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Effects of supra-nutritional levels of vitamin E and vitamin C on growth performance and egg production traits of Japanese quails

Samantha Sigolo, Roshanak Khazaei, Alireza Seidavi, Antonio Gallo and Aldo Prandini

ABSTRACT
A study was conducted to evaluate growth performance and egg production traits of Japanese quails fed diets containing different supra-nutritional levels of vitamin E and C (600, 800 and 1000 mg/kg). A completely randomised design was adopted and main effects (vitamin E and C) were arranged in a $3^2$ factorial approach. Throughout the study (42 to 105 d), the highest feed intake (vitamin C, $p = .01$) and weight gain (vitamin E and/or C, tendency $p = .06$) were obtained with vitamin E and/or C at 800 mg/kg whereas the highest dietary efficiency with 600 mg/kg of vitamin E plus 600 or 1000 mg/kg of C (vitamin E and C, tendency $p = .06$). The highest final body weight was achieved with 1000 mg/kg of both the vitamins (vitamin E and C, $p = .02$). Vitamin E and/or C at 1000 mg/kg increased egg production, weight of produced eggs (vitamin E and C, $p = .04$), number of produced eggs to feed ratio, weight of produced eggs to feed ratio (vitamin E and C, $p < .01$), average egg volume (vitamin E and C, $p = .03$), and egg shape index (vitamin E, $p < .01$; vitamin C, $p = .01$). Current findings showed that feeding vitamin E and C at supra-nutritional levels can be a good management practice in Japanese quail nutrition to promote growth performance and egg production traits under thermoneutral condition.

HIGHLIGHTS
- Supra-nutritional levels of vitamin E and C can promote growth performance and egg production traits of Japanese quails.
- Vitamin E plus C at high doses (1000 mg/kg) can synergistically act in promoting quail growth.
- Dietary supplementation with vitamin E at 800 or 1000 mg/kg plus vitamin C at 1000 mg/kg can improve egg production traits.

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Egg production traits; growth performance; Japanese quail; vitamin C; vitamin E

Introduction
The avian egg is an important source of nutrients containing all of the proteins, lipids, vitamins and minerals as well as growth and defence factors necessary for embryo development (Tolik et al. 2014). Moreover, eggs and egg components are of great importance for human health and in disease prevention and treatment thanks to their content in numerous substances with biological functions and activities (i.e. antioxidants, antimicrobials, immunomodulators, anticancer, etc.; Kovacs-Nolan et al. 2005). Although chicken eggs are most commonly eaten by humans, a lot of people, especially in Asian countries, consume quail eggs (Tunsaringkarn et al. 2013). Moreover, the consumption of quail eggs is becoming more popular also in Europe and America (Tolik et al. 2014) because quail eggs, despite their small size, have a nutritional value three or four times greater than chicken eggs (Tunsaringkarn et al. 2013). To meet the increasing demand for quail eggs as well as meat, the rearing of commercial quails, such as Japanese quail (Coturnix coturnix Japonica) selected for rapid growth and high body weight, has been raising worldwide (Hemid et al. 2010).

The birds’ survival rate, egg-laying level and egg physical traits are the most crucial factors in quail production (Lukaszewicz et al. 2007; Lin et al. 2004). Natural antioxidants are important in promoting the health of birds with high level of egg laying (Lukaszewicz et al. 2007). Vitamin E and vitamin C play...
a major role as antioxidants in biological systems and act individually or synergistically such that vitamin E explicates its antioxidant function in lipid phases whereas vitamin C in aqueous compartments by reacting with peroxyl radicals and by restoring the antioxidant properties of vitamin E (Cotelle et al. 2003). Studies on poultry have reported that dietary supplementation with vitamins E and C, alone or together, can improve growth performance (Sahin and Kucuk 2001; Ajakaiye et al. 2010), egg production and egg quality (Ajakaiye et al. 2011; Caurez and Olo 2013). Because the most of studies were conducted under heat stress condition, further research is necessary to study the effects of additional vitamin E and C in diet of quails reared under standard environmental condition.

Therefore, the aim of this work was to evaluate the effects of different supra-nutritional levels of vitamins E and C on growth performance and egg production traits of Japanese quail during the laying period under thermoneutral environmental condition.

