Dietary vitamin E improves meat quality and antioxidant capacity in broilers by upregulating the expression of antioxidant enzyme genes

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ABSTRACT

A total of 240 one-day-old broiler chicks were divided into 3 treatments with 4 replicates of 20 birds. The birds were fed a corn–soybean meal diet supplemented with 0, 100, and 200 mg/kg vitamin E (VE), respectively. The results indicated that VE supplementation led to a significant decrease in shear force ($P < .05$), and showed higher $b^*$ values and lower $a^*$ values ($P < .05$). Dietary supplementation with VE significantly increased VE concentration in serum compared to that in the control group ($P < .05$). Total antioxidant capacity activity in breast muscle or serum was increased with the increase in dietary VE supplementation in a dose-dependent manner ($P < .05$). Total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activity were linearly increased with the increase in dietary VE ($P < .05$), while malondialdehyde content decreased linearly ($P < .05$). The mRNA expression of SOD and GSH-Px in liver was linearly increased with the increase in dietary VE ($P < .05$). These findings suggest that dietary VE could increase meat quality by upregulating the expression of antioxidant enzyme genes in broilers.

1. Introduction

In the recent decades, the broiler industry has rapidly grown and the global consumption of broiler chickens currently ranks the second following pork, as these are considered the major sources of the most important protein for human beings. Due to its higher level of unsaturated fatty acids, poultry meat is susceptible to lipid oxidation, resulting in meat spoilage and adverse changes in the flavour and texture of meat. During the meat marketing process, lipid stability, colour, and water capacity are known to be the major quality properties influencing the desirability of the product (Cannon et al. 1996). Vitamin E acts as a bio-antioxidant in the membranes of cells and sub-cellular organelles (Machlin & Bendich 1987). Studies have shown that higher allowance of VE had beneficial effects in poultry (Mcllroy et al. 1993; Haq et al. 1996; Khan et al. 2016; Shah et al. 2016). Similarly, the addition of α-tocopherol (VE) to animal diets is an effective means of improving the oxidative stability of meat, in addition to improving flavour (De Winne & Dirinck 1996; Sheldon et al. 1997).

2. Materials and methods

2.1. Treatments

Three dietary treatments that differed in the levels of VE supplementation were used in the present study; the diets were primarily based on corn and soybean meal and were formulated to meet or exceed National Research Council (1994) nutrient recommendations. The diets were offered in two feeding phases, starter and grower, from 0 to 21 d and 22 to 42 d of age. Table 1 displays the ingredients and nutrient composition of the broilers. The three treatments were supplemented with 0, 100, and 200 mg/kg VE, respectively. The basal $V_t$ level in the diet was 17 and 26 mg/kg, respectively, according to the actual determination of the dietary ingredients. The VE supplement used in the present study was α-tocopherol acetate ($ω = 50%$) (Vitamin Pharmaceuticals Co., Ltd, Shanghai, China). The present study did not provide any additives containing VE during the following rearing process.

2.2. Bird husbandry

This experiment was conducted according to protocols approved by the Northwest A&F University Animal Care and Use Committee. A total of 240 one-day-old avian broiler chicks were obtained from a commercial hatchery and distributed equally across 24 pens, with each treatment consisting of four replicates of 20 broilers each. The birds were provided food and water ad libitum. The photoperiod was set at 24 L throughout the whole experimental period. The birds were vaccinated with the Newcastle disease-infective bronchitis (ND-IB) combined vaccine by eye and nose dripping at 7 d. When the birds reached 14- and 19-day-old, they were vaccinated by drinking water with the Infectious Bursal Disease Vaccine, Live (Strain B87), respectively. When the birds reached 26-day-old, they were vaccinated by drinking water with the ND-IB combined inactive vaccine.

