated to meet or exceed National Research Council (NRC, 1994; Table 1) for the starter (1-7 d), grower (8-21 d), and finisher (22-28 d) periods. All broilers were given *ad libitum* access to diet and water throughout the experiments.

ANALYSIS ITEM AND MEASUREMENTS

1. Growth Performance

All broilers were weighed at days 0, 7, 21, and 28 for assessing body weight gain (BWG). Feed intake (FI) was calculated by subtracting the remaining amount from the diet supply amount when measuring weight. The feed conversion ratio (FCR) was calculated by dividing FI by BWG.

2. Blood Profile

Blood samples were collected from the brachial wing vein into a sterile syringe using a serum separate tube on day 28 (5 broilers/treatment). After collection, the blood tubes were wrapped with aluminum foil to avoid light and centrifuged at 12,500 × g at 4℃ for 20 min. Vitamin E content in serum was analyzed using a high-performance liquid chromatography (HPLC) method.

3. Meat Quality

The broilers were slaughtered, and the breast meat was collected on day 28 (5 broilers/treatment). Moisture, fat, and ash contents among general components were analyzed according to the AOAC (2007) method. Vitamin E content in breast meat was analyzed using the HPLC method. The pH was measured with a pH meter (Thermo Orion 535A, Thermo, IL, USA) after adding 100 mL of distilled water to 10 g of breast meat and then homogenizing at 68,400 × g for 30 seconds using a homogenizer (Bihon seiki, Ace, Osaka, Japan). To analyze the cooking loss (CL), breast meat with a thickness of 3 cm was shaped into a circle, immersed in a 70℃-water bath, and cooled for 30 minutes. Then, the weight ratio (%) of the initial sample was measured. The DL was calculated as the weight ratio (%) of the initial sample by measuring the loss generated after 2 cm-thick breast meat was cut into a circle, vacuum-packed in a polypropylene bag, and stored at 4℃ for 24 hours. The WHC was analyzed according to Laakkonen et al. (1970) method. Shearing force was analyzed through a shear force cutting test using a rheometer (Compac-100, Sun Scientific co., Tokyo, Japan). Meat color was measured using a spectro colorimeter (Model JX-777, Color Techno. System Co., Tokyo, Japan) standardized with a white plate (L*, lightness 94.04; a*, redness 0.13; b*, yellowness -0.51).

4. Meat Storage Characteristics

2-Thiobarbituric (TBA) values were analyzed using the modified method of Witte et al. (1970). The 10 g of breast meat sample was homogenized with 15 mL of 10% PCA solution and 25 mL distilled water using a homogenizer (Bihon seiki, Ace, Osaka, Japan). After homogenization, the entire eluate was transferred to Whatman No. 2 filter paper using ø 150 nm filter paper. The filtrate and the TBA solution were transferred to numbered tubes (5 mL each) and

Table 1. Ingredient composition of basal diets[1]

Items (%)	Starter (1-7 d)	Grower (8-21 d)	Finisher (22-28 d)
Corn	40.645	47.357	53.292
Soybean meal (CP 45%)	33.263	26.750	23.675
Wheat	10.000	10.000	10.000
DDGS 28%	4.000	5.000	3.000
Tankage ML-60	3.000	3.000	1.900
MBM 50%	1.800	1.700	1.700
Wheat flour	2.000	2.000	2.000
Poultry oil	2.633	1.700	2.108
L-Lysine-SO_4	0.501	0.510	0.392
DL-Methionine	0.418	0.367	0.411
L-Threonine	0.141	0.141	0.105
L-Tryptophan	0.010	0.010	0.100
Salt	0.224	0.250	0.234
Limestone	0.445	0.450	0.438
Mono-dicalcium phosphate	0.435	0.300	0.200
Mineral premix[2]	0.220	0.220	0.220
Vitamin premix[3]	0.150	0.130	0.130
Choline	0.100	0.100	0.080
Phytase1000	0.010	0.010	0.010
Xylanase	0.005	0.005	0.005
Total	100.00	100.00	100.00
Calculated value (%)			
ME (kcal/kg)	3,000	3,020	3,100
CP (%)	22.000	20.500	19.000
Available lysine	1.310	1.170	1.010
Available methionine	0.570	0.540	0.560
Available threonine	0.850	0.770	0.680
Available tryptophan	0.210	0.190	0.160
Available SAA	0.990	0.900	0.900
Arginine	1.510	1.350	1.160
Iso-leucine	0.970	0.880	0.780
Valine	1.120	1.010	0.890
Calcium	0.850	0.800	0.700
Available P	0.480	0.450	0.400
Na	0.150	0.150	0.150

[1] DDGS, dried distiller's grains with solubles; MBM, meat and bone meal; ME, metabolizable energy; CP, crude protein; SAA, sulphur-containing amino acid.

