

## **The effects of vitamin E and vitamin C on sexual maturity body weight and hatching characteristics of Japanese quails (*Coturnix coturnix japonica*) reared under heat stress**

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Used were a total of 810 seven-day-old Japanese quails. The birds received a basal diet with three levels of two vitamins – vitamin E (ROVIMIX® E-50 SD; stable source of vitamin E in feed, DL- $\alpha$  Tocopheryl acetate) 60, 120 and 240 mg/kg of diet, vitamin C (ROVIMIX® Stay-C 35; stable source of vitamin C in feed, L ascorbic acid) 60, 120 and 240 mg/kg of diet. Birds were reared at 33°C during the treatment period (week 0 to week 16 of age). At weeks 15-16 eggs were collected from the pens and put into incubators. The highest mean sexual maturity body weight (SMBW) and egg weight (EW) values were determined in a combination of 240 mg of Vitamin E and 240 mg of vitamin C group. The effect of treatment groups on fertility (F) ratio was found to be significant ( $P \leq 0.01$ ). When the fertility ratios are compared both combinations of 240 mg of vitamin E and 240 mg of vitamin C group and combinations of 240 mg of vitamin E and 120 mg of vitamin C group had higher values than the other treatment groups. The effect of treatment on the hatchability of fertile eggs (HFE), hatchability of total eggs (HTE) and embryo mortalities were significant ( $P \leq 0.01$ ). Lower HFE was observed in a combination of 60 mg of vitamin E and 60 mg of vitamin C group.

**KEY WORDS:** hatchability / heat stress / Japanese quail / vitamin C / vitamin E

To reduce the effects of heat stress many practical applications have been developed: increasing ventilation rates, using evaporative cooling systems in enclosed houses, lowering stocking densities or changing diets. Changing diets usually cover

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altered needs of stressed birds for protein and energy or for providing some additional nutrients such as vitamins and minerals [Çiftçi *et al.* 2005].

An increase of one unit of the hatchability of total eggs (a primary criterion of productivity in breeder farms) converts into a great financial value. Economic losses in heat stressed poultry birds such as high morbidity and mortality, immune suppression, poor FCR and reduced growth rate are well known [Siegel 1995, Utomo *et al.* 1994]. Heat stress decreases the reproductive ability of poultry [McDaniel *et al.* 2004]. It is well known that high ambient temperature coupled with high humidity has a detrimental effect on the poultry industry by decreasing fertility. Keirs [1982] noted that during summer months broiler breeder fertility can be decreased by as much as 15 %. Kevin [1982] observed that dietary supplementation of vitamin E increased the fertility and hatchability of breeder eggs.

Several studies have shown that vitamin C and vitamin E are used in the poultry diet because of their antioxidant properties and antistress effects and also because their synthesis is reduced during heat stress [Şahin and Küçük 2001, Şahin and Küçük 2002, Ramnath *et al.* 2008]. It was reported that heat stress increases lipid peroxidation in poultry [Bollengier-Lee *et al.* 1998]. It was speculated that vitamin E protected the liver from lipid peroxidation and damage to cell membranes [Whitehead *et al.* 1998]. Vitamin E is essential for optimum fertility and hatchability [Narahari *et al.* 2002]. Vitamin C is an anti-stress agent [Brake and Pardue 1998]. In the form of ascorbic acid is regarded as effective source of vitamin C when added to feed or drinking water [EFSA 2013]. Although poultry can synthesise vitamin C, its quantity becomes insufficient during heat stress [Şahin *et al.* 2002, Ajakaiye *et al.* 2011]. On the other hand, poultry cannot synthesise vitamin E, their vitamin E concentration is reduced under heat stress conditions [Bollengier-Lee *et al.* 1998] and the vitamin E requirement must be met from the diet [Chan and Decker 1994].

Japanese quail is becoming more popular as a source of meat and eggs in some parts of the world. The nutrient requirements of these strains and the optimum housing temperature along with their responses to heat stress environments are still obscure. The aim of this study, therefore, was to investigate the possible beneficial effects of different vitamin E and vitamin C supplementation as a combination on sexual maturity body weight, egg weight and incubation performance in Japanese quails reared under heat stress.

