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*M Abdulmohsen, MI Al-Rasheedb, M Shawky, SM Abdel-Raheem*

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# Impact of Dietary Supplementation of Vitamins C and E on the Productive Traits, Egg Quality and Immune Response of Guinea Fowl in Al-Ahsa Province, Saudia Arabia

**M. Abdulmohsen[1,2], M. Al-Rasheed[3], M. Shawky[4,5], S.M. Abdel-Raheem[1,6]**

[1]*Department of Public Health, Collegeof Veterinary Medicine, King Faisal University, Al-Ahsa, P.O. Box: 400, Al Hofuf 31982, Saudi Arabia*

[2]*Department of Animal Behavior, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt*

[3]*College of Veterinary Medicine, Avian Research Center, King Faisal University, Al-Ahsa,Saudi Arabia*

[4]*Avian Research Center, King Faisal University, Al-Ahsa, Saudi Arabia*

[5]*Veterinary Serum and Vaccine Research Institute, Cairo, Egypt*

[6]*Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt*

**ABSTRACT:** Guinea fowl production makes significant contributions to animal protein through production of cheap meat and eggs, which serve as a buffer to the shortages of poultry products. This study aims to investigate the effect of vitamin C and vitamin E either solely and together on laying performance, egg quality, and some blood parameters of Guinea fowl. A total of 180, 8-month-oldblack Guinea fowls were equally divided into 4 groups, 45 birds each. Each group was subdivided into 3 replicates, 15 birds each.. The dietary treatments included the control group (basal diet); basal diet plus 500 mg /kg of diet vitamin C(T1); basal diet plus 200 mg/kg of diet vitamin E and basal diet plus 500 mg vitamin C and 200 mg vitamin E /kg diet. The results revealed that the overall production parameters including livability of laying Guinea fowl were significantly ($P<0.05$) improved by the dietary inclusion of vitamin C and E singly or in combinations. The hen day egg production %, egg mass, albumen height, albumen weight, egg length, egg width, and egg shell thickness were significantly ($P < 0.01$) higher in group supplemented with the combination of vitamin C and E in comparison with other treatment groups. In addition, supplementation with the dietary combination of vitamin C and E not only significantly ($P<0.01$) increased the level of antibody titer against Newcastle virus vaccine and serum total protein but also significantly ($P<0.01$) decreased serum total cholesterol than single supplementation of each vitamins. In conclusion, the combined dietary supplementation of 500 mg /kg vitamin C and 200 mg/kg vitamin E had proved to be successful in improving the productive performance, egg quality, the immune responses, and the general health of black Guinea fowl under present experiment conditions. This study proposes the synergistic beneficial action between vitamin C and vitamin E when supplemented together to Guinea fowl.

***Keywords:*** Vitamins C; vitamin E; immune response; Guinea fowl; laying performance; egg quality

*Corresponding Author:*
Sherief Mohamed Abdel-Raheem, Department of Public Health, College of Veterinary Medicine, King Faisal University, P.O Box 400, Al-Ahsa, 31982, Saudi Arabia.
E-mail address: sdiab@kfu.edu.sa

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## INTRODUCTION

Guinea fowl is domesticated 2000 years B.C. in Greece, and descends from wild Guinea fowl (*Numida meleagris*) were found in South Africa and Madagascar. They are raised for meat and egg production, and known for their taste and nutritional value (Ayorinde, 1991).

Vitamin E and vitamin C are important antioxidants in biological systems, and they can work alone or in tandem. Vitamin E performs its antioxidant effect in lipid phases, while vitamin C performs its antioxidant function in aqueous compartments by reacting with peroxyl radicals and restoring vitamin E antioxidant ability (Cotelle et al., 2003). Moreover, Vitamin C ensures the health and integrity of various cells including lymphocytes and prevents the cell damage caused by liberated free radicals from infections or toxins (Nimse and Pol, 2015).