[2] Provided per kg of diet: 37.5 mg Zn (as $ZnSO_4$), 37.5 mg of Mn (MnO_2), 37.5 mg of Fe (as $FeSO_4 \cdot 7H_2O$), 3.75 mg of Cu (as $CuSO_4 \cdot 5H_2O$), 0.83 mg of I (as KI), and 0.23 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).

[3] Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 24 μg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of pantothenic acid.

mixed using a vortex mixer. A mixture of 5 mL of distilled water and 0.02 M TBA solution was used as a blank test tool. After sealing the surface with parafilm and incubating it in a cool dark place for 16 hours, absorbance was measured at 529 nm using a spectrophotometer (Model JX-777, Color Techno. System Co., Tokyo, Japan). The volatile basic nitrogen (VBN) value was measured by the Conway (1950) method. The 3 g of breast meat samples were homogenized with 3 mL of distilled water and 6 mL of 10% TCA solution for 1 minute and then centrifuged at 2,090 × g for 15 minutes. After filtering the supernatant using Whatman No. 4 filter paper, the filtrate was put into a test tube and the final volume was made to 30 mL with 5% TCA solution. 0.01 N boric acid as a VBN absorbent was placed in the inner section of a Conway micro-diffusion cell (Sibata Ltd. Tokyo, Japan), and 1 mL sample solution and 1 mL saturated K_2CO_3 were placed in the outer section of the same cell. A 5% TCA solution was used as a blank test tool. The cell was incubated for 120 minutes at 37℃ and then titrated against 0.02 N sulfuric acid. The VBN concentration was calculated using the following equation: VBN value = [0.28 × (titration volume of sample solution – titration volume of blank) × 10] × 100.

5. Statistical Analysis

All data were statistically processed using the GLM procedures of SAS (SAS Institute, Cary, NC, USA), using each pen as the experimental unit. Differences among all treatment means were determined using Duncan's multiple-range test. A probability level of $P<0.05$ was indicated to be statistically significant, and a level of $0.05 \leq P<0.10$ was considered to have such a tendency.

RESULTS

1. Growth Performance

On day 7, the T1 and T2 groups showed significantly higher ($P<0.05$) BW than the CON group (Table 2). On days 0-7, the T1 and T2 groups significantly increased ($P<0.05$) BWG compared to the CON group.

2. Blood Profile

The T1 and T2 groups significantly increased ($P<0.05$) vitamin E content in blood compared to the CON group

Table 2. Effect of supplementing vitamin E and nanoparticle-sized vitamin E on growth performance in broilers[1]

Items	CON	T1	T2	SE	*P*-value
BW (g)					
d 0	37.00	37.25	36.87	0.163	0.280
d 7	138.05[b]	144.39[a]	148.14[a]	1.731	0.005
d 21	750.67	788.67	774.00	28.248	0.642
d 28	1,341.33	1,388.40	1,370.00	30.654	0.565
d 0 to 7					
BWG (g)	101.05[b]	107.15[a]	111.28[a]	1.707	0.004
FI (g)	117.74	115.94	127.84	4.469	0.170
FCR	1.17	1.08	1.15	0.053	0.715
d 7 to 21					
BWG (g)	612.62	644.27	625.86	28.691	0.741
FI (g)	869.29	874.00	855.33	7.539	0.231
FCR	1.42	1.36	1.37	0.065	0.752
d 21 to 28					

Table 2. Continued

Items	CON	T1	T2	SE	*P*-value
BWG (g)	590.67	599.73	596.00	38.803	0.986
FI (g)	937.50	949.00	961.44	8.256	0.165
FCR	1.59	1.58	1.61	0.121	0.932
d 0 to 28					
BWG (g)	1,304.33	1,351.15	1,333.13	30.646	0.568
FI (g)	1,924.53	1,938.94	1,944.61	14.968	0.631
FCR	1.48	1.44	1.46	0.036	0.872

[1] CON, basal diet; T1, basal diet+380 mg/kg of vitamin E; T2, basal diet+380 mg/kg of nanoparticle-sized vitamin E; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; SE, standard error.
Each value is the mean value of 5 replicates.
[a,b] Means within column with different superscripts differ significantly ($P<0.05$).

(Table 3).

3. Meat Quality

There were no significant differences ($P>0.05$) in breast meat's moisture, ash, and fat content among treatment groups (Table 4). The T1 and T2 groups showed significantly higher ($P<0.05$) vitamin E content in breast meat than the CON group. In shearing force, the T2 group showed a lower tendency ($P=0.070$) than the CON group. The T1 group showed a higher tendency ($P=0.086$) in the b* value than the T2 group. There were no significant differences ($P>0.05$) among treatment groups in pH, CL, DL, WHC, L*, and a* value.