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Table 1. Composition of basal diets.

| Ingredient, % | Starter phase (0–21 d) | Grower phase (22–42 d) |
|--------------|-------------------------|------------------------|
| Corn         | 60.87                   | 65.97                  |
| Soybean meal | 30.01                   | 24.28                  |
| Corn oil     | 0.91                    | 1.40                   |
| Corn gluten meal | 3.00            | 4.00                   |
| Dicalcium phosphate | 2.01   | 1.39                   |
| Limestone     | 1.25                    | 1.30                   |
| Salt          | 0.38                    | 0.33                   |
| Choline       | 0.10                    | 0.18                   |
| Lys           | 0.19                    | 0.10                   |
| Met           | 0.29                    | 0.05                   |
| Premixa       | 1.00                    | 1.00                   |
| Total         | 100                     | 100                    |

Nutrient analysis, %

ME (MJ/kg) | 12.20 | 12.60 |
CP          | 20.00 | 18.60 |
Calcium     | 1.00  | 0.90  |
Non-phytate phosphorus | 0.45  | 0.35  |
Lysine       | 1.10  | 0.90  |
Methionine   | 0.59  | 0.35  |
Met + Cys    | 0.90  | 0.86  |

*Provided per kilogram of diet: Vitamin A 11000 IU, Vitamin D3 3740 IU, Vitamin K3 5.1 mg, Vitamin B1 2.2 mg, Vitamin B2 6.6 mg, Calcium pantothenate 13.5 mg, Nicotinic acid 44 mg, Folic acid 1.1 mg, Biotin 0.2 mg, Mn 108 mg, Fe 100 mg, Zn 88 mg, Cu 9.6 mg, I 0.3 mg, Se 0.23 mg.

2.3. Sample preparation

At 42 d of age, six individuals from each of the three treatments were selected, venous blood samples were collected, the serum immediately separated, sub-packaged, and then stored at −20°C for further determination. After sacrificing the broilers, both sides of the breast muscles were stripped, packaged, and divided into three parts randomly; the first part was stored at −20°C for determining antioxidant enzyme (AOE) activity, the second part was stored at 4°C for determining the shelf life, and the third part was used for determining the meat quality and measured as soon as possible. Liver samples were stored at −80°C for the determination of AOE gene expression.

2.4. Meat quality parameters

Parameters for the breast meat quality were measured, including water-loss rate, shear force, pH value, and colour (L*, a*, b*). Water-loss rate was measured as pressurization within 2 h after slaughter. Shear force was measured three times using a C-LMS digital shear force tester every 1 h and the means were calculated. Meat pH was measured after 1 h using a pH metre (Nova-Tech International, Inc, Houston, USA). Colour measurements were carried out using a colorimeter within 2 h. The Lab system provides the values of three colour components: L*(black–white component, lightness), and the chromaticity coordinates a*(red to green component) and b*(yellow to blue component).

2.5. Biochemical analyses

Minced meat was mixed with physiological saline (1:9) precooled at 4°C and prepared as a tissue homogenate. Then the supernatant was collected for measuring AOEAs [total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px)] activity and T-AOC (total antioxidant capacity) using the test kit purchased from Nanjing Jiancheng Bioengineering Institute following the instructions of the test kit, and for VE determination after centrifugation by high performance liquid chromatography according to the method described by McGeachin and Bailey (1995).

To assess the amount of lipid oxidation, the thiobarbituric acid reactive substances (TBARS) level was determined using the thiobarbituric acid method. Results were expressed as mmol of malondialdehyde (MDA) equivalents/mg protein of fresh meat. The concentration of MDA in the breast muscle was determined at the zeroth, second, fourth, sixth, and eighth days after storing at 4°C by using the test kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) following the instructions of the test kit.

2.6. Determination of AOE mRNA levels

Total RNAs from liver were prepared using RNAiso Reagent (TaKaRa, Dalian, China). Total RNA was isolated from these frozen tissues according to the manufacturer’s instructions. The concentration of RNA was determined using NanoDrop® ND-1000 (Thermo, Pittsburgh PA, USA). Five hundred nanograms of RNA was transcribed into single-stranded cDNA using the PrimerScript™ RT reagent (TaKaRa, Japan) and used for PCR amplification. qPCR was performed using the synergy brands premix ExTaq II (TaKaRa, Japan) and the IQ5 real-time PCR detection system (Bio-Rad, CA, USA). The sequences of the primers are listed in Table 2.

2.7. Statistical analysis

All the data were analysed statistically using the general linear model procedure (SAS Institute 1989) and the treatment means were separated by Duncan’s multiple range test (P = .05).