Vitamins E and C are crucial for the immune system (Shojadoost et al., 2021) and their deficiency affects antibody-mediated responses and immune regulation. Consequently, vitamins supplementation promotes in the fight against microbial pathogens and boosts vaccine immune responses. In fact, vitamins deficiency causes immunological failure, which consequently increases the susceptibility to infections and decreases the growth parameters (Koutsos et al., 2006).

Vitamins and other dietary supplements have productive and reproductive functions. This because of their stress-relieving, anti-inflammatory, antioxidant, anti-aging, and immunity-related properties (Abedi et al., 2017;El-Senousy et al.,2018; Dalia et al., 2018; Liu et al., 2019; Amevor et al., 2021).Supplementation of vitamins E and C, individually or together induced improvement in both egg production and quality of chickens (Ajakaiye et al., 2011; Caurez and Olo, 2013). However, the information about the effect of vitamin C and E on the laying performance and immune response of Guinea fowl is lacking. Therefore, this study aims to investigate the effects of vitamin C and E solely and together on the productive performance, egg quality, and immune response of Guinea fowl at Al-Ahsa province in Saudi Arabia.

## MATERIALS AND METHODS

### *Statement of animal rights*

According to the Ethical Committee for Laboratory Animal Research at King Faisal University's advice. The institutional and national guidelines that ensure the animal rights were strictly followed during this study.

### *Animals, diets and experimental protocol*

One hundred and eighty, 8-mounth-old non-vaccinated black Guinea fowl hens were obtained from Agriculture Research Station, Poultry Research Unit, and King Faisal University, Saudi Arabia. They were randomly divided into 4 groups, 45 bird of each. Each group was divided into 3 replicates cages (15 each). Each replicate was raised in a separate chamber with a diameter of 1.5 by 2 meters with clean water and feeder. Throughout the trial, hens were housed at 21°Cand 45% relative humidity.The experiment was carried out during the summer with special lighting schedules 16 hours of light: 8 hours of darkness (16L: 8D).

### *Dietary treatments*

The first treatment was assigned as a control (T0) in which no supplement was added to the basal diet,while other treatments (T1, T2, and T3) were fed on the basal diet supplemented with 500 mg vitamin C, 200 mg Vitamin E and selenium, 500 mg vitamin C plus 200 mg vitamin E and selenium per kg of feed, respectively. The additives were applied next day after vaccination with Newcastle disease virus vaccine. The treatments were continued for 3 months from 33 to 45 weeks of age. The basal diet composition was shown in Table 1. Feed (offered in a mash form), and water were available *ad libitum* throughout the experiment. The hens were fed a standard layer diet with 2800 metabolizable energy (ME) kcal/kg and 18% crude protein which met or slightly exceeded the NRC's (1996) dietary requirements (Table1). At 08:00 and 17:00 hours each day, basal feed was provided *ad libitum*, and clean tap water was available all the time. Every day, the difference between the amount of offered feed and left one for each pen was determined to calculate the amount of the consumed feed.

### *Newcastle disease virus (NDV) vaccine*

Vaccination against NDV was carried out using living LaSota vaccine in the drinking water before the addition of vitamins to Guinea fowl diet.

### *Blood samples*

Blood samples were collected from wing vein for all groups at four time intervals 0, 30, 60 and 90 days from the start of the experiment.

**Table 1.** Composition and analysis (%, as fed) of the experimental diets.

| Ingredient and analysis (%) | Basal laying diet |
|---|---|
| Yellow corn | 61.06 |
| Soybean meal | 26.3 |
| Sunflower oil | 1.2 |
| Limestone flour | 8 |
| Calcium dibasic phosphate | 2.5 |
| Common salt | 0.4 |
| DL-methionine | 0.1 |
| L-Lysine HCL | 0.1 |
| Vitamin & mineral premix* | 0.3 |
| Antioxidant | 0.04 |
| Total | 100 |
| Chemical analysis (% on as fed basis) | |
| ME, Kcal/kg | 2800 |
| Crude protein | 18.00 |
| Dry matter | 89.76 |
| Crude fiber | 2.86 |
| Ether extract | 3.17 |
| Ash | 13.36 |
| Nitrogen free-extract | 52.36 |
| Organic matter | 76.4 |
| Calcium | 3.67 |
| Total phosphorus | 0.80 |
| Methionine | 0.39 |
| Lysine | 1.04 |
| Methionine+ cysteine | 0.69 |