4. Meat Storage Characteristics

On day 0, TBA and VBN showed no significant difference ($P>0.05$) among treatment groups (Table 5). On day 7, the T2 group significantly decreased ($P<0.05$) TBA values compared to the CON group.

DISCUSSION

In our study, supplementing vitamin E or NVE significantly improved broiler BW and BWG on day 7 compared to basal diet feeding. According to Rebolé et al. (2006), the addition of 200 mg/kg of α-tocopheryl acetate to a diet did not affect broiler FI, although it increased BWG and feed efficiency. Calik et al. (2022) have also reported that supplementation of vitamin E could improve BWG and FI in broilers under heat-stress conditions. Vitamin E affects both humoral and cell-mediated immune responses in broilers and has immunomodulatory effects by modulating cyclooxygenase and lipoxygenase pathways (Leshchinsky and Klasing, 2003; Liu et al., 2014).

Supplementation of vitamin E in a diet could also positively modulate the immune function of poultry, including innate cellular oxidative immunity (Perez-Carbajal et al., 2010). In our study, it seemed that the immunity of broilers was improved during the starter period through

Table 3. Effect of supplementing vitamin E and nanoparticle-sized vitamin E on vitamin E content in blood in broilers[1]

Items	CON	T1	T2	SE	*P*-value
Vitamin E (mg/L)	15.19[b]	66.09[a]	62.65[a]	1.264	<0.001

[1] CON, basal diet; T1, basal diet+380 mg/kg of vitamin E; T2, basal diet+380 mg/kg of nanoparticle-sized vitamin E; SE, standard error.
Each value is the mean value of 5 replicates.
[a,b] Means within column with different superscripts differ significantly ($P<0.05$).

Table 4. Effect of supplementing vitamin E and nanoparticle-sized vitamin E on breast meat quality in broilers[1]

Items	CON	T1	T2	SE	*P*-value
Content					
Moisture (%)	83.14	83.70	83.83	0.573	0.669
Ash (%)	0.75	0.96	0.84	0.085	0.276
Fat (%)	1.01	0.93	1.10	0.087	0.407
Vitamin E (mg/kg)	7.35[b]	9.69[a]	9.38[a]	0.186	<0.001
Characteristic					
pH	5.85	5.86	5.86	0.043	0.981
CL (%)	12.32	12.06	13.93	0.603	0.100
DL (%)	4.49	5.54	4.42	0.540	0.374
WHC (%)	54.00	53.79	61.65	2.713	0.106
Shearing force (g/g)	2,489.00	2,243.00	2,009.00	117.776	0.070
Hunter color					
L^*	45.26	48.23	46.19	1.389	0.336
a^*	4.51	4.45	4.07	0.411	0.722
b^*	11.16	11.66	10.46	0.348	0.086

[1] CON, basal diet; T1, basal diet+380 mg/kg of vitamin E; T2, basal diet+380 mg/kg of nanoparticle-sized vitamin E; WHC, water holding capacity; CL, cooking loss; DL, drip loss; L^*, lightness; a^*, redness; b^*, yellowness; SE, standard error.
Each value is the mean value of 5 replicates.
[a,b] Means within column with different superscripts differ significantly ($P<0.05$).

Table 5. Effect of supplementing vitamin E and nanoparticle-sized vitamin E on meat storage in broilers[1]

Items	CON	T1	T2	SE	*P*-value
d 0					
TBA (mg MDA/kg)	0.18	0.15	0.17	0.009	0.272
VBN (mg%)	14.53	14.39	13.95	0.235	0.231
d 7					
TBA (mg MDA/kg)	0.27[a]	0.24[ab]	0.21[b]	0.011	0.004
VBN (mg%)	20.42	19.68	19.26	0.374	0.125

[1] CON, basal diet; T1, basal diet+380 mg/kg of vitamin E; T2, basal diet+380 mg/kg of nanoparticle-sized vitamin E; TBA, thiobarbituric acid; MDA, malondialdehyde; VBN, total volatile basic nitrogen; SE, standard error.
Each value is the mean value of 5 replicates.
[a,b] Means within column with different superscripts differ significantly ($P<0.05$).

supplementation of vitamin E or NVE, resulting in increased BW and improved BWG. However, there was no significant difference in BWG, FI, or FCR among treatment groups during the entire period in our study. The results of this study are consistent with those of Cheng et al. (2016), suggesting that additional vitamin E supplementation does not affect growth performance since vitamin E requirements in a basal diet for broilers are already met.

