Effects of dietary natural vitamin E supplementation on laying performance, egg quality, serum biochemical indices, tocopherol deposition and antioxidant capacity of laying hens

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ABSTRACT
This study was to evaluate the effects of dietary natural vitamin E supplementation on laying performance, egg quality, serum biochemical indices, tocopherol deposition and antioxidant capacity of laying hens. A total of 360 46-week-old Hyline Brown hens were randomly assigned into five treatments consisting of six replicates with 12 hens each for a 9-week feeding trial. Birds were fed a basal diet supplemented with 0, 25, 50, 75, and 100 mg/kg natural vitamin E, respectively. Natural vitamin E linearly increased egg production and egg mass \((p < .05)\), linearly decreased feed conversion ratio \((p < .05)\) and linearly and quadratically increased feed intake \((p < .05)\). The total tocopherol concentration in liver and yolk increased quadratically and linearly with increasing levels of natural vitamin E, respectively \((p < .05)\). A linear decrease in content of serum low-density lipoprotein cholesterol was observed \((p < .05)\). Supplementation of natural vitamin E linearly decreased serum malondialdehyde (MDA) level, linearly increased hepatic total antioxidant capacity (T-AOC) and catalase (CAT) activities, quadratically increased serum CAT activity and linearly and quadratically decreased hepatic MDA accumulation \((p < .05)\). In conclusion, natural vitamin E can enhance laying performance, regulate serum cholesterol concentration, promote tocopherol deposition and improve antioxidant capacity, and dietary supplementation at a dosage of 100 mg/kg was recommended.

HIGHLIGHTS
- Dietary supplementations of natural vitamin E significantly improved laying performance of hens.
- Dietary supplementations of natural vitamin E significantly increased tocopherol concentration of liver and yolk.
- Dietary supplementations of natural vitamin E significantly improved antioxidant capacity of hens.

Introduction
Vitamin E is an important fat-soluble nutrient. Its roles in domestic animal production are indispensable since animal are not capable of vitamin E synthesis (Shakeri et al. 2020). Dietary supplementation of vitamin E has been reported to have beneficial effects on antioxidant status (Traber 2007), anti-inflammatory (Panda et al. 2008), as well as hatchability and fertility (Ipek and Dikmen 2014). Moreover, it has been revealed that vitamin E could improve meat and egg quality, and carcase characteristics (Idamokoro et al. 2020).

Generally, ‘vitamin E’ has eight analogues, including \(\alpha\), \(\beta\), \(\gamma\), \(\delta\)-tocopherols and tocotrienols. Among these structures, only \(\alpha\)-tocopherol has been used to set the recommendation of dietary allowance for vitamin E, and it has been identified as the most efficient lipophilic antioxidant in protecting integrity of cell membrane and possesses the highest bioavailability in blood and tissues when compare with other forms (Jiang 2018). There are two main sources of \(\alpha\)-tocopherol, naturally occurring form of RRR-\(\alpha\)-tocopherol, which is widely available in plants and photosynthetic organisms, and the synthetic form of all rac \(\alpha\)-tocopherol, which is from the reaction between trimethyl hydroquinone and synthetic isophytol (Clemente et al. 2015; Idamokoro et al. 2020).
Research has demonstrated that the bioavailability of synthetic vitamin E (*all rac* α-tocopherol) is only half of that of natural vitamin E (RRR-α-tocopherol) at the equivalent mass dose (Ranard and Erdman 2018). Cheng et al. (2018) found that bioavailability of natural vitamin E was higher than synthetic in broiler chickens at an early age. Natural vitamin E was superior to the synthetic form in the aspects of antioxidant capacity and immune function (Cheng et al. 2017). In turkey, natural vitamin E exerted higher antioxidant capacity, and dosages at 80 mg/kg of natural vitamin E was recommended (Rey et al. 2015). The two forms of vitamin E are absorbed in different patterns that contribute to discrepancy of bioavailability. Natural vitamin E is usually hydrolysed by pancreatic lipase in small intestine and then absorbed by the body, while the synthetic one needs to be hydrolysed and reduced to alcohol form in the digestive tract prior to absorption (Scherf et al. 1996). Besides, the existence of α-tocopherol transfer protein, which can effectively bind RRR-tocopherol, also results in a higher bioavailability of natural vitamin E (Traber 2007).