*Each kg contains: vitamin A 40000 IU; vitamin D 100000 IU; vitamin E 233.3 mg; vitamin $K_3$ 166.7 mg; 166.6 mg Vit. B1, 66.7 mg Vit. B2; 200 mg Vit. B6; 1 mg Vit. B12; 150 mg Vit. C; 1000 mg Niacin; choline chloride 3333.3 mg; folic acid 100 mg; biotin 2 mg; pantothenic acid 223.3 mg; magnesium sulfate 1000 mg; iron sulfate 333.3 mg; zinc sulfate 500 mg; cobalt sulfate 100 mg.

### *Body weight*

At the beginning and the end of experiment, each hen was individually weighed using a sensitive electronic balance, and the difference between the initial and final weights was used to calculate body weight change.

### *Egg parameters*

At 08:00 and 17:00 hours, eggs laid in each pen were collected twice daily. The average egg weight was calculated by dividing the total egg weights by the number of gathered eggs each week from each pen. Assessment of egg quality was performed by using NABEL (Digital Egg Tester 6000, Japan) and the process was repeated every week. An electronic caliper was used to measure egg length (along the longitudinal axis) and egg width (along the equatorial axis).A micrometer screw was used to measure the thickness of the egg shell (mm) at the equator to the nearest 0.001 mm. The color of the yolk was evaluated using a La Roche 15-point scale fan. Haugh units (HU) were calculated using the height of thick albumen (H) and egg weight (W) as a main basis, using Haugh's formula (1937):

HU = 100 log (H + 7.57 - 1.7 W0.37). The Egg mass (Per hen per day in grams) was calculated by this formula:

Average egg mass = Per cent HDEP × Average egg weight in grams

Feed conversion ratio (FCR) was estimated by dividing the gram of feed consumed by the gram of eggs produced (egg mass).

Hen-day egg production was determined according to Hunton (1995) as follows.

$$\text{HDEP} = \frac{\text{Total number of eggs produced on a day}}{\text{Total number of hens present on that day}} \text{ x } 100$$

### *Enzyme-Linked immunosorbent Assay (ELISA)*

The antibody titers against ND were determined using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (ID Screen® Newcastle Disease Nucleoprotein Indirect, France.

### *Statistical analysis*

The statistical analysis was carried out using SPSS (version 19). To see if the variables were normally distributed, the Kolmogorov-Smirnov normality test was used. To compare the means of the groups, a one-way analysis of variance (ANOVA) was used. The effect of treatments on the studied parameters was investigated using the Duncan multiple range test (Steel and Torrie, 1980). Statistical significance was estimated at ($p < 0.05$).

## RESULTS

### *Laying performance and egg quality*

Overall production performances including livability of laying Guinea fowl were significantly improved ($P<0.05$) by the dietary addition of vitamins (Table 2).In the period of 33 to 45 weeks of age, the hen day egg production %, the egg mass, albumen height, albumen weight, egg length, egg width, and egg shell thickness of vitamin C +E supplemented group were higher than those of the other treatment groups (P < 0.01).Birds of the group supplemented by combinations of both vitamin C and E showed the highest values (P<0.01) in most of laying performance and egg quality parameters, followed in order by the group supplemented solely by vitamin E or C.There were no significant differences (P > 0.05) among the treatment groups in terms of initial and final body weight, yolk height, yolk weight, yolk color, and egg shell weight. Feed conversion ratio was significantly improved (P<0.01) by addition of vitamins and the highest values were recorded in the control group.