## Material and methods

A total of 810 seven-day-old Japanese quails (*Coturnix coturnix japonica*) were sexed according to their cloacal view under sexascope. The chicks were weighed on a digital balance with 0.1 g precision and their body weights were found between 24.8 g and 26.1 g. The birds were randomly assigned to nine treatment groups, three replicates of 30 quails (1:2 male to female ratios) in each. Wing marks bearing running numbers were attached to the wings of all chicks. Treatment groups were fed with

basal diet supplemented with three levels of vitamin E (ROVIMIX® E-50 SD; stable source of vitamin E in feed, DL- $\alpha$  Tocopheryl acetate) 60, 120 and 240 mg/kg of diet and vitamin C (ROVIMIX® Stay-C 35; specifically produced for use as a stabilized source of vitamin C in feed; L ascorbic acid ) 60,120 and 240 mg/kg of diet. Water and feed were supplied *ad libitum*.

The birds were kept in a storey cage system in which each sub cage unit (90x48 cm) contained 30 birds (1:2 male to female ratios). The room temperature was maintained at 33±2°C during the treatment period (0 to 16 weeks of age). The overhead ruby infrared heating lamp was used to keep the temperature constant. The groups were subjected to continuous lighting for 24 h a day for the first two weeks. The lighting period was gradually reduced to 12 hours a day between weeks 2 and 4. These 12 h lighting was kept constant between weeks 4 and 8. After week 8 the lighting period was gradually increased to 17 h. The environmental conditions were the same for all groups.

**Table 1.** Composition of diets

Ingredients (%)	Starter (0-5 wks)	Layer (6-16 wks)
Ground corn	50.70	66.08
Soybean meal	34.00	29.6
Fish meal	5.00	-
Plant oil	7.50	1.2
Dicalcium phosphate	1.33	1.34
Sodium chloride	0.11	0.2
Limestone	0.51	0.58
Calcium carbonate	0.10	0.16
DL-methionine	0.22	0.27
Lysine	0.10	0.14
Vitamin premix*	0.27	0.27
Trace mineral premix**	0.16	0.16
Constituents (g/kg DM)		
organic matters	934.9	912.5
crude protein	230.0	180.0
ether extract	71.5	51.3
crude fibre	88.7	124.0
crude ash	65.1	87.5
nitrogen free extractives	544.7	469.7
ME, MJ/kg	12.8	11.3

\*Ingredients in 2 kg of premix (Rovimix 124/v): vitamin A 15 000 000 IU; cholecalciferol 3 000 IU; vitamin E 15 IU; Menadione 2500 mg; vitamin B1 1000 mg; vitamin B2 10 000 mg; niacin 70 000 mg; d-Pantothenic acid 20 000 mg; vitamin B12 4 000 mg; folic acid 2 000 mg; biotin 100 mg. Vitamin E (DL- $\alpha$  Tocopheryl acetate) and vitamin C (L ascorbic acid) were added to the basal diet according to treatment.

\*\*Premix (Remineral CH) supplied for 2 kg: Mn 80 000 mg; Fe 25 000 mg; Zn 50 000 mg; Cu 7000 mg; Iodine 300 mg; Se 150 mg; choline chloride 350 000 mg.

All treatment groups were fed with broiler starter diet containing 230.0 g/kg CP and 12.8 MJ ME/kg during the growth period. Layer diet containing 180.0 g/kg CP and 11.3 MJ ME/kg was used from the week 6 onwards. The composition and nutritive value of the basal diet were ascertained by the Weende analysis according to the indications of Akyildiz [1984] – Table 1.

The age of the sexual maturity was determined as the quails laid their first egg in each pen and quails weighed on a digital balance with 0.1 g precision. Eggs were collected from the pens between week 15 and 16 of age that shown mean egg production period. A total of 972 eggs were stored at 16-18°C and 65-75 % RH for 3 days. They were incubated in a incubator (Çimuka, Ankara) at a temperature of 37.5°C and 65% RH for 15 days and turned 45° every hour. The eggs were transferred to a hatcher (Çimuka, Ankara) maintained at 37.0°C and 70 % RH until hatching (day 17).