Dietary supplementation of vitamin E for laying hens should be 5–10 U/kg, according to the recommendations set by National Research Council (NRC 1994). However, in modern poultry industry, 25 U/kg or more are favoured, because intensive production may result in unexpected situation, including unusual ambient temperature changes, nutrition imbalance or inflammatory challenges, and strategies, such as the use of antioxidants like vitamin E can be applied to relieve these adverse consequences (Jiang W et al. 2013; Attia et al. 2016; Liu et al. 2019). Sahin et al. (2002) have demonstrated that an extra supplementation of 250 mg/kg commercial vitamin E (ROVIMIX®E-50 SD, main component is dl-α-tocopheryl acetate) could relieve lipid peroxidation of laying hens subjected to high ambient temperature. Similarly, Kirunda et al. (2001) have reported that extra vitamin E (dl-α-tocopheryl acetate) could alleviate the heat-induced deterioration of egg quality. Moreover, egg production and egg weight of Hyline White Leghorn hens have been significantly improved when supplementing an extra 125 mg/kg vitamin E (proportional to-tocopheryl acetate) to the basal diet (Ciftci et al. 2005).

However, materials applied in the aforementioned researches are mainly synthetic vitamin E, and little is known about the effects of natural vitamin E supplementation on laying hens. Based on previous research on broiler (Cheng et al. 2017), turkey (Rey et al. 2015), swine (Yang et al. 2009) or even in rumen animal (Weiss et al. 2009), it was convinced that natural vitamin E has a superior bioavailability than synthetical form. Therefore, this study was conducted to evaluate the beneficial consequences of supplementing extra natural vitamin E at different levels on laying hens by measuring laying performance, egg quality, serum biochemical indices, deposition of tocopherol and antioxidant capacity. The results of this study could provide scientific reference for further utilisation of natural vitamin E in laying hens’ feed.

**Materials and methods**

**Animals, diets and experimental design**

All experiments involving animals in this study were conducted in accordance with the protocol approved by the Ethics Committee of Nanjing Agricultural University (SYXK (SU) 2017-0007).

A total of 360 46-week-old Hyline Brown hens with similar body weight and egg production were allocated into five groups with six replicates and each replicate consisted of two adjacent cages (40 × 40 × 35 cm) with 12 birds. The vitamin E level of the basal diet was set to be 30 U in the form of *all rac* α-tocopherol. After 1-week preliminary feeding and adaption period, all birds were fed with a basal diet supplemented with 0 (Control group), 25, 50, 75, and 100 mg/kg natural vitamin E products for 9 weeks, respectively. The natural vitamin E applied in this study was provided by Yichun Dahaigui Life Science Co., Ltd (Yichun City, Jiangxi Province, China). Natural vitamin E was extracted from deodoriser distillate generated during soybean oil production and then purified by molecular distillation. After that, using silica as carrier, natural vitamin E in the form of powder was obtained. Total tocopherol content was greater than 20%, and the remaining was carrier. The natural vitamin E in the form of powder was mixed thoroughly with premix used in feed formulation prior to feed preparation. The formulation and nutrient composition of the basal diet are presented in Table 1. The mash feed and water were offered *ad libitum* throughout the experiment and birds were strictly following the commercial lighting programme. The intensity of the light was 30 lx, using ‘warm’ lights (2700–3500 K) in laying flocks to ensure sufficient red spectrum light, and the experiment was under a 16:8 light: dark cycle. Egg number and egg weight were recorded daily and feed consumption on replicate basis was recorded weekly. Egg production was represented through the ratio between the number of daily eggs and the number of hens on the same day. Average egg mass was calculated by dividing the total egg weight by the
number of eggs. Feed conversion ratio was calculated as the ratio between total feed consumption and total egg weight.

Sample collection
At the end of experiment, one bird per replicate was selected and blood samples were collected from wing vein, serum were then separated by centrifugation at 3500 \( \times g \) for 15 min at 4 \( ^\circ \)C and stored at −20 \( ^\circ \)C. After blood sampling, birds were euthanised by cervical dislocation. The jejunum was dissected and washed with ice cold phosphate-buffered saline (pH = 7.4). Jejunal mucosa were carefully collected into sterile frozen tubes and stored at −80 \( ^\circ \)C until analysis. The right lobe of liver was removed and stored at −20 \( ^\circ \)C for analysis of hepatic total tocopherol concentration and hepatic antioxidant status.