### *Biochemical parameters*

During the three periods (30, 60 and 90 days post vaccinations), serum total protein (TP) levels ranged from 21 to 46 g/l. the highest (P < 0.05) levels of serum TP were observed in the vitamin C and E supplemented group however the lowest (P < 0.05) levels was recorded in the control group (Fig 1.). Furthermore, serum total cholesterol level was significantly decreased (P<0.01) in vitamin C+E group in comparison with the control and the other treatment during the three times post-vaccination.

### *Immune response against NDV*

The combination of both vitamin C + E significantly improved the serum NDV antibody titer in vaccinated Guinea fowl groups on day 30 post-vaccination (Table 3). On numerical base, There was an increase numerically in serum NDV antibody titer at 60 and 90 days post-vaccination in all treatment groups in comparison with the control, however these elevation in titers was not statically significant (p>0.05).

## DISCUSSION

The production of layers is crucial to the world's population's food security. Both vitamin C and E are essential for the performance and egg quality of laying hen (Sahin et al., 2006; Ajakaiye et al., 2010; Ajakaiye et al., 2011; Caurez and Olo 2013; Abedi et al., 2017). It is essential to keep the body's metabolic activity and to meet physiological needs. This study indicated that dietary supplementation of vitamin C or vitamin E alone or together had improved most of the laying performance parameters. Similar findings were reported in this regard (Dalia et al., 2018; Liu et al., 2019,Sigolo et al., 2019, Amevor et al., 2021).

The highest values of laying performance and egg quality traits were recorded in the group supplemented with a combination of vitamin C and E in comparison with other groups. Several studies revealed that feeding Japanese quails, broilers, and laying hens with supra-nutritional quantities of vitamin E and

**Table 2.** Effect of dietary treatment on laying performance and egg quality parameters of guinea fowl (Mean± SE)

| | Control | Vit. C | Vit. E | Vit.C+E | P- value |
|---|---|---|---|---|---|
| Daily feed intake/g/hen | 150.75±3.23[a] | 138.87±2.21[b] | 132.22±2.64[b] | 136.42±1.06[b] | <0.001 |
| Initial BW, g | 1402.5±5.5 | 1407.67±5.94 | 1420±5.94 | 1421.25±6.88 | 0.09 |
| Final body weight | 1568.5±12.5 | 1593.25±15.15 | 1594.91±14.09 | 1614.83±13.43 | 0.14 |
| Body weight change (g/hen) | 166.00±7.7[b] | 185.58±9.82[ab] | 174.92±9.13[ab] | 193.58±8.39[a] | 0.15 |
| Hen-day egg production (%) | 49.81±1.54[c] | 49.76±1.89[c] | 55.56±2.06[b] | 61.67±1.86[a] | <0.001 |
| Egg weight (g) | 40.05±0.69[c] | 41.89±0.30[a] | 42.12±0.69[b] | 44.36±0.4[a] | <0.001 |
| Egg mass (g/hen/day) | 19.89±0.5[a] | 20.86±0.84[c] | 23.47±1.01[b] | 27.37±0.95[a] | <0.001 |
| Feed conversionratio(g feed /g egg) | 7.64±0.27[a] | 6.81±0.33[b] | 5.75±0.27[c] | 5.05±0.17[c] | <0.001 |
| Livability (%) | 94.00±0.58[a] | 97.33±0.33[b] | 98.00±0.57[b] | 100.00±00[a] | <0.001 |
| Albumen height (mm) | 4.89±0.1[c] | 5.15±0.07[bc] | 5.27±0.097[b] | 5.64±0.10[a] | <0.001 |
| Albumen weight (g) | 19.34±0.26[b] | 20.93±0.26[a] | 21.02±0.25[a] | 20.42±0.26[a] | <0.001 |
| Yolk height (mm) | 13.6±0.29 | 14.76±0.32 | 13.86±0.58 | 14.57±0.44 | 0.18 |
| Yolk weight (g) | 16.25±0.76 | 17.83±0.76 | 18.28±0.76 | 18.98±0.75 | 0.08 |
| Yolk color (score) | 6.16±0.61 | 8.08±0.26 | 7.62±0.78 | 7.87±0.61 | 0.23 |
| Egg length (mm) | 43.42±0.26[c] | 47.09±0.17[b] | 48.13±0.19[a] | 48.36±0.22[a] | <0.001 |
| Egg width (mm) | 35.41±0.11[d] | 36.38±0.09[c] | 36.75±0.19[b] | 37.15±0.06[a] | <0.001 |
| Egg shell weight (g) | 6.17±0.33 | 6.27±0.25 | 6.15±0.19 | 6.91±0.33 | 0.21 |
| Egg Shell thickness (mm) | 0.48±0.02[b] | 0.57±0.01[a] | 0.56±0.01[a] | 0.61±0.01[a] | <0.001 |
| Hauf unit | 76.24±0.83[b] | 77.41±0.52[b] | 78.18±0.50[ab] | 79.88±0.69[a] | <0.01 |