Eggs that failed to hatch were opened for macroscopic observation, thus they were classified according to time of embryo mortality. Fertility (F), hatchability of fertile eggs (HFE), hatchability of total eggs (HTE) and early-term embryo mortality (EEM) (1 up to 4 days), and late-term embryonic mortality (LEM) (16 up to 18 days) were determined. Mean term embryo mortalities at day 5 up to 15 were observed very rarely in this study; therefore, these mortalities were classified as late-term embryo mortalities (LEM) – Pedroso *et al.* [2006].

Data were analysed by the general linear model program of SAS [1989] using Duncan's multiple range test to compare treatment means. Analyses for percentage data were conducted after arc sine transformation.

## Results and discussion

The effect of vitamin E and vitamin C on the sexual maturity body weight and hatching characteristics of Japanese quails reared under heat stress are given in Table 2. The difference between the treatment groups with respect to SMBW and EW were found to be significant ( $P \leq 0.01$ ). The highest mean SMBW and EW values were determined in a combination of 240 mg of vitamin E and 240 mg of vitamin C group. The supplementation of vitamin E and vitamin C improved certain physiological and biochemical parameters of quails and this was reflected in a reduction in puberty age [Abdulrahman and Alrahawi 2012]. Şahin and Küçük [2001] found that high level dietary vitamin E and vitamin C supplementation resulted in a higher body weight in quails. Supplementary vitamin C may improve heat tolerance and thereby reduce body weight losses that are normally associated with stress conditions [El-Daly *et al.* 2013]. In contrast to our findings Bardakcioglu *et al.* [2005] suggest that vitamin C supplementation appears to have no prominent effect on egg weight. However, some authors found positive effect of dietary vitamin C supplementation on egg weight [Altan *et al.* 1999, Konca and Yazgan 1999]. Also single or combined dietary supplementation with vitamin C and vitamin E of laying hens exposed to heat stress had significantly improved egg weight [Ajakaiye *et al.* 2011].

Vitamins C and E are used in poultry diet because of their antistress effects, and also because their synthesis is reduced during heat stress [Şahin and Küçük 2001, Şahin *et al.* 2009]. Considered separately, both are primary antioxidants in biological systems and break the chain of lipid peroxidation in cell membranes. However, overall antioxidant potential has been reported to possibly be more efficient and crucial than single antioxidant nutrients [Gallo-Torres 1980]. In this respect, vitamin C and vitamin E work together in such a way that vitamin E is the major chain breaking antioxidant in lipid phases such as cellular membrane or low density lipoproteins, and the oxidizing free radical chain reactions are terminated in aqueous compartments, with vitamin C as the terminal reductant [Gey 1998].

Franchini *et al.* [1991] reported that dietary vitamin E increases the level of sex hormones. The research with different avian species has shown that increased level of dietary vitamin E increased hatchability and fertility [El-Latif 1999, Lin *et al.* 2004, Fitri *et al.* 2012]. However, in contrast, Hooda *et al.* [2007] found that feeding higher rates of dietary vitamin E did not affect the fertility and hatchability in quails. Vitamin

C was accounted to have a positive impact on the fertility and hatchability in Pekin ducks [Kontecka *et al.* 2001] and pheasants [Nowaczewski and Kontecka 2005]. In the present study the effect of group treatment on F ratio was found to be significant ( $P \leq 0.01$ ). When the F ratios were compared to both combinations of 240 mg of vitamin E and 240 mg of vitamin C group and that of 240 mg of vitamin E and 120 mg of vitamin C group were found higher than those of other treatment groups. The effect of treatment on the HFE, HTE and EEM-LEM were significant ( $P \leq 0.01$ ). Lower HFE was observed

**Table 2.** The effect of diet supplementation with vitamin E and vitamin C on sexual maturity body weight and hatching characteristics of Japanese quails reared under heat stress. (mean $\pm$ SEM)