Egg quality measurement
At 28 and 63 d of this experiment, two eggs from each replicate were randomly collected. Eggshell strength was measured by the compression tester (Model-II, Robotmation, Tokyo, Japan). Eggshell thickness was calculated from the average of three sites of egg (air cell, equator and the sharp end of egg) by spiral micrometre. Yolk colour, Haugh unit, and albumen height were measured by the egg tester (EMT-5200, Robotmation, Tokyo, Japan). The albumen and yolk were separated by commercially separator according to method described by Scherf et al. (1996). And egg yolks were pooled in sterile dishes, mixed and freeze-dried according to the method of Gao et al. (2020), and put into 5mL sterile tubes and stored at −80 \( ^\circ \)C for the analysis of tocopherol concentration.

Measurement of total tocopherol concentration
The concentrations of total tocopherol in serum, liver, jejunal mucosa, and yolk were measured with commercial kits (cat. No. A008-1-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). In the presence of phenanthroline, tocopherol can reduce trivalent iron ions to divalent iron ions, and the latter can form pink complex with phenanthroline under specific circumstances. The content of total tocopherol can be calculated by spectrophotometer at absorbance of 533 nm. All the measurements were performed strictly following the manufacturer’s guidance.

Measurement of serum biochemical indices
The glucose (GLU, cat. No. F006-1-1), total protein (TP, cat. No. A045-4-1), albumin (ALB, cat. No. A028-2-1), total cholesterol (TCHO, cat. No. A111-1-1), triglyceride (TG, cat. No. A110-2-1), high-density lipoprotein cholesterol (HDL-C, cat. No. A112-1-1) and low-density lipoprotein cholesterol (LDL-C, cat. No. A113-1-1) concentrations in the serum were analysed using commercial test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with spectrophotometer (1510, Thermo Fisher Scientific Inc., Vantaa, Finland) to observe absorbance.

Measurement of antioxidant capacity
The liver and jejunal mucosa samples were homogenised with ice-cold physiological saline solution (4 \( ^\circ \)C) in an ice-cold bath at a ratio of 1: 9 (wt/vol), using a homogeniser (PRO-PK-02200D, Pro Scientific, Inc., Monroe, CT). The homogenate was then centrifugated at 3500 \( \times g \) for 10 min at 4 \( ^\circ \)C. The supernatant was collected and stored at −20 \( ^\circ \)C immediately for the subsequent analysis.

The total antioxidant capacity (T-AOC, No.A015-1-2), the activities of superoxide dismutase (T-SOD, cat. No. A001-1-1), catalase (CAT, No.A007-1-1) and glutathione peroxidase (GSH-Px, No.A005-1-2), and the concentrations of glutathione (GSH, No.A006-1-1) and

| Table 1. Composition and nutrient level of basal diet (g/kg, air dry). |
|-----------------|-----------------|
| Items           | Basal diet      |
| Ingredients     |                 |
| Maize           | 618             |
| Soybean meal    | 249             |
| Soybean oil     | 8               |
| Limestone       | 85              |
| Premix          | 40              |
| Calculated nutrient levels |       |
| Apparent metabolisable energy (MJ/kg) | 11.15 |
| Crude protein   | 164             |
| Ether extract   | 35              |
| Calcium         | 38              |
| Total phosphorus| 6.2             |
| Available phosphorus | 3.7  |
| Lysine          | 8.2             |
| Methionine      | 3.6             |
| Total sulphur amino acids | 6.4 |
| Analysed nutrient levels |       |
| Crude protein   | 159             |
| Ether extract   | 31              |