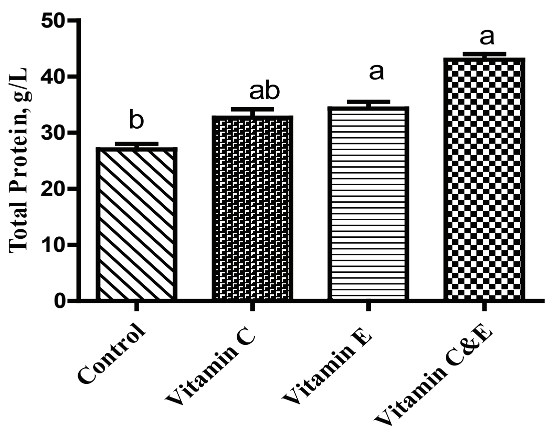
Vit., Vitamin

**Table 3.** Mean values of Newcastle disease (ND) antibody titer in vaccinated groups (Mean± SE, anti-log10)

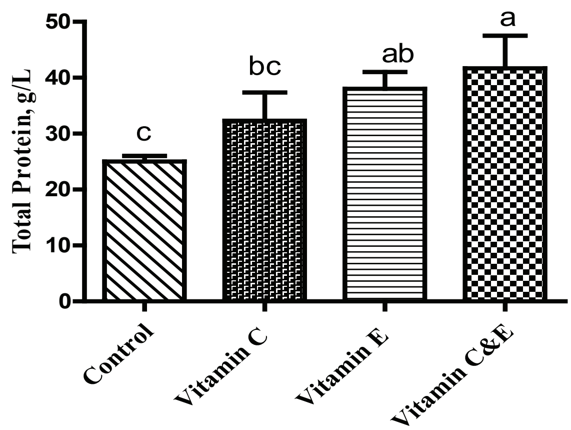
| | Control | Vit. C | Vit. E | Vit. C+E | P- value |
|---|---|---|---|---|---|
| **0 time** | 250.19±41.58 | 257.29±22.41 | 327.92±51.45 | 201.6±24.68 | 0.17 |
| **30 day** | 4674.2±670.02[b] | 6329.00±548.81[b] | 5283.57±486.99[b] | 7898.01±207.22[a] | 0.03 |
| **60 day** | 4181.87±679.32 | 6683.39±571.52 | 6643.99±516.12 | 6836.26±147.92 | 0.95 |
| **90 day** | 2397.67±1507.41 | 4626.82±710.05 | 3666.17±502.01 | 4546.82±789.27 | 0.36 |