Treatment	SMBW	EW	F	HFE	HTE	EEM	LEM
Vit E (mg/kg)	Vit C (mg/kg)	(g)	(%)	(%)	(%)	(%)	(%)
60	60	165.8 <sup>a</sup> $\pm$ 5.7	82.7 <sup>a</sup> $\pm$ 1.1	85.8 <sup>a</sup> $\pm$ 1.4	71.0 <sup>d</sup> $\pm$ 1.3	6.7 <sup>a</sup> $\pm$ 0.3	7.5 <sup>a</sup> $\pm$ 0.4
120	120	165.2 <sup>a</sup> $\pm$ 5.4	87.3 <sup>a</sup> $\pm$ 1.2	87.3 <sup>a</sup> $\pm$ 1.3	76.2 <sup>c</sup> $\pm$ 1.1	6.0 <sup>b</sup> $\pm$ 0.3	7.0 <sup>b</sup> $\pm$ 0.4
240	240	176.0 <sup>c</sup> $\pm$ 6.0	90.4 <sup>b</sup> $\pm$ 1.5	89.0 <sup>b</sup> $\pm$ 1.7	80.5 <sup>b</sup> $\pm$ 1.6	4.8 <sup>c</sup> $\pm$ 0.2	6.1 <sup>c</sup> $\pm$ 0.3
120	60	165.4 <sup>a</sup> $\pm$ 5.6	88.3 <sup>a</sup> $\pm$ 1.3	87.1 <sup>c</sup> $\pm$ 1.1	76.8 <sup>c</sup> $\pm$ 1.2	5.9 <sup>b</sup> $\pm$ 0.3	7.0 <sup>b</sup> $\pm$ 0.4
120	120	166.3 <sup>a</sup> $\pm$ 5.3	90.7 <sup>b</sup> $\pm$ 1.6	89.5 <sup>b</sup> $\pm$ 1.5	81.2 <sup>b</sup> $\pm$ 1.7	4.4 <sup>c</sup> $\pm$ 0.2	6.1 <sup>c</sup> $\pm$ 0.4
240	240	187.1 <sup>b</sup> $\pm$ 6.8	90.1 <sup>b</sup> $\pm$ 1.5	90.0 <sup>b</sup> $\pm$ 1.7	81.2 <sup>b</sup> $\pm$ 1.6	4.1 <sup>c</sup> $\pm$ 0.2	5.8 <sup>c</sup> $\pm$ 0.3
60	60	162.9 <sup>a</sup> $\pm$ 5.2	90.4 <sup>b</sup> $\pm$ 1.7	89.8 <sup>b</sup> $\pm$ 1.6	81.1 <sup>b</sup> $\pm$ 1.6	4.4 <sup>c</sup> $\pm$ 0.3	5.8 <sup>c</sup> $\pm$ 0.4
120	120	183.5 <sup>b</sup> $\pm$ 6.0	92.0 <sup>b</sup> $\pm$ 2.0	91.9 <sup>a</sup> $\pm$ 1.9	84.6 <sup>a</sup> $\pm$ 2.1	3.4 <sup>d</sup> $\pm$ 0.2	4.7 <sup>d</sup> $\pm$ 0.2
240	240	211.4 <sup>a</sup> $\pm$ 7.3	92.6 <sup>a</sup> $\pm$ 2.2	92.3 <sup>a</sup> $\pm$ 2.0	85.5 <sup>a</sup> $\pm$ 2.0	3.3 <sup>d</sup> $\pm$ 0.2	4.4 <sup>d</sup> $\pm$ 0.2
P		**	**	**	**	**	**

<sup>abc</sup>Means within columns with no common letter differ significantly.

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ .

SMBW - sexual maturity weight; EW - egg weight; F - fertility; HFE - hatchability of fertile eggs; HTE - hatchability of total eggs; EEM - early term embryo mortalities; LEM-late term embryo mortalities.

in a combination of 60 mg of vitamin E and 60 mg of vitamin C group. Combination of 240 mg of vitamin E and 240 mg of vitamin C group and of 240 mg of vitamin E and 120 mg of vitamin C group affected the HFE and THE. Shanawany [1992] and Hargis [1993] suggest that supplementation of vitamin E on breeder diet reduced embryonic mortality. In contrast to our findings, Lin *et al.* [2004] suggest that diet supplemented with vitamin E did not significantly influence embryo survival.

In conclusion, it is known that stress increased vitamin E and C requirements of birds. Vitamin E and C can be used to attenuate the negative effects of heat stress. Results of the present study suggests that a dietary combination of vitamin E (240 mg) and vitamin C (240 mg) supplementation may affect positively sexual maturity body weight, egg weight and hatching characteristics of quails reared under heat stress. Further