*The vitamin E level of the basal diet was set to be 30 U in the form of all rac α-tocopherol; Premix provided per kilogram of diet: transretinyl acetate, 10,000 U; cholecalciferol, 3000 U; all-rac-α-tocopherol, 30 U; menadione, 1 mg; thiamine, 1 mg; riboflavine, 6 mg; nicotinamide, 40 mg; choline chloride, 350 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 3 mg; biotin, 0.1 mg; folic acid, 0.3 mg; cobalamin, 0.01 mg; Cu: copper sulphate, 8 mg; Fe: ferrous sulphate, 80 mg; Zn: zinc sulphate, 50 mg; I: NaI; manganese sulphate, 100 mg; I: calcium iodate, 1 mg; Se: sodium selenite, 0.3 mg; calcium, 6.25 g; P, 3 g; DL-methionine, 1 g; salt, 3 g.
malondialdehyde (MDA, No. A003-1-2) were determined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, PR China), strictly following the instructions of manufacture’s manuals. The activity of T-SOD was determined by the hydroxylamine method (Kono 1978), monitoring the degree of hydroxylamine oxidised by superoxide anion radical. The T-AOC activity was determined by the method of ferric-reducing assay. 5’-5-dithiobis-(2-nitrobenzoic acid) method was applied to determine the GSH level and GSH-Px activity (Owens and Belcher 1965). The concentrations of MDA were measured by thiobarbituric acid method (Placer et al. 1966). And the activities of CAT were measured using ammonium molybdate method (Góth 1991).

**Statistical analysis**

All data were analysed by one-way analysis of variance using SPSS statistical software (2010, SPSS Inc., Chicago, IL, USA). Differences among treatments were examined using Tukey’s multiple range tests. Polynomial contrasts were applied to test the linear and quadratic effects of dietary natural vitamin E supplementation level. Statistical significance was considered when p value was less than .05.

### Results

**Laying performance**

As shown in Table 2, during the whole experiment period, dietary natural vitamin E supplementation linearly increased egg production (p = .01) and egg mass (p = .001), linearly and quadratically increased feed intake (linear, p = .003; quadratic, p = .006) and linearly decreased feed conversion ratio (p = .001). Compared with the control group, feeding a basal diet supplemented with 100 mg/kg of natural vitamin E increased egg production (p = .015), egg mass (p = .004), feed intake (p = .001), but decreased feed conversion ratio (p = .003). However, 25, 50, and 75 mg/kg of natural vitamin E treatment had no effects on laying performance of the whole experiment period (p > .05).

**Egg quality**

Dietary supplementation of natural vitamin E did not alter eggshell thickness, eggshell strength, egg albumen height, Haugh unit or egg yolk colour at both 28 and 63 d of the trial (Table 3, p > .05).

**Deposition of total tocopherol**

Dietary supplementation of natural vitamin E quadratically increased total tocopherol concentration in liver.

### Table 2. Effects of dietary supplemented with natural vitamin E on laying performance of laying hens.

| Items                  | Levels of dietary natural vitamin E (mg/kg) | p Value         | SEM | ANOVA | Linear | Quadratic |
|------------------------|--------------------------------------------|-----------------|-----|-------|--------|-----------|
|                        | 0   | 25  | 50  | 75  | 100   |           |           |
| Egg production (%)     | 83.49<sup>a</sup>                          | 87.48<sup>ab</sup> | 84.26<sup>ab</sup> | 86.92<sup>ab</sup> | 88.98<sup>a</sup> | 0.620 | .015 | .010 | .648 |
| Egg mass (g)           | 57.96<sup>b</sup>                          | 58.51<sup>b</sup> | 58.61<sup>b</sup> | 58.92<sup>ab</sup> | 60.94<sup>a</sup> | 0.285 | .004 | .001 | .115 |
| Feed intake (g/hen/d)  | 123.76<sup>b</sup>                         | 124.39<sup>ab</sup> | 122.93<sup>b</sup> | 124.38<sup>ab</sup> | 126.21<sup>a</sup> | 0.280 | .001 | .003 | .006 |
| Feed conversion ratio  | 2.59<sup>a</sup>                           | 2.49<sup>ab</sup> | 2.55<sup>b</sup> | 2.45<sup>ab</sup> | 2.34<sup>b</sup> | 0.024 | .003 | .001 | .338 |

Means within a row with different superscripts are different at p < .05.