Vit., Vitamin

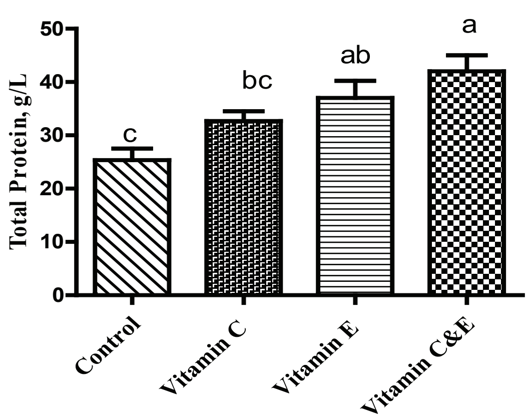
**Fig1.** Effect of dietary treatments on serum total protein and total cholesterol (A, B and C) for total protein, (D, E and F) for total cholesterol (means+SD)



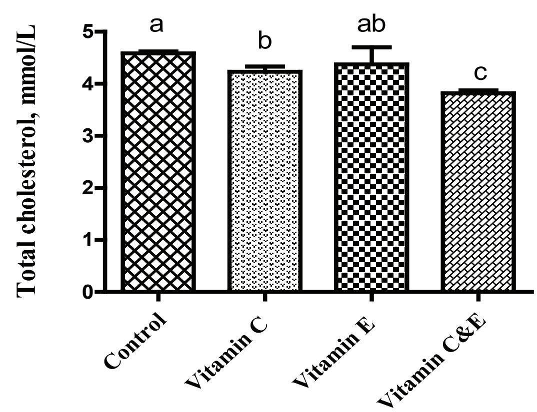

A) (Total protein at day 30)



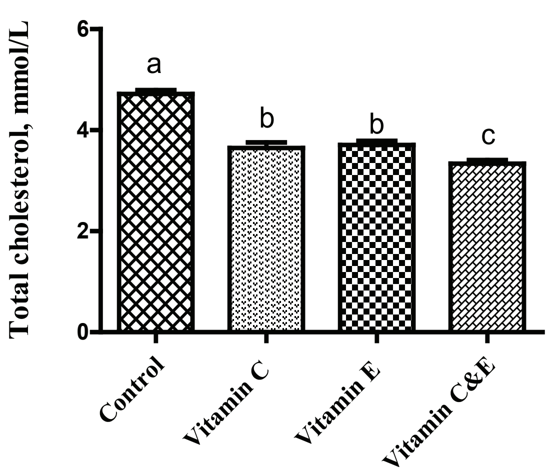

B) (Total protein at day 60)



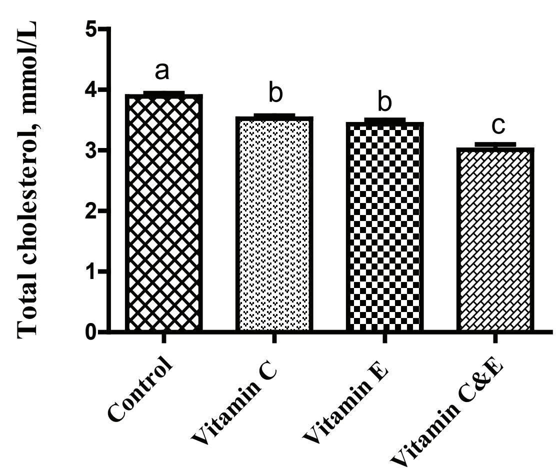

C) (Total protein at day 90)




D) (Total Cholesterol at day 30)




E) (Total Cholesterol at day 60)




F) (Total Cholesterol at day 30)

C in thermoneutral conditions improved the growth performance, egg production qualities, and overall animal wellbeing (Sahin et al., 2006; Ajakaiye et al., 2010; Caurez and Olo, 2013; Abedi et al. 2017; Sigolo et al. 2019a; Sigolo et al., 2019b, Amevor et al., 2021).Vitamin E stimulates the liver to produce the yolk precursor (vitellogenin)and also it acts as an anti-stressor by eliminating free radicals and preventing lipid peroxidation.This in turn improves the egg production and quality in laying hens(Jiang et al., 2013; Rahman et al. 2017).