Materials and methods

Birds, housing and diets

All procedures were approved by the Animal Care and Welfare Committee of Islamic Azad University.

The current work was conducted at a quail farm (Amol, Iran) during March–May 2016 and represents the continuation of our previous study (Sigolo et al. 2018). At the end of the trial described by Sigolo et al. (2018), the same quails (a total of 360 Japanese female quails (Coturnix coturnix Japonica), 6 weeks old) were used in the current study. Housing and experimental design were as reported by Sigolo et al. (2018). Briefly, there were 9 dietary treatments and birds were subdivided into 4 replicates for each dietary treatment (10 birds/replicate) for a total of 36 experimental units. Each replicate was housed in a ground cage (0.70 m × 0.50 m). The trial was conducted under controlled environmental conditions. Temperature and relative humidity were respectively maintained at 24 °C and 55–65% and were near to those used by Minvielle et al. (1999) and Şahin et al. (2001). Throughout the experimental trial, the lighting programme consisted of 17 h of light and 7 h of dark in accordance with Şahin et al. (2001) and Sahin et al. (2002a).

Quail responses to different dietary levels of vitamin E and C were evaluated from 42 to 105 d of age. The standard diet was formulated to meet the nutritional requirements of breeding Japanese quails as recommended by NRC (1994). Ingredients, chemical composition and energy of the standard diet are shown in Table 1. The standard diet was supplemented with vitamin E (DL-α-tocopheryl acetate) and C (l-ascorbic acid) at 3 different levels (600, 800 and 1000 mg/kg of diet) in a 3 × 3 factorial design. All the experimental diets were iso-energetic and iso-nitrogenous. Water and experimental diets were offered ad libitum.

Growth performance

Body weight and feed intake were measured at d 42, d 63, d 84, and d 105 by the cage. The average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) were calculated for each replicate and for the periods 42 to 63 d, 64 to 84 d, 85 to 105 d, and throughout the study (i.e. 42 to 105 d).

Egg production traits

At the farm, the eggs were manually collected two times per day. The number, weight and volume of eggs were recorded at d 63, d 84 and d 105 by the cage. The egg production, number of produced eggs to feed ratio (N:F), weight of produced eggs, weight of produced eggs to feed ratio (W:F), average egg weight and average egg volume were calculated for each replicate and for the periods 42 to 63 d, 64 to 84 d, 85 to 105 d and throughout the study (i.e. 42 to 105 d). For

Table 1. Ingredients, chemical composition, and energy of the standard quail diet (from 42 to 105d of age).

| Ingredient                        | % of Diet | Calculated Analysis, % as Fed |
|-----------------------------------|-----------|------------------------------|
| Corn                              | 50.00     | ME, kcal/kg: 3000.00         |
| Soybean meal, 44% protein         | 30.17     | CP: 20.00                    |
| Wheat                             | 4.42      | Ca: 2.50                     |
| Barley                            | 2.96      | Available P: 0.55            |
| Rapeseed oil                      | 4.75      | Met: 0.45                    |
| Salt                              | 0.27      | Met + Cys: 0.76              |
| Mineral oysters                   | 3.79      | Lys: 1.15                    |
| Bone meal                         | 2.91      |                             |
| Vitamin and mineral premix<sup>1</sup> | 0.50  |                             |
| α-methionine                      | 0.10      |                             |
| Lysine hydrochloride              | 0.08      |                             |
| Enzyme                            | 0.05      |                             |

<sup>1</sup> Supplied the following per kilogram of diet: Cu, 8 mg; Fe, 50 mg; Mn, 70 mg; Zn, 50 mg; I, 1.2 mg; Se, 0.2 mg; vitamin A, 14000 U; vitamin D<sub>3</sub>, 4000 U; vitamin E, 10 mg; vitamin K<sub>3</sub>, 3.2 mg; vitamin B<sub>2</sub>, 6 mg; vitamin B<sub>12</sub>, 16 μg; niacin, 40 mg; pantothenic acid, 10 mg; antioxidant, 30 mg.
the same periods, the egg shape index was also calculated by the following formula (Alkan et al. 2010):

\[
\text{shape index} = \frac{\text{eggwidth} (\text{mm})}{\text{egglength} (\text{mm})}
\]