### Table 3. Effects of dietary supplemented with natural vitamin E on egg quality of laying hens at 28 and 63 d of the experiment.

| Items                  | Levels of dietary natural vitamin E (mg/kg) | p Value         | SEM | ANOVA | Linear | Quadratic |
|------------------------|--------------------------------------------|-----------------|-----|-------|--------|-----------|
|                        | 0   | 25  | 50  | 75  | 100   |           |           |
| 28, d<sup>b</sup>      |                                             |                 |     |       |        |           |
| Eggshell thickness (mm)| 0.31 | 0.33 | 0.32 | 0.30 | 0.32  | 0.004    | .365 | .887 | .902 |
| Egghshell strength (kg/m<sup>2</sup>) | 2.59 | 2.39 | 2.73 | 2.65 | 2.68  | 0.047    | .163 | .174 | .871 |
| Egg albumen height (mm)| 8.50 | 9.22 | 8.18 | 8.92 | 9.19  | 0.146    | .095 | .267 | .441 |
| Haugh unit             | 93.07 | 95.63 | 91.39 | 94.38 | 95.22  | 0.073    | .266 | .316 | .498 |
| Egg yolk colour        | 2.88 | 2.79 | 2.92 | 2.72 | 2.78  | 0.045    | .661 | .410 | .983 |

### 63, d<sup>b</sup>

| Items                  | Levels of dietary natural vitamin E (mg/kg) | p Value         | SEM | ANOVA | Linear | Quadratic |
|------------------------|--------------------------------------------|-----------------|-----|-------|--------|-----------|
|                        | 0   | 25  | 50  | 75  | 100   |           |           |
| Eggshell thickness (mm)| 0.37 | 0.38 | 0.38 | 0.36 | 0.39  | 0.003    | .154 | .566 | .541 |
| Egghshell strength (kg/m<sup>2</sup>) | 2.79 | 2.85 | 2.96 | 2.76 | 3.20  | 0.057    | .082 | .062 | .320 |
| Egg albumen height (mm)| 6.99 | 7.35 | 7.03 | 7.78 | 6.73  | 0.205    | .565 | .947 | .327 |
| Haugh unit             | 81.99 | 84.45 | 82.68 | 83.35 | 78.63  | 1.426    | .779 | .463 | .347 |
| Egg yolk colour        | 3.69 | 4.38 | 3.94 | 3.71 | 3.44  | 0.133    | .229 | .210 | .126 |

<sup>a</sup>SEM: total standard error of means (n = 6).

<sup>b</sup>28 and 63 d, sampling time.
Table 4. Effects of dietary supplemented with natural vitamin E on deposition of total tocopherol of laying hens.

| Items            | Levels of dietary natural vitamin E (mg/kg) | SEMc | p Value |
|------------------|--------------------------------------------|------|---------|
| Serum (µg/mL)    | 0 25 50 75 100 AVONA Linear Quadratic       |      |         |
|                  | 5.11 5.19 4.21 5.54 5.24 0.376 .878 .855 .665 |
| Jejunal mucosa (µg/g) | 59.58 54.64 60.28 51.31 54.13 1.312 .140 .116 .930 |
| Liver (µg/g)     | 43.06b 49.65ab 63.84a 43.65b 48.10th 2.249 .013 .764 .022 |
| Yolk (µg/g)      | 10.58b 11.40b 13.25ab 14.34a 14.66a 0.408 .001 .000 .488 |

Means within a row with different superscripts are different at p < .05.
cSEM: total standard error of means (n = 6).

Table 5. Effects of dietary supplemented with natural vitamin E on serum biochemical of laying hens.

| Items            | Levels of dietary natural vitamin E (mg/kg) | SEMc | p Value |
|------------------|--------------------------------------------|------|---------|
| Glucose (mmol/L) | 0 25 50 75 100 ANOVA Linear Quadratic       |      |         |
|                  | 15.27 16.68 13.88 15.23 15.17 0.573 .693 .698 .807 |
| Total protein (g/L) | 36.12 35.04 33.80 33.43 35.74 0.656 .657 .621 .187 |
| Albumin (g/L)    | 22.86 26.15 23.71 22.00 23.76 0.676 .398 .625 .683 |
| Total cholesterol (mmol/L) | 4.56 4.13 4.16 4.55 4.42 0.775 .991 .928 .995 |
| Triglyceride (mmol/L) | 9.92 10.78 9.57 10.52 10.32 0.985 .673 .939 |
| High-density lipoprotein cholesterol (mmol/L) | 1.38 1.39 1.42 1.39 1.42 0.026 0.036 0.003 .547 |
| Low-density lipoprotein cholesterol (mmol/L) | 0.62a 0.43ab 0.40ab 0.42ab 0.27b |

Means within a row with different superscripts are different at p < .05.
cSEM: total standard error of means (n = 6).

