Impact of dietary supplementation of vitamin E (alpha-tocopherol acetate) on genetic expression of inflammatory cytokines and growth efficiency of broiler chickens

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1. INTRODUCTION

Vitamin E is an antioxidant fat-soluble vitamin that protects the membranes of the cells from oxidation (Traber and Atkinson, 2007). Two vitamin E families exist (tocopherol and tocotrienol). Alpha tocopherol is the most biologically active source of vitamin E involved in the pathway of glutathione peroxidase and protects species against oxidative damage by responding in fat peroxidation with lipid radicals (Shakeri et al., 2020). Dietary vitamin E has an immune-modulatory effect on T-cells that can benefit the immune system and well-being of chickenbroilers (Min et al., 2018). The addition of vitamin E to broiler diets reduced the expression of pro-inflammatory (IFN-γ and IL-1β) cytokines in chickens that had acquired intravenous lipopolysaccharide (Leshchinsky and Klaasning, 2003). Zhang et al. (2010) found that the dietary supplementation with alpha tocopherol reduced plasma protein levels of both pro-inflammatory cytokines (IFN-γ, IL-1β), and IL-6) and anti-inflammatory cytokines (IL-4 and IL-10). Habibian et al. (2014) confirmed that a 250 mg/kg vitamin E supplement had high titers against NDV in thermo-neutral conditions.

It is important to keep broiler chickens in good health. The immune system is critical to defend against infectious agents (Dalia et al., 2018). The key proteins of immunity cytokines were known as endogenous signaling molecules which mediate the cellular mechanism against inflammatory responses (Hietbrink et al., 2006). The cytokines can be classified according to their functionality in the control of inflammation and immunity into pro-inflammatory (IL-1β, IFN-γ) and anti-inflammatory (IL-4 and IL-10) cytokines in spleen and liver. In Conclusions: vitamin E supplementation (100 mg/kg diet) can enhance growth efficiency, serum total protein, albumin, globulin, and humoral immunity, down-regulate pro-inflammatory and anti-inflammatory cytokines gene expression in broiler chickens.
against NDV, total protein, albumin globulin and growth efficiency of broiler chickens.

2. MATERIAL AND METHODS

2.1. Chickens, management, and housing:
The current study was undertaken at Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University in accordance with guidelines of institutional Animal Care and Use Committee Research Ethics Board (No. BUFVTM 02-12-20).

Seventy-two one-day-old Cobb broiler chicks bought from El-Dakahlia Company, and were allotted randomly into two groups, each containing three replicates of 12 chicks. The house was clean, disinfected, well-ventilated space with proper environmental temperature according to the age of chicks. Lightening was provided for 24 hours throughout the experimental period. The litter consists of fresh wood shaving. Feed and water were ad libitum. Vitamin E was obtained from AB Chemical Raw Materials Company, El-Dakahlia Governorate, Mansoura City. The broiler chicks were randomly allocated into two groups: Group I: supplemented with the basal diet as shown as in table 1. Group II: supplemented with basal diet + vitamin E (100 mg/kg ration) according to (Dalia et al., 2018). The chicks were given a well-balanced diet (NRC, 1994). The experimental diets were fed in four phases: starter (0-7 day), grower (9 to 18 day (growing), 19 to 28 day (finisher1) and 29 to 35 day (finisher2).

Table 1 Ingredients and nutrient composition of starter, grower, and finisher diets (Basal diet).

| Ingredient       | Starter | Grower | Finisher1 | Finisher2 |
|------------------|---------|--------|-----------|-----------|
| Yellow corn      | 53.97   | 52.17  | 58.66     | 62.38     |
| Soybean meal     | 33.40   | 32.70  | 31.50     | 22.40     |
| Corn gluten meal | 5.70    | 2.60   | 1.80      | 5.70      |
| Vegetable oil    | 2.30    | 3.40   | 4.40      | 4.20      |
| Lysine           | 1.14    | 1.35   | 1.23      | 2.70      |
| DL calcium phosphate | 1.43 | 1.23 | 1.00 | 1.05 |
| L-threonine      | 0.39    | 0.29   | 0.21      | 0.37      |
| DL-methionine    | 0.31    | 0.31   | 0.29      | 0.24      |
| Vitamin premix   | 0.30    | 0.30   | 0.30      | 0.30      |
| Sodium chloride  | 0.29    | 0.29   | 0.29      | 0.29      |
| Sodium bicarbonate | 0.16 | 0.12 | 0.13 | 0.14 |
| L-threonine      | 0.15    | 0.10   | 0.05      | 0.08      |
| Anti-esteroidal  | 0.05    | 0.05   | 0.05      | 0.05      |
| Anti-microtubin  | 0.05    | 0.05   | 0.05      | 0.05      |
| Choline chloride | 0.05    | 0.05   | 0.05      | 0.05      |
| Energy enzyme    | 0.02    | -      | -         | -         |
| Phosphate enzyme | 0.01    | 0.01   | 0.01      | 0.01      |