Statistical analysis

Data were tested for normality with the Shapiro–Wilk test and variance homogeneity with the Levene’s test before statistical analysis. Then, data were analysed according to a completely randomised design using the GLM procedure of SAS (2003) according to the model reported below:

\[
Y_{ijk} = \mu + \text{vitamin E}_i + \text{vitamin C}_j + (\text{vitamin E} \times \text{vitamin C})_ij + e_{ijk}
\]

where \(Y_{ijk}\) is the response variable, \(\mu\) is overall mean, vitamin E\(_i\) is the fixed effect of dietary level of vitamin E \((i = 3; \text{being } 600, 800 \text{ and } 1000 \text{ mg/kg}), \text{vitamin C}_j\) is the fixed effect of dietary level of vitamin C \((j = 3; \text{being } 600, 800 \text{ and } 1000 \text{ mg/kg}), (\text{vitamin E} \times \text{vitamin C})_ij\) is first-order interaction of main tested fixed effects, and \(e_{ijk}\) is random residual error. The growth performance parameters and egg production traits were analysed both within experimental periods (i.e. 42 to 63 d, 64 to 84 d or 85 to 105 d) and on whole period (i.e. 42 to 105 d). Post hoc least-squares means tests were performed with the LSMEANS option of SAS (2003), using the Tukey method for multiple testing correction. The experimental unit was the pen. Significance was considered at \(p < 0.05\) and tendency was declared at \(0.05 < p \leq 0.10\).

Results

Growth performance

The effects of different supra-nutritional levels of vitamin E and C on growth performance of Japanese quails are shown in Table 2.

Effects of interaction between vitamin E and C were observed on BW, ADFI, ADG, and G:F. As reported by Sigolo et al. (2018), after 42 d of trial the highest BW \((p < .01)\) were obtained with 1000 mg/kg of both vitamin E and C. After 63 \((p < .01)\) and 84 d \((p = .05)\), the highest BW were achieved with levels of vitamin E plus C of 800 and/or 1000 mg/kg. However, the highest final BW \((i.e. 105 d; p = .02)\) was exclusively obtained with 1000 mg/kg of both the vitamins.

In the 42 to 63 d period, the highest ADFI \((i.e. 704.55 \text{ g}; p < .01)\) was observed at 800 mg/kg of vitamin E and 1000 mg/kg of vitamin C, whereas in the 64 to 84 d period at 800 mg/kg of both vitamin E and C \((i.e. 745.35 \text{ g}; p < .01)\). In the 85 to 105 d period, no treatment effects were observed on ADFI. Throughout the study \((i.e. 42 \text{ to } 105 \text{ d}), \text{a vitamin C effect} (p = .01)\) was found on ADFI with the highest value observed at 800 mg/kg level \((i.e. 2175.77 \text{ g})\).

The highest ADG were achieved with: 800 mg/kg of vitamin E plus 800 or 1000 mg/kg of vitamin C in the 42 to 63 d period \((p < .01)\); 600 mg/kg of vitamin E plus 600 or 800 mg/kg of vitamin C in the 64 to 84 d period \((i.e. 26.08 \text{ and } 25.55 \text{ g}; p = .04)\); 1000 mg/kg of vitamin E plus 600 mg/kg of vitamin C in the 85 to 105 d period \((i.e. 21.38 \text{ g}; \text{vitamin E} \times \text{C interaction, tendency } p = .06)\); and 800 mg/kg of both the vitamins throughout the study \((i.e. 100.63 \text{ g}; \text{vitamin E} \times \text{C interaction, tendency } p = .06)\).

The highest G:F values were observed at 800 mg/kg of vitamin E plus 800 or 1000 mg/kg of vitamin C in the 42 to 63 d period \((i.e. 0.094; p < .01)\) and at 600 mg/kg of both the vitamins in the 64 to 84 d period \((i.e. 0.037; p = .03)\). In the 85 to 105 d period, vitamin E and vitamin C effects \((p < .01)\) were found on G:F. In particular, the supplementation of the diet with vitamin E at 600 or 1000 mg/kg level or vitamin C at 600 mg/kg level resulted in the highest G:F. Throughout the study, a vitamin E \(\times C\) interaction effect \((tendency, p = .06)\) was found on G:F with the highest values observed at the following levels of vitamins: 600 mg/kg of vitamin E plus 600 or 1000 mg/kg of vitamin C \((i.e. 0.047)\) and 800 mg/kg of vitamin E plus 600 or 800 mg/kg of vitamin C \((i.e. 0.047 \text{ and } 0.046, \text{respectively})\).