3. Results and discussion

3.1. Meat quality

The effect of VE supplementation on meat quality attributes of breast fillets of broilers is presented in Table 3. The results showed that pH was not affected by supplementation with VE (P > .05), which is consistent with the findings involving Beijing-You chicken (Li et al. 2009). No significant difference in water-loss rate was observed (P > .05), whereas the use of 100 mg/kg VE led to a significant decrease in shear force (P < .05). The colour of the breast fillets was significantly affected by VE supplementation, particularly the a* and b* values, which were higher with 100 mg/kg VE.

Table 2. Primer sequences of the related genes assessed in this study.

| Gene name | Primer (5′ – 3′) |
|-----------|-----------------|
| SOD1      | F:CATGATATCCCGACAGTTGAC |
|           | R:CCCCCTGACCCCAGGGTCAA |
| GSH-Px    | F:GCACTCGGTCTACGACGCTC |
|           | R:CCGGCTCATGCCGCGTCACAT |
| β-actin   | F:TCTTGCGTTATGGAAGTGCTC |
|           | R:TAGAGCCATTTCGCGGTG |

Table 3. The effect of VE supplementation on meat quality attributes of breast fillets of broilers.

| Treatment | pH  | Water-loss rate | Shear force | a* | b* |
|-----------|-----|-----------------|-------------|----|----|
| Control   | 5.75| 10.92%          | 4.24 N/mm   | 7.7| 11.4|
| 50 mg/kg  | 5.73| 10.78%          | 4.22 N/mm   | 7.9| 11.7|
| 100 mg/kg | 5.71| 10.66%          | 4.20 N/mm   | 8.0| 11.8|
times of storage, the TBARS values increased in each treatment. After 7 d, the TBARS values of 100 and 200 mg/kg treatments were significantly lower than those of the control treatment. These results showed that the addition of 100 mg/kg VE to the diet was sufficient in reducing lipid oxidation in chicken meat during storage. The present findings were in agreement with those of Li et al. (2009), which also support the results of Young et al. (2003), who reported that dietary VE helps prolong the flavour and shelf life of chicken meat products by reducing lipid oxidation. Our findings are in agreement with those of Guo et al. (2001), who reported that the content of TBARS in thigh meat and dietary VE levels are inversely correlated ($R^2 = 0.93$, $P < .01$), and dietary VE supplementation significantly substantially improves the stability of meat quality against oxidative deterioration. Dietary VE supplementation resulted in a significant decrease in the content of MDA in fresh meat or meat refrigerated for 8 months (Smet et al. 2008); stored at 4°C for 1, 4, or 7 d (Brenes et al. 2008); and stored at −20°C for 0, 3, 5, or 7 d (Grau et al. 2001). Previous studies have shown that supplementation with high levels of VE than the normal requirement in poultry could increase consumer acceptance of the poultry meat stored at 4°C for 4 d (Kennedy et al. 2005). Supplementation with 200 mg/kg α-tocopherol acetate resulted in lower C16:1 n-7 levels and higher proportions of C18:1 n-9 and C18:1 n-7 in the polar muscle lipids compared to that with 10 mg/kg VE supplementation; and the TBARS value significantly decreased after refrigeration for 3, 6, or 9 d compared to that in the control group, and elevated lipid oxidative stability by 15% (Rey et al. 2004). However, Formanek et al. (2001) reported that supplementation of VE increased the oxidative stability of minced beef.

There is considerable debate over specifically what level of VE supplementation is optimal for poultry meat quality. The recommended concentration of VE supplementation for the

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**Table 3. Effect of VE supplementation on meat quality attributes of breast fillets in broilers.**

| Treatment         | Ph   | $L^*$  | $a^*$ | $b^*$  | Water-loss rate | Warner-Bratzler shear force ($W^*$) |
|-------------------|------|--------|-------|--------|-----------------|-----------------------------------|
| 0 mg/kg vitamin E | 6.34 ± 0.06 | 45.29 ± 1.43 | 11.16 ± 0.38$^{ab}$ | 20.83 ± 0.75$^b$ | 12.77 ± 0.65 | 18.55 ± 0.92$^a$ |
| 100 mg/kg vitamin E | 6.37 ± 0.13 | 42.66 ± 1.18 | 10.30 ± 0.33$^b$ | 23.56 ± 0.53$^a$ | 11.54 ± 0.19 | 12.96 ± 0.90$^b$ |
| 200 mg/kg vitamin E | 6.30 ± 0.06 | 45.20 ± 1.15 | 11.34 ± 0.26$^a$ | 20.91 ± 0.73$^b$ | 12.00 ± 1.17 | 19.37 ± 0.73$^a$ |

Notes:
- $L^*$, lightness; $a^*$, redness; $b^*$, yellowness; N, Newton, an unit for force.
- $^{ab}$Means within a column without a common superscript differ significantly ($P < .05$).