(Table 4, p = .002) and linearly increased total tocopherol concentration in yolk (p < .001). Compared with the control group, the supplementation of natural vitamin E (50 mg/kg) increased hepatic total tocopherol concentration (p = .013). The concentration of total tocopherol in yolk was increased by both 75 and 100 mg/kg natural vitamin E (p = .001). However, the total tocopherol concentration in the serum and jejunal mucosa was similar among the five groups (p > .05).

**Serum biochemical indices**

The supplementation of natural vitamin E linearly decreased serum LDL-C concentration (p = .003, Table 5). Compared with the control group, natural vitamin E supplementation at a level of 100 mg/kg significantly decreased serum LDL-C concentration (p = .027). However, there was no significant difference in concentration of serum GLU, TP, ALB, TC, TG or HDL-C among the five groups (p > .05).

**Antioxidative status**

Supplementing natural vitamin E linearly decreased serum MDA content (p = .002, Table 6), linearly increased hepatic T-AOC activity (p = .003) and hepatic CAT activity (p < .001), but decreased serum MDA concentration (p = .032). Supplementing 75 mg/kg of natural vitamin E increased jejunal GSH concentration (p = .009) and decreased hepatic MDA concentration (p = .033). The supplementation of natural vitamin E at a level of 50 mg/kg increased serum CAT activity (p = .044). Besides, activities of SOD and GSH-Px were similar among the five treatments (p > .05). In serum, activities of T-AOC and concentrations of GSH were not changed (p > .05). Furthermore, treatment did not affect jejunal T-AOC and CAT activities, and MDA concentrations (p > .05).

**Discussion**

In this study, supplementing 100 mg/kg of natural vitamin E improved egg production, egg mass, feed intake and feed conversion ratio of laying hens. These results were corresponding to the findings of Jiang et al. (2013), who found that only 200 mg/kg of vitamin E could improve egg production. And Ciftci et al. (2005) concluded that egg production and egg mass were improved when fed White Leghorn with diet containing 200 mg/kg vitamin E under heat stress condition. The beneficial effects of vitamin E can be attributed to facilitating the release of vitellogenin, thus increasing the egg production (Bollengier-Lee et al. 1998). However, as has demonstrated by Hosain et al. (1998), broiler breeder fed with graded levels of vitamin E (25, 50, 75, and 100 mg/kg) had no difference on egg production among groups. Liu et al. (2019)
also found that there was no difference among vitamin E treatments for the egg production of laying hens. Probably because in this study, extra supplementing natural vitamin E exceeds the basic nutritional requirements of laying hens, thus natural vitamin E exert its antioxidant capacity (Traber 2007), and ultimately promote laying performance.

In this study, dietary supplemented of natural vitamin E did not influence egg quality parameters, including eggshell thickness, eggshell strength, egg albumen height, Haugh units and egg yolk colour. In line with this study, Urso et al. (2015) found no difference in egg quality when broiler breeder was fed with 30 and 120 mg/kg of vitamin E. However, slight increase of eggshell thickness was found by Ciftci et al. (2005). It cannot be excluded that differences in type of vitamin E, since Ciftci et al. (2005) used 
\[\alpha\]-tocopheryl acetate as vitamin E source, which were differed in this study. Finally, ambient factors, including heat stress, difference in feed raw material and pathogen stress may also contribute to different conclusions.

Serum biochemical indices including GLU, TP and ALB are involved in animal health status. In this study, serum concentrations of GLU, TP and ALB were not influenced by supplementation of natural vitamin E, which can be supported by the finding of Mu et al. (2019), who have reported that natural vitamin E supplementation from 25 to 100 mg/kg have no influence on these parameters. In addition, lipid metabolism parameters including serum TC, TG and HDL-C concentration were not changed in this study, but serum LDL-C concentration was decreased by 100 mg/kg of natural vitamin E. LDL-C, which is harmful to organism to some extent, is thought to be main factor of causing cardiovascular disease, and many research groups were working to protect animal health by reducing LDL-C levels, including the usage of vitamin E in animal feed (Katzmann and Laufs 2019). Casamassima et al. (2014) found that supplementation of vitamin E could decrease serum LDL-C concentration of lacaune ewes, which were consist with the research of Dou et al. (2009). The presence of vitamin E in lipoprotein may contribute to the decreased level of LDL-C, because vitamin E is considered as suppressor of LDL lipid oxidation (Miyazawa et al. 2019).