Egg production traits

The effects of different supra-nutritional levels of vitamin E and C on egg production traits of Japanese quails are shown in Table 3.

In the 42 to 63 d period \((p = .03)\) and throughout the study \((p = .04)\), a vitamin E \(\times C\) interaction effect was found on egg production with the highest number of eggs achieved with 1000 mg/kg of both the vitamins \((i.e. 138 \text{ and } 504, \text{respectively})\). In the 64 to 84 d period, the highest number of eggs was obtained with 1000 mg/kg of vitamin E or C, whereas in the 85 to 105 d period with 1000 mg/kg of vitamin E \((i.e. 190)\) or 800 or 1000 mg/kg of vitamin C \((\text{vitamin E and vitamin C, } p < .01)\).

In the 42 to 63 d \((\text{vitamin E and vitamin C, } p < .01)\) and 85 to 105 d \((\text{vitamin E and vitamin C, } p = .01)\) periods, and throughout the study \((\text{vitamin E and vitamin C, } p < .01)\), the highest N:F ratios, were obtained with 1000 mg/kg of vitamin E \((i.e. 0.188, 0.258, \text{and } 0.224, \text{respectively in the three periods})\) or C \((i.e. 0.193, 0.259, \text{and } 0.228, \text{respectively})\). In the 64 to 84 d
| Treatment | Vitamin E (mg/kg) | Vitamin C (mg/kg) | 42d BW | 63d BW | 84d BW | 105d BW | 42 to 63d ADG | 64 to 84d ADG | 85 to 105d ADG | 42 to 105d ADG | 42d BW | 63d BW | 84d BW | 105d BW | 42 to 63d ADFI | 64 to 84d ADFI | 85 to 105d ADFI | 42 to 105d ADFI | 42d BW | 63d BW | 84d BW | 105d BW | 42 to 63d G:F | 64 to 84d G:F | 85 to 105d G:F | 42 to 105d G:F |
|-----------|------------------|------------------|--------|--------|--------|--------|-------------|-------------|--------------|--------------|--------|--------|--------|--------|----------------|----------------|--------------|--------------|--------|--------|--------|--------|---------------|---------------|--------------|--------------|
| 600       | 600              | 248.980          | 275.030 | 295.630 | 680.350 | 714.150 | 733.850     | 2128.35     | 53.330       | 99.980       | 99.980 |       |       |       | 20.580        | 20.580        | 99.980       |       | 0.0790     | 0.0790     | 0.0280     | 0.0470     |
| 600       | 800              | 251.380          | 276.930 | 296.730 | 679.600 | 714.730 | 781.500     | 2173.83     | 54.630       | 99.980       | 99.980 |       |       |       | 19.800        | 19.800        | 99.980       |       | 0.0810     | 0.0810     | 0.0250     | 0.0460     |
| 600       | 1000             | 257.280          | 278.800 | 297.030 | 674.400 | 723.030 | 739.280     | 2136.70     | 62.230       | 99.980       | 99.980 |       |       |       | 18.230        | 18.230        | 99.980       |       | 0.0890     | 0.0890     | 0.0250     | 0.0470     |
| 800       | 600              | 263.730          | 282.800 | 298.850 | 693.700 | 743.500 | 735.200     | 2174.25     | 59.800       | 99.980       | 99.980 |       |       |       | 19.750        | 19.750        | 99.980       |       | 0.0870     | 0.0870     | 0.0270     | 0.0470     |
| 800       | 800              | 278.150          | 282.800 | 298.850 | 693.700 | 743.500 | 735.200     | 2174.25     | 65.500       | 100.630      | 100.630 |       |       |       | 19.750        | 19.750        | 99.980       |       | 0.0940     | 0.0940     | 0.0220     | 0.0460     |
| 800       | 1000             | 266.180          | 284.530 | 298.400 | 704.550 | 726.980 | 727.380     | 2158.90     | 66.580       | 98.800       | 98.800 |       |       |       | 13.880        | 13.880        | 98.800       |       | 0.0940     | 0.0940     | 0.0250     | 0.0460     |
| 1000      | 600              | 258.330          | 278.150 | 299.530 | 691.150 | 736.380 | 712.950     | 2140.48     | 56.850       | 98.050       | 98.050 |       |       |       | 21.380        | 21.380        | 98.050       |       | 0.0820     | 0.0820     | 0.0300     | 0.0460     |
| 1000      | 800              | 258.550          | 282.350 | 300.580 | 696.700 | 737.800 | 745.750     | 2177.23     | 57.300       | 99.530       | 99.530 |       |       |       | 23.800        | 23.800        | 99.530       |       | 0.0850     | 0.0850     | 0.0300     | 0.0460     |
| 1000      | 1000             | 265.950          | 284.400 | 302.250 | 701.050 | 736.800 | 748.630     | 2180.35     | 60.480       | 98.800       | 98.800 |       |       |       | 19.150        | 19.150        | 98.800       |       | 0.0840     | 0.0840     | 0.0280     | 0.0460     |