**3.2. Biochemical analyses**

Table 4 shows that dietary supplementation with VE significantly increases serum VE levels compared to that in the control group ($P < .05$). The VE levels in the breast muscle were significantly higher after supplementation with 200 mg/kg of VE compared to that in the control group or the 100 mg/kg treatment group ($P < .05$). These results were in agreement with the findings of previous studies (Arnold et al. 1992; Wulf et al. 1995). Liu et al. (1995) showed that VE supplementation facilitates in achieving sufficient serum and muscle VE levels, thereby maximizing antioxidant efficacy. The ideal VE concentration for supplementation will be the minimum quantity within the tissue that provides for the maximal suppression of lipid and protein oxidation in meat. VE as a part of the cellular antioxidant system, which played the part role coordinating with the other cellular antioxidant especially ascorbic acid and the glutathione system intimately, some composition of this system could regenerate mutually (Packer et al. 1996).

T-AOC activity in the breast muscle or serum increased with the supplementation of VE, whereas no significant difference was observed ($P > .05$) between the control treatment and 100 mg/kg VE treatment groups ($P > .05$). However, the T-AOC activity of the breast muscle or serum in birds fed with 200 mg/kg VE diet was significantly higher than that in the control treatment group ($P < .05$). T-SOD and GSH-Px activity in the breast muscle or in serum was positively correlated to dietary VE supplementation ($P < .05$). MDA content significantly decreased linearly with the addition of VE, which indicates that the addition of VE alleviated lipid oxidation; this result is similar to the findings of Machlin and Bendich (1987). Supplementation of VE (200 mg/kg) decreased the lipid oxidation product (the concentration of MDA) in the breast muscle in comparison to that in the control group (Goni et al. 2007). Carreras et al. (2004) reported that dietary VE supplementation increased the content of VE in the muscle tissue. The present study indicated that the content of VE in the breast muscle tissue increased with the increase in dietary VE supplementation; however, the content of VE in the breast muscle was significantly higher than that of the control only when supplemented with 200 mg/kg VE.

Oxidation of lipid components in muscle tissues is the major cause for the deterioration of quality and the short shelf life of broiler meat after slaughter. The results of the present study (Figure 1) indicated that the oxidative stability of breast muscle lipids during storage was favourably affected by dietary VE supplementation. While no differences were observed from 0 to 4 days, the TBARS values of the control group were higher ($P < .05$) than those of chickens that received 100 or 200 mg/kg VE from 6 to 8 days. With longer

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**Figure 1. Effect of dietary VE supplementation on TBARS values in the breast muscle of chickens during storage at 4°C (n = 6 per treatment).**
control of off-flavour ranges from 200 to 400 mg/kg (Galvin et al. 1998; Bou et al. 2001). However, the results of the present study have demonstrated that dietary supplementation of 100 mg/kg VE could significantly improve the antioxidant capacity in broilers. Such roles may have been mediated by an increase in AOE activities and the content of VE in the body, as well as a decrease in lipid peroxidation.