2.2. Groups and treatments:
Vitamin E was obtained from AB chem Pharmaceutical Raw Materials Company, El-Dakahlia Governorate, Mansoura City. The broiler chicks were randomly allocated into two groups: Group I: supplemented with the basal diet as shown as in table 1. Group II: supplemented with basal diet + vitamin E (100 mg/kg ration) according to (Dalia et al., 2018). The chicks were given a well-balanced diet (NRC, 1994). The experimental diets were fed in four phases: starter (0-7 day), grower (9 to 18 day (growing), 19 to 28 day (finisher1) and 29 to 35 day (finisher2).

2.3. Determination of pro-inflammatory and anti-inflammatory cytokines gene expression:
2.3.1. Samples collection:
At day 35, 18 representative randomly selected birds (n= three birds/replicate) had been slaughtered for sampling. Samples of spleen and liver had been collected and saved at -80°C for further analysis.

2.3.2. RNA isolation and Real-time PCR for cytokines gene expression:
Extraction of overall RNA was performed according to manufacturer's procedure with Trizol Reagent (Invitrogen, Korea). Concentration and purity of RNA was tested by Spectro Star Nanodrop (BMG Lab Tec. GmbH, Germany) at 260/280 nm absorbance. Then by using 2X Reverse Transcriptase Master Mix (Applied Bio system, USA) according to manufacturer instructions, approximately 2 μg of total RNA had been reverse transcribed to cDNA. With the support of NCBI Primer-BLAST software, primers had been planned. Table 2 illustrates primers used for quantitative real time PCR (qRT-PCR).

Table 2 Primers used for qRT-PCR

| Primer name | Sequence | Accession number |
|-------------|----------|-----------------|
| F-actin     | F-ACCCCAAGGCAACAGA | EU309690 |
|             | R-CCAGGATCCATACACGATCC |           |
| IFN-γ       | F-CTGAAAGACTGGACGAGAGG | FF788637 |
|             | R-CACCACTGCTGAAAGATGC |           |
| IL-1β       | F-GTAGGCGTCACATGGCCTGTA | HM179638 |
|             | R-TGTCAGCGGCTAGAAAGTGAAG |           |
| IL-4        | F-TGCCTCCAGCTGCTTCTGCTG | GU119892 |
|             | R-ACGACATGGAGAGGACGAC |           |
| IL-10       | F-ACGAGTCAGGGAGAGCTTC | EF554720 |
|             | R-ATCACAGGATCTCCTCGAT |           |

2.4. Determination of HI antibody titers against NDV: Haemagglutination inhibition (HI) was applied to determine antibodies to NDV. Samples of blood have been obtained from five birds from each group at 7th, 14th, 21st, 28th, 35th day of age. Clotted blood samples had been centrifuged in order to extract pure serum at 3000 rpm for 15 minutes. The serum samples were kept in labeled sterile Eppendorf tubes and stored at -20 °C till used, using microtite U-shape plate of 96 wells (Majiyagbe and Hitchner, 1977).

2.5. Determination of total protein, albumin, and globulin: Total proteins (g/dl) were detected at day 35 with 10 serum samples (five samples from each group) according to the method designated by Weichselbaum (1946). The colorimetric approach for the identification of plasma albumin (g/dl) as defined in Doumas et al., (1971). For calculation of globulin, make subtraction of serum albumin from serum whole protein. Globulin = overall protein – albumin.

2.6. Growth parameters:
2.6.1. Body weight (BW):
The chicks had been weighed individually (in gram) at day 1, and then the live body weight was recorded every week till 8th week (Omar, 2014).
2.6.2. Body weight gain (BWG): 
Body weight gain was calculated by subtracting the body weight between two successive weights every week.

2.6.3. Feed Intake (FI): 
Weekly feed intake was estimated by subtracting the amount of feed remained from total amount offered in each group (in grams).

2.6.4. Feed Conversion Ratio (FCR): according to Lambert et al., (1936).
FCR = Feed intake (g/chick/week) / Body Weight Gain (g/chick/week).