Main effect

Vitamin E

- 600: 196.480
- 800: 198.380
- 1000: 202.730

Vitamin C

- 600: 198.140
- 800: 198.740
- 1000: 200.710

SEM: 0.366, 0.690, 0.711, 0.315, 2.325, 2.254, 14.678, 15.50, 0.749, 0.968, 0.694, 0.412, 0.0012, 0.0014, 0.0010, 0.0003

p-values

Vitamin E

- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010

Vitamin C

- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010

Vitamin E × C

- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010
- <0.010: 0.0010

**BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; G:F, gain to feed ratio.**

Means within a column with different superscript letters differ (p ≤ .05).
Egg production traits of Japanese quails fed diets with different dietary levels of vitamin E and C.

| Treatment | Number of produced eggs | N:F | Weight of produced eggs, g | W:F |
|-----------|-------------------------|-----|-----------------------------|-----|
| Vitamin C |                         |     |                             |     |
| 600       | 116.00                  | 177.00 | 445.00                     |     |
| 800       | 124.00                  | 183.00 | 465.00                     |     |
| 1000      | 129.00                  | 186.00 | 475.00                     |     |
| 800       | 117.00                  | 177.00 | 445.00                     |     |
| 1000      | 135.00                  | 168.00 | 497.00                     |     |
| 1200      | 122.00                  | 181.00 | 458.00                     |     |
| 1000      | 134.00                  | 166.00 | 495.00                     |     |
| 1000      | 138.00                  | 172.00 | 504.00                     |     |
| Main effect |                       |     |                             |     |
| Vitamin E |                         |     |                             |     |
| 600       | 123.00                  | 156.00 | 462.00                     |     |
| 800       | 128.00                  | 160.00 | 484.00                     |     |
| 1000      | 131.00                  | 164.00 | 485.00                     |     |
| Vitamin C |                         |     |                             |     |
| 600       | 118.00                  | 153.00 | 449.00                     |     |
| 800       | 130.00                  | 162.00 | 480.00                     |     |
| 1000      | 134.00                  | 167.00 | 492.00                     |     |
| SEM       | 1.1                     | 2.0   | 3.60                       |     |
| p-values  |                         |     |                             |     |
| Vitamin E | .01                      | .01   | .01                         | .01 |
| Vitamin C | .01                      | .01   | .01                         | .01 |
| Vitamin E x C | .03                | .14   | .04                         | .04 |