Concentration of 
\[\alpha\] tocopherol in tissue and plasma was sensitive to dietary supplementation of vitamin E. Jiang et al. (2013) demonstrated that 
\[\alpha\] tocopherol concentration in plasma was higher in laying hens fed a diet containing 200 mg/kg of vitamin E when compare with the control group. In an experiment conducted in White Pekin ducks, a linearly or quadratically increase of tocopherol concentration in plasma and liver was observed (Xie et al. 2018). Furthermore, several studies also reported that dietary supplementation of vitamin E could increase the hepatic tocopherol content (Goni et al. 2007; Reza et al. 2016), which was consistent with finding of this study that hepatic tocopherol concentration was increased.

### Table 6. Effects of dietary supplemented with natural vitamin E on antioxidative status of laying hens.

| Items | Levels of dietary natural vitamin E (mg/kg) | p Value |
|-------|---------------------------------------------|---------|
|       | 0 | 25 | 50 | 75 | 100 | SEM | ANOVA Linear Quadratic |
| Serum | GSH-PX (U/mL) | 1113.67 | 1194.3 | 1089.2 | 1093.82 | 1103.89 | 15.521 | .183 | .263 | .802 |
|       | GSH (mg/L) | 6.60 | 5.98 | 4.50 | 5.30 | 5.67 | 0.294 | .215 | .217 | .076 |
|       | T-AOC (U/mL) | 3.42 | 2.93 | 3.32 | 3.54 | 3.72 | 0.145 | .527 | .255 | .346 |
|       | T-SOD (U/mL) | 399.89 | 409.79 | 396.1 | 385.85 | 415.56 | 5.347 | .447 | .852 | .343 |
|       | MDA (nmol/mL) | 2.71ab | 2.54ab | 2.46ab | 2.39ab | 2.10b | 0.065 | .032 | .002 | .666 |
|       | CAT (U/mL) | 21.64b | 24.13ab | 25.39b | 22.20ab | 22.88ab | 0.448 | .044 | .847 | .023 |
| Jejunal mucosa | GSH-PX (U/mg protein) | 13.65 | 13.87 | 14.53 | 17.65 | 15.28 | 0.767 | .486 | .237 | .614 |
|       | GSH (mg/g protein) | 6.38ab | 8.44ab | 6.52ab | 8.58b | 6.95ab | 0.276 | .009 | .436 | .090 |
|       | T-AOC (U/mg protein) | 1.77 | 1.78 | 1.82 | 1.84 | 1.80 | 0.017 | .734 | .304 | .472 |
|       | T-SOD (U/mg protein) | 420.75 | 457.55 | 448.22 | 451.59 | 427.95 | 6.567 | .142 | .820 | .019 |
|       | MDA (nmol/mg protein) | 0.51 | 0.47 | 0.51 | 0.36 | 0.41 | 0.027 | .366 | .129 | .937 |
|       | CAT (U/mg protein) | 2.70 | 2.68 | 2.37 | 2.28 | 2.40 | 0.088 | .502 | .148 | .476 |
| Liver | GSH-PX (U/mg protein) | 29.81 | 42.90 | 31.54 | 35.84 | 30.90 | 1.748 | .116 | .642 | .149 |
|       | GSH (mg/g protein) | 14.13 | 15.61 | 13.17 | 15.34 | 13.83 | 0.668 | .778 | .862 | .813 |
|       | T-AOC (U/mg protein) | 1.72ab | 1.70b | 1.77ab | 1.93ab | 1.98b | 0.032 | .003 | .000 | .300 |
|       | T-SOD (U/mg protein) | 1013.58 | 1006.96 | 1018.81 | 1016.15 | 1025.87 | 10.495 | .990 | .685 | .865 |
|       | MDA (nmol/mg protein) | 0.58ab | 0.47ab | 0.41ab | 0.35b | 0.41ab | 0.024 | .033 | .014 | .040 |
|       | CAT (U/mg protein) | 44.02ab | 44.52b | 52.14ab | 50.67b | 60.41b | 1.486 | .000 | .000 | .281 |

Means within a row with different superscripts are different at \(p < .05\).