### 3.3. mRNA expressions

The results in Table 5 show that there was an increase in mRNA expression of SOD and GSH-Px in liver with the increase in dietary VE (P < .05). However, no significant difference between birds fed 100 mg/kg VE diet or 200 mg/kg VE diet was observed (P > .05). Modulation of transcriptional factor activity such as activator protein-1 (AP-1) and nuclear factor B (NF-B) is related to VE antioxidant potency. Furthermore, AP-1 and NF-B DNA binding sites have been located in the regulatory regions of inflammatory genes such as adhesion molecules, cytokines, and AOE. Thus, it could be postulated that VE could modulate AOE expression and activity by altering the cell redox status (Maggi-Capeyron et al. 2002). In our study, birds supplemented with VE showed improved antioxidant capability through upregulating the relative expression of SOD and GSH-Px mRNA. Administration of VE to hypothyroid rats resulted in elevated catalase (CAT) mRNA level (Jena et al. 2012). VE treatment resulted in significant increases in Cu/Zn SOD and CAT mRNA levels in human umbilical vein endothelial cells (Nakamura & Omaye 2008). These findings indicate that VE may play a role not only in preventing against oxidative damage as an exogenous antioxidant by scavenging free radicals and superoxide, but also in modulating the expression of endogenous AOE as gene regulators. The results of the present study indicate that supplementation of VE significantly upregulates the expression of AOE genes to increase antioxidant capacity, thereby improving meat quality.

### Table 4. Biochemical indices in breast muscle and serum of broilers.

| Treatment | Breast fillets | Serum |
|-----------|----------------|-------|
|           | T-AOC1 (U/mg of protein) | T-SOD2 (U/mg of protein) | MDA (nmol/ml) | T-AOC1 (U/mg of protein) | T-SOD2 (U/mg of protein) | MDA (nmol/ml) |
| 0 mg/kg vitamin E | 3.96 ± 0.14<sup>b</sup> | 9.61 ± 0.94<sup>b</sup> | 30.96 ± 0.94<sup>b</sup> | 342.30 ± 1.47<sup>b</sup> | 0.27 ± 0.03<sup>b</sup> | 2.47 ± 0.11<sup>b</sup> | 17.62 ± 1.04<sup>b</sup> | 17.62 ± 1.28<sup>b</sup> | 331.74 ± 2.56<sup>b</sup> | 4.02 ± 0.21<sup>b</sup> |
| 100 mg/kg vitamin E | 4.34 ± 0.18<sup>b</sup> | 10.21 ± 1.20<sup>b</sup> | 37.42 ± 1.52<sup>b</sup> | 359.58 ± 1.36<sup>b</sup> | 0.24 ± 0.04<sup>b</sup> | 3.25 ± 0.14<sup>b</sup> | 18.93 ± 1.11<sup>b</sup> | 19.87 ± 1.13<sup>b</sup> | 339.51 ± 1.69<sup>b</sup> | 3.76 ± 0.17<sup>a</sup> |
| 200 mg/kg vitamin E | 4.86 ± 0.16<sup>a</sup> | 12.46 ± 1.24<sup>b</sup> | 39.40 ± 1.17<sup>a</sup> | 362.21 ± 1.25<sup>a</sup> | 0.19 ± 0.04<sup>b</sup> | 3.98 ± 0.12<sup>a</sup> | 21.14 ± 0.74<sup>a</sup> | 24.37 ± 1.11<sup>a</sup> | 359.35 ± 1.43<sup>a</sup> | 2.96 ± 0.14<sup>b</sup> |

1Total antioxidative capabilities.
2Total superoxide dismutase.
3Glutathione peroxidase.
4<sup>ab</sup>Means within a column without a common superscript differ significantly (P < .05).

### Table 5. Effects of VE on mRNA expression of SOD and GSH-Px in broilers.

| Items | 0 mg/kg vitamin E | 100 mg/kg vitamin E | 200 mg/kg vitamin E |
|-------|-------------------|---------------------|---------------------|
| T-SOD | 0.36 ± 0.05<sup>b</sup> | 0.71 ± 0.02<sup>b</sup> | 0.92 ± 0.03<sup>b</sup> |
| GSH-Px | 0.25 ± 0.008<sup>b</sup> | 0.82 ± 0.006<sup>b</sup> | 0.89 ± 0.00048<sup>b</sup> |

<sup>1</sup>In the same row, values with different small letter superscripts indicate a significant difference (P < .05), whereas with no letter superscripts show no significant differences (P > .05).

### 4. Conclusion

The results of the present research suggest that dietary supplementation with VE could increase lipid stability and improve the shelf life of chicken meat by upregulating the expression of AOE genes to increase antioxidant capacity in broilers. Under these experimental conditions, the efficacy of improving the meat quality and antioxidant capacity in broiler chickens fed with 200 mg/kg VE diet is greater compared to that of those fed with 100 mg/kg VE diet.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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