Average egg weight, g/egg

| Treatment | Average egg volume, cc/egg | Egg shape index |
|-----------|-----------------------------|-----------------|
| Vitamin E |                             |                 |
| 600       | 12.030                      | 12.130          |
| 800       | 12.010                      | 12.120          |
| 1000      | 12.020                      | 12.180          |
| 800       | 12.040                      | 12.120          |
| 1000      | 12.060                      | 12.080          |
| 800       | 12.090                      | 12.140          |
| 1000      | 12.050                      | 12.080          |
| Main effect |                             |                 |
| Vitamin E |                             |                 |
| 600       | 12.050                      | 12.120          |
| 800       | 12.070                      | 12.120          |
| 1000      | 12.070                      | 12.120          |
| Vitamin C |                             |                 |
| 600       | 12.040                      | 12.110          |
| 800       | 12.090                      | 12.110          |
| 1000      | 12.090                      | 12.110          |
| SEM       | .038                       | .052            |
| p-values  | .450                       | .310            |

N:F, number of produced eggs to feed ratio; W:F, weight of produced eggs to feed ratio.
1Number of samples = 10 eggs/replicate.
2Egg width/egg length; number of samples = 5 eggs/replicate.
Means within a column with different superscript letters differ (p < 0.05).
period, a vitamin E × C interaction effect (p = .05) was found on N:F with the highest value (i.e. 0.235) observed at 1000 mg/kg of both the vitamins.

A vitamin E × C interaction effect was found on the weight of produced eggs in the 42 to 63 d period (p = .05) and throughout the study (p = .04) with the highest weights observed at 1000 mg/kg of both the vitamins (i.e. 1664 and 6088 g, respectively). In the 64 to 84 d and 85 to 105 d periods, there were vitamin E and vitamin C effects (p < .01) and the highest egg weights were obtained with 1000 mg/kg of vitamin E or C both at the level of 800 mg/kg. However, the quails fed the diet supplemented with both the vitamins at the highest tested dose (1000 mg/kg) raised the highest weights at the end of the trial. In agreement with our findings, Sahin and Kucuk (2001), and Ipek and Dikmen (2014) found that high dietary level of vitamin E and C resulted in increased body weight of quails. Previous studies have demonstrated that dietary supplementations with vitamin E and C, alone or in combination, may improve growth performance of quails reared under heat stress condition (Sahin et al. 2002b; Ipek et al. 2007; Hemid et al. 2010).

Stressful conditions, such as high environmental temperature, bring to the formation of cell-damaging cytotoxic free radicals, increased protein catabolism and reduced protein biosynthesis, and depletion of vitamin C (Ipek et al. 2007). Therefore, in poultry nutrition, vitamin E and C, thanks to their antioxidant properties, play a key role in the alleviating some of the physiological responses and improving thermo-tolerance (Sahin and Kucuk 2001; Çiftçi et al. 2005; Ipek et al. 2007). In particular, vitamin C promotes performance associated with the suppressed-stress responses as indicated by lowering the plasma corticosterone level and adrenocorticotropic hormone (Lin et al. 2006; Ahmadu et al. 2016). Nevertheless, it has been suggested that vitamin C effectiveness on poultry performance expresses only in environmental stress condition whereas is not detectable under normal temperature condition (Newman and Leeson 1999; Saki et al. 2010). However, there is growing evidence that the interaction between the two major antioxidant vitamins (i.e. E and C) is also of pathological importance (Subasree 2014). Oxidative lesions, leading to conformational modifications of proteins, could induce pancreatic enzyme inhibition and/or dietary protein resistance to digestion (Ahmadu et al. 2016). Consequently, antioxidants, such as vitamin E and/or C, could contribute in preserving the proteins from oxidative denaturation improving digestibility of nutrients and their metabolic utilisation (Panda et al. 2008; Ahmadu et al. 2016). As already observed in our previous study (Sigolo et al. 2018), under thermoneutral condition, vitamin E and C added together at high doses (1000 mg/kg) to quail diet seem to interact in promoting a general animals’ welfare which results in raised growth.