In the present study, vitamin E content in blood was significantly increased when vitamin E or NVE was supplemented compared to that in the basal diet. It seemed that supplementation of vitamin E in the diet increased vitamin E accumulation in broiler blood. According to Surai (1999), the accumulation of vitamin E from diet occurs

simultaneously in the blood, liver, and kidneys, and is involved in antioxidant activity throughout the reproductive process. Vitamin E can reduce the harmful effects of oxygen on cell membranes, increase cell membrane stability, and prevents oxidation of unsaturated fatty acids (Bast et al., 1991; Arslan et al., 2001).

In our study, vitamin E or NVE supplementation significantly improved vitamin E content in broiler breast meat. Previous studies have reported that the addition of vitamin E to a diet can increase vitamin E content in broiler meat (Habibian et al., 2016; Albergamo et al., 2022). Selenoprotein is present in almost all broiler tissues including the digestive tract mucosa. It can enhance the action of vitamin E and by reducing the degree of oxidation of vitamin E by providing a primary barrier to the absorption of ingested hydroperoxide (Skřivan et al., 2008). For this reason, vitamin E content in breast meat was increased when vitamin E or NVE was added in this study. To predict meat quality, pH, DL, CL, WHC, and meat color are commonly used factors. Shearing force is known to indicate the degree of toughness of cooked meat (Hashizawa et al., 2013). Supplementation of vitamin E in broiler diets can improve resistance to pale, soft, and exuding PSE meat (Petracci and Cavani, 2012). It has been reported that supplementation of vitamin E can improve cellular integrity, reduce lipid oxidation, decrease pigment oxidation, and positively affect WHC (Jensen et al., 1998; Choct and Naylor, 2004). Muscle antioxidant levels play an important role in determining color stability during slaughter (Ponnampalam et al., 2012). According to previous studies (Khatun et al., 2020; Vieira et al., 2021), supplementation of vitamin E in a diet can improve a* value and prevent discoloration of meat by reducing lipid oxidation, which can indirectly retard myoglobin oxidation and reduce cellular damage caused by the oxidative process. However, in our study, supplementation of vitamin E in the diet did not show a significant effect on pH, DL, CL, or WHC. Supplementation of NVE showed a tendency to reduce shearing force. Supplementation of vitamin E in the diet also tended to result in higher b* values than supplementation of NVE. Similarly, previous studies have shown that vitamin E supplementation in a diet does not show a significant effect on broiler meat pH or DL (Vieira et al., 2021; Pečjak et al., 2022). Niu et al. (2018) have reported that vitamin E supplementation at 100 mg/kg in a broiler diet can significantly reduce shearing force. However, most studies have reported that vitamin E supplementation does not affect shear force (Choi et al., 2010; Hu et al., 2015; Zdanowska-Sasiadek et al., 2016). For meat color, when 200 mg/kg of vitamin E was supplemented into the diet, L* and b* values were significantly decreased while a* values were significantly increased (Zhang et al., 2013). On the other hand, there is also a study that supplementation of vitamin E in the diet does not affect L*, a*, or b* values (Leonel et al., 2007; Zhang et al., 2011). As such, the results of previous studies are inconsistent. The effects of NVE on broiler meat quality characteristics have not been elucidated yet. Thus, additional study is needed.

The TBA assay is one of the efficient methods for measuring the antioxidant activity of meat. This assay is also an indicator of malondialdehyde, an oxidation product (Yesilbag et al., 2011). Therefore, the TBA value increases as the storage period pass. In our study, supplementation of NVE to broiler diets significantly reduced TBA values at day 7. It has been reported that vitamin E supplementation in a broiler diet can reduce lipid oxidation in meat by protecting polyunsaturated fatty acids in meat cell membranes from oxidation, thereby increasing the shelf life of meat (De Winne and Dirinck, 1997; Barroeta, 2007). Imik et al. (2012) have reported that vitamin E supplementation in broilers exposed to heat stress can reduce lipid oxidation and meat TBA values. Results of the present study indicate that NVE could reduce lipid oxidation in broilers, decrease TBA values, and increase shelf life. The VBN value is an indicator of spoilage. It is increased by proteolysis carried out by microorganisms and enzymes in the meat (Jung et al., 2010). In the present study, VBN values increased over time. However, supplementation of vitamin E or NVE numerically showed a VBN value within 20 mg%, which was the range of fresh meat presented in the Korea Food Code (2018). This showed that protein deterioration could be suppressed by supplementation with vitamin E or NVE compared to that in control without such supplementation.

SUMMARY

Supplementation of vitamin E or NVE in broiler diets improved broiler BW and BWG in the starter period. Also, vitamin E content in blood and breast meat was significantly increased. Meat quality characteristics did not show significant differences among the treatment groups. However, it was shown that the TBA values were significantly reduced in the storage characteristics, and the antioxidant action of vitamin E could prevent lipid oxidation and increase the shelf life when vitamin E or NVE supplementation in the diet.

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