In laying hens under thermoneutral or heat stress, nutritional supplementation with vitamin C alone improved laying performance and egg quality features in terms of albumen index, increased yolk height, yolk index, egg shape index, and Haugh unit )Sahin et al., 2003; Saki et al., 2010, Skrivan et al., 2013).Vitamin C stimulates 1, 25 dihydroxy cholecalciferol, which improves calcium absorption and mobilization from bone, implying that it has a role in eggshell development and quality, as well as perhaps improving bone characteris ) Leeson et al., 1995;Sahin and Sahin, 2002; Chung et al., 2005). On the other hand, Wang et al. (2011) found that vitamin C had no influence on egg weight or production in laying hens.

Many previous studies have highlighted the effectiveness of vitamins C and E in enhancing laying performance and egg quality traits when used together than separately Abedi et al., 2017;Sigolo et al., 2019a; Sigolo et al., 2019b; Amevor et al., 2021).Vitamin E and C, due to their antioxidant properties, play an important role in improving poultry thermotolerance. They promote protein production by suppressing the cytotoxic impact of free radicals, which protects proteins from oxidative denaturation and improving digestibility and metabolic utilization of nutrients (Ipek et al., 2007; Ahmadu et al., 2016).

In accordance with the present results, previous studies reported that supplementation of vitamins C and E together resulted in achieving better results and improved immune responses against NDV than the situation of individual supplementation of each vitamin.

Our results indicated that vitamin C and E had beneficial effects on serum levels of NDV antibodies in Guinea fowl when used separately or in combination and the highest values were observed in the group received a combination of both vitamins. In accordance with the present results, previous researches have established that supplementation of vitamin C and E together improves the humoral immune response to NDV more effectively than supplementing these vitamins individually (Mohiti-Asli et al., 2010; Habibian et al., 2014, Khan et al., 2014, Bhatti et al., 2016). Vitamin E promotes antibody production, whereas vitamin C increases macrophage activity, resulting in a synergistic effect (Raja and Qureshi, 2000; Aengwanich et al., 2003).One possible explanation is that vitamin E antioxidant properties enhance antibodies production by improving plasma cell function for increased antibody production. However, because of the effect of vitamin E on T cells, it's become hard to say whether vitamin E stimulates antibody production directly by activating B cells or indirectly through T cells (Lee and Han, 2018).However, the antioxidant properties of vitamin C protect immature cells from free radical damage and promote the immune response, resulting in higher antibody titers against the NDV when various levels of ascorbic acid are supplemented (Aengwanich et al., 2003; Elagib et al., 2012).

When vitamins C and E were supplemented to the diet individually or combined, serum cholesterol levels were significantly ($p <0.05$) decreased, and the serum total protein levels were significantly ($p < 0.05$) increased. However, the highest values were found with dietary combinations of both vitamin C and E. These findings are consistent with those of prior investigations (Sahin et al., 2002,Kucuk et al., 2003, Imik et al. 2009, Jiang et al., 2013, Sigolo et al., 2019a). These researchers revealed that supplementing the diets of laying hens and quail with vitamin E and C, either separately or together, reduces the oxidative stress, increases total protein concentration in serum, and lowers serum total cholesterol. Thus, increased concentrations of total protein are accompanied by increased levels of serum albumin and globulin because albumin and globulin make up the majority of serum total protein in avian species (Scholtz et al. 2009). In contrast, previous research found that vitamin C and E supplementation had no effect on serum total cholesterol concentration in laying hens (Mohiti-Asli and Zaghari, 2010, Mirfendereski and Jahanian, 2015)

## CONCLUSIONS

The finding of the current study indicated that, the dietary supplementations of both 500 mg vitamin C and 200 mg/kg vitamin E could improve the laying performance, egg quality, the immune responses, and the general health of black Guinea fowl. This study proposes the synergistic beneficial action between vitamin C and vitamin E when supplemented together to Guinea fowl.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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