2.7. Statistical analysis:
Data analysis was carried out using the SPSS statistical software package (Version 23; SPSS Inc., Chicago, IL, USA). The results achieved were found by the independent sample t-test study to be mean ± SE. Meaningful significance (P<0.05).

3. RESULTS
Data herein indicated a significant (P< 0.05) improvement of growth efficiency (BW, BWG, FI and FCR) in group enriched with vitamin E in relation to control one (Table 3). Results of total protein, albumin and globulin as influenced by dietary supplementation of vitamin E (Table 4) indicated that serum total protein, albumin and globulin for chicks enriched with vitamin E have considerably (P< 0.05) higher values than those of their control group.

Table 3 Effect of dietary supplementation of vitamin E on BW, BWG, FI and FCR of broiler chickens.

| Parameter     | Control group | Vitamin E suppl. group |
|---------------|---------------|------------------------|
| B. Wt. (g/chick) | Initial wt. | LSm ± SE | LSm ± SE |
| 1st week      | 47.11 ± 0.48  | 48.22 ± 0.52 |
| 2nd week      | 140.22 ± 1.43 | 155.56 ± 1.44 |
| 3rd week      | 326.89 ± 1.86 | 363.11 ± 1.42 |
| 4th week      | 497.33 ± 1.56 | 753.56 ± 1.79 |
| 5th week      | 1088.67 ± 2.26| 1205.56 ± 2.42 |
| Final BWW     | 1672.22 ± 2.53| 1840.00 ± 2.43 |

| BWG (g/chick) | Initial wt. | LSm ± SE | LSm ± SE |
| 1st week      | 93.11 ± 1.46 | 107.33 ± 1.33 |
| 2nd week      | 186.67 ± 2.19 | 207.56 ± 2.13 |
| 3rd week      | 370.44 ± 1.56 | 390.44 ± 1.48 |
| 4th week      | 451.33 ± 1.41 | 452.00 ± 1.20 |
| 5th week      | 583.56 ± 1.60 | 634.44 ± 1.56 |
| Final BWG     | 1625.11 ± 2.81| 1791.78 ± 2.50 |

| FI (g/chicks) | Initial wt. | LSm ± SE | LSm ± SE |
| 1st week      | 119.44 ± 0.22 | 127.09 ± 0.43 |
| 2nd week      | 372.90 ± 0.76 | 365.13 ± 0.38 |
| 3rd week      | 706.17 ± 0.66 | 619.51 ± 0.33 |
| 4th week      | 772.12 ± 1.28 | 745.12 ± 1.09 |
| 5th week      | 960.28 ± 2.10 | 912.07 ± 1.22 |
| Final FI      | 2880.91 ± 1.51| 2708.93 ± 1.02 |

| FCR           | Initial wt. | LSm ± SE | LSm ± SE |
| 1st week      | 1.39 ± 0.04  | 1.28 ± 0.02 |
| 2nd week      | 2.07 ± 0.05  | 1.77 ± 0.02 |
| 3rd week      | 1.79 ± 0.05  | 1.59 ± 0.02 |
| 4th week      | 2.01 ± 0.05  | 1.68 ± 0.01 |
| 5th week      | 1.67 ± 0.05  | 1.50 ± 0.02 |
| Final FCR     | 1.78 ± 0.02  | 1.56 ± 0.02 |

Values are means ± standard error. Mean values with different letters within the same row significantly varied P<0.05.

Table 4 Effect of dietary supplementation of vitamin E on serum total protein, albumin, and globulin of broiler chickens.

| Parameter       | Control group | Vitamin E suppl. group |
|-----------------|---------------|------------------------|
| Total protein   | 3.04 ± 0.02   | 4.44 ± 0.01           |
| Albumin         | 1.39 ± 0.01   | 1.56 ± 0.01           |
| Globulin        | 1.65 ± 0.04   | 2.86 ± 0.05           |

Values are means ± standard error. Mean values with different letters within the same row significantly varied P<0.05.

Table 5 showed results of dietary vitamin E supplements on antibody titers for NDV. This result showed that there was no significance difference between control group and group supplemented with vitamin E at week 1 and week 2, whereas the vitamin E uptake group significantly (p<0.05) increased antibody titers against NDV at week 3, week 4 and week 5 compared to control one.

The effect of dietary vitamin E addition on pro-inflammatory (IFN-γ, IL-1β) and anti-inflammatory (IL-4, IL-10) gene expression cytokines in spleen and liver was shown in figs. 1 and 2. These findings revealed the significant (p<0.05) decrease in expression of both pro- and anti-inflammatory cytokine genes in the vitamin E group (p<0.05) compared to the control group.

4. DISCUSSION
This study showed that addition of vitamin E in the diet of broilers resulted in a significant improvement in growth efficiency compared to control group. This result was consistent with Maimi et al. (2007), who noted that supplementation of 200 mg/kg of vitamin E in broiler diet cause an increase of body weight compared to the basal diet. Using of vitamin E as a supplement revealed a significant (P<0.05) and better FCR (Abd El-Hack et al., 2017). Also, Hedayati et al. (2021) explained that vitamin E enriched group had a better growth performance than control one.

Table 5 Effect of dietary supplementation of vitamin E on serum H2 titers (Log2) against NDV of broiler chicks.

| Parameter     | Control group | Vitamin E suppl. group |
|---------------|---------------|------------------------|
| Day 7         | 5.60 ± 0.51*  | 6.20 ± 0.37*           |
| Day 14        | 2.60 ± 0.25*  | 3.00 ± 0.32*           |
| Day 21        | 4.60 ± 0.25*  | 6.80 ± 0.66*           |
| Day 28        | 5.60 ± 0.40*  | 7.40 ± 0.60*           |
| Day 35        | 4.80 ± 0.37*  | 5.80 ± 0.37*           |

Values are means ± standard error. Mean values with different letters within the same row significantly varied P<0.05.
similar to Rashidi et al. (2010), who found that vitamin E supplementation improved plasma total protein, albumin and globulin. Also, Gouda et al. (2019), who found that supplementation of vitamin E (200 IU/kg diet) increase the total plasma protein and globulin values (P < 0.05) at 42 day old chicks. Moreover, Atta et al. (2020) explained the same result. The increase in gamma-globulins is caused by the increase in immunoglobulin production (Gružauskas et al., 2014).

Regarding the HI titers against NDV, the result showed a significant increase of antibody titers against NDV at the 3rd, 5th weeks in the group supplemented with vitamin E relative to control. These results were acceptable with Swain et al. (2000), who found that broiler chicks, which were fed with vitamin E, have considerably enhanced antibody titers against NDV. In the community fortified with Vitamin E (200 mg/kg). Desoky (2018) documented a significantly improved humoral immune response against Newcastle. Sheikh et al. (2020) found that at week 3 of vitamin C (500 mg/kg) and vitamin E (200 mg/g) significantly enhance (P < 0.05) antibody titers. Vitamin E treatment showed the highest titer while the lowest titer was in the control.

The current study showed that the gene expression of pro- and anti-inflammatory cytokines in the vitamin E enriched group was significantly (P < 0.05) lower compared to the basal dietary group. This finding is in agreement with Leshchinsky and Klasing (2003), who noted that the addition of Vit E decreased the expression of proinflammatory cytokines in lipopolysaccharide-receiving chickens. Also, Zhang et al. (2010) observed a reduction in plasma protein levels of both inflammatory cytokines (IFN-γ, IL-1β) and (IL-4 and IL-10) in alpha-tocopherol dietary supplementations. McCary et al. (2011), found that the expression of some cytokines, such as the IL-10, is decreased by higher doses of tocopherols. The same trend was reported by El-Senousy et al. (2018), who found that the dietary addition of vitamin C, vitamin E or alpha lipoic acid (ALA) had greatly decreased the mRNA expression levels of IL-1β, IL-6 and IFN-γ in the spleens of broilers in relative to the control group. Moreover, Khatun et al. (2020) indicated that the IFN-γ pro-inflammatory cytokine was decreased by 0.25 percent L-Arginine and 50–150 mg/kg vitamin E supplementations. The reason of our observation for the down-regulation of both pro- and anti-inflammatory cytokines may be due to the increase vitamin E level in broiler diet cause maintaining the (T-helper cell1/T-helper cell2) balance leading to increase balance of inflammatory response (Kaiser et al., 2012). When the Th1/Th2 equilibrium is disrupted, the cytokines secreted by Th1/Th2 cells are abnormally expressed, causing the inflammation to develop (Zhao et al., 2020). Alteration the expression of cytokines in broiler chickens that could have a beneficial impact on immune function (Khatun et al., 2020). This explains the immunomodulatory effect of vitamin E.

5. CONCLUSION

From these results, it could be concluded that supplementation of vitamin E (alpha-tocopherol acetate 100 mg/kg diet) in the diet of broiler chickens may cause a down-regulation of inflammatory cytokines (pro and anti-), as well as an increase of HI titers against NDV, total protein, albumin, globulin, and growth efficiency of broiler chickens.

6. REFERENCES

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