**Discussion**

**Growth performance**

Current results showed that dietary supplementation with vitamin E and C may positively affect the growth performance of Japanese quails during the egg production period under controlled environmental conditions. Taking into account all the parameters of growth performance obtained throughout the study (i.e. from d 42 to d 105), it emerges that feed intake, weight gain, and dietary efficiency were at the same time improved by using a combination of vitamin E and C both at the level of 800 mg/kg. However, the quails fed the diet supplemented with both the vitamins at the highest tested dose (1000 mg/kg) raised the highest weights at the end of the trial. In agreement with our findings, Sahin and Kucuk (2001), and Ipek and Dikmen (2014) found that high dietary level of vitamin E and C resulted in increased body weight of quails. Previous studies have demonstrated that dietary supplementations with vitamin E and C, alone or in combination, may improve growth performance of quails reared under heat stress condition (Sahin et al. 2002b; Ipek et al. 2007; Hemid et al. 2010). Stressful conditions, such as high environmental temperature, bring to the formation of cell-damaging cytotoxic free radicals, increased protein catabolism and reduced protein biosynthesis, and depletion of vitamin C (Ipek et al. 2007). Therefore, in poultry nutrition, vitamin E and C, thanks to their antioxidant properties, play a key role in the alleviating some of the physiological responses and improving thermo-tolerance (Sahin and Kucuk 2001; Çiftçi et al. 2005; Ipek et al. 2007). In particular, vitamin C promotes performance associated with the suppressed-stress responses as indicated by lowering the plasma corticosterone level and adrenocorticotropic hormone (Lin et al. 2006; Ahmadu et al. 2016). Nevertheless, it has been suggested that vitamin C effectiveness on poultry performance expresses only in environmental stress condition whereas is not detectable under normal temperature condition (Newman and Leeson 1999; Saki et al. 2010). However, there is growing evidence that the interaction between the two major antioxidant vitamins (i.e. E and C) is also of pathological importance (Subasree 2014). Oxidative lesions, leading to conformational modifications of proteins, could induce pancreatic enzyme inhibition and/or dietary protein resistance to digestion (Ahmadu et al. 2016). Consequently, antioxidants, such as vitamin E and/or C, could contribute in preserving the proteins from oxidative denaturation improving digestibility of nutrients and their metabolic utilisation (Panda et al. 2008; Ahmadu et al. 2016). As already observed in our previous study (Sigolo et al. 2018), under thermoneutral condition, vitamin E and C added together at high doses (1000 mg/kg) to quail diet seem to interact in promoting a general animals’ welfare which results in raised growth.
**Egg production traits**

Taking into account all the results related to the egg production traits obtained throughout the study (i.e. from d 42 to d 105), it emerges that dietary supplementation with a combination of vitamin E and C both at 1000 mg/kg level increased egg production, weight of produced eggs, N:F and W:F ratios, average egg volume, and egg shape index. However, similar results were also obtained by a combination of vitamin E at 800 mg/kg level and C at 1000 mg/kg level.

In agreement with our findings, several studies have reported increased egg production when Japanese quails fed diets supplemented with vitamin E and/or C (Bardakcioglu et al. 2005; Sahin et al. 2006; Ajakaiye et al. 2010; Caurez and Olo 2013; Abedi et al. 2017). In the current study, although the dietary addition of vitamin E plus C raised the total weight of produced eggs, no treatment effect was observed on the average egg weight in disagreement with Ipek and Dikmen (2014). Nevertheless, Bardakcioglu et al. (2005) suggested that vitamin C supplementation seems to have no prominent effect on egg weight. However, in our study higher average egg weights were obtained compared with those found by Bardakcioglu et al. (2005), and Ipek and Dikmen (2014). The increased egg shape indices, obtained throughout our study by supplementing quail diet with vitamin E or C, were in contrast with Chitra et al. (2016). The latter found no effect of dietary supplementation with vitamin E on egg shape index. The literature is still controversial about the effects of vitamin E and/or C on the egg production traits. However, the reported discrepancies could depend on the different conditions of work, such as dose of vitamin E and/or C used and environmental temperature. On the base of our results, it deduces that vitamin E and C, added together to the diet of quails at high doses, can improve egg production traits under thermoneutral environmental condition.

**Conclusions**

Current findings showed that feeding vitamin E and C at supra-nutritional levels can be a good management practice in Japanese quail nutrition to promote growth performance and egg production traits under thermoneutral environmental condition. When these vitamins are added together to quail diet, both at high doses (1000 mg/kg), they seem synergistically act in promoting animal growth. Dietary supplementation with vitamin E at 800 or 1000 mg/kg level plus vitamin C at 1000 mg/kg could improve egg production traits in terms of egg production, total weight of produced eggs, N:F and W:F ratios, average egg volume, and egg shape index.

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