\(\text{GSH-PX: glutathione peroxidase; GSH: reduced glutathione; T-AOC: total antioxidative capacity; T-SOD: total superoxide dismutase; MDA: malondialdehyde; CAT: catalase}

\(\text{SEM: total standard error of means (n = 6).}\)
Besides, in this study, the concentration of total tocopherol in yolk was significantly improved, which was in agreement with the results of Jiang et al. (2013) and Scheideler et al. (2010), both of which showed the similar change of yolk tocopherol concentration when supplementing vitamin E in the diet. Vitamin E is an essential fat-soluble micronutrient, which may explain the tocopherol concentration in yolk is susceptible to it. In addition, the serum total tocopherol concentration was not changed, probably due to natural vitamin E is preferentially adsorbed by the liver, and then secreted into plasma.

The antioxidant system includes enzymatic antioxidants, such as CAT, SOD, GSH-Px and non-enzymatic antioxidants including GSH and vitamin E (Ighodaro and Akinloye 2018). Of these, vitamin E was considered as a chain-breaking antioxidant and plays an important role in regulating redox balance (Miyazawa et al. 2019). It has been demonstrated that vitamin E can indirectly affect antioxidant system by elevating GSH concentration (Surai et al. 2019). Supplementation of vitamin E to White Leghorn resulted in improvement of antioxidant capacity by enhancing serum GSH level (Panda et al. 2008). Likewise, in newly hatched chicken, dietary vitamin E can increase hepatic GSH concentration (Surai 2000). The antioxidant enzymes, such as SOD, GSH-Px and CAT were considered as main components of the first-level antioxidant defence of cells, therefore, the improved antioxidant capability by vitamin E in laying hens also related to increase of GSH-Px, SOD and CAT activity (Panda et al. 2008; Ding et al. 2021). Similarly, at early age of broiler, SOD and GSH-Px activity in liver and plasma was increased by dietary vitamin E (El-Senousey et al. 2018). Cheng et al. (2018) also found that plasma SOD activity as well as hepatic and ileal GSH-Px activity were increased when supplemented with vitamin E to early age of broilers. Furthermore, vitamin E has been shown to have effects on terminating lipid peroxidation in tissue. MDA was considered as an end product of lipid peroxidation. Min et al. (2016) found that supplementation of 300 mg/kg vitamin E could significantly reduce plasma MDA in breeder roaster. Cheng et al. (2017) also found that the accumulations of MDA in plasma and liver were decreased in broiler chicken fed with vitamin E under cyclophosphamide challenged condition. In this study, dietary supplementation of natural vitamin E could improve antioxidant capacity of laying hens by elevating serum and hepatic CAT activity, increasing GSH levels in jejunal mucosa and enhancing T-AOC activity in liver. Besides, this study showed that concentrations of MDA in serum and liver were decreased. The improvement of lipid peroxidation was also associated with tocopherol regulation. These results revealed that elevating hepatic tocopherol concentration associated with the decrease concentration of MDA in liver. In line with this research, Sahin et al. (2002) revealed that serum MDA concentration was decreased in response to increase of serum vitamin E. These results suggest that dietary supplementation of natural vitamin E can improve antioxidant capability of laying hens directly by reducing MDA content and enhancing T-AOC activity, and indirectly by improving enzymatic or non-enzymatic antioxidants activity, including CAT and GSH. These beneficial effects could reduce oxidative stress and lipid peroxidation, and finally improve laying performance of hens.

**Conclusion**

In conclusion, natural vitamin E supplementation into laying hens can elevate tocopherol concentration in tissues, decrease serum LDL-C concentration and MDA content. Besides, dietary supplementation of natural vitamin E could improve antioxidant capacity by enhancing CAT activity, GSH level and T-AOC capability. These positive effects may eventually lead to improved laying performance of laying hens. Therefore, supplementing natural vitamin E could have a good effect on laying performance of laying hens, and natural vitamin E at a dosage of 100 mg/kg was recommended.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Data availability statement**

The data that support the findings of this study are openly available in figshare at [https://doi.org/10.6084/m9.figshare.16558314.v1](https://doi.org/10.6084/m9.figshare.16558314.v1). Link to Datasets: [https://figshare.com/articles/journal_contribution/the_effects_of_natural_vitamin_E_on_laying_hens/16558314](https://figshare.com/articles/journal_contribution/the_effects_of_natural_vitamin_E_on_laying_hens/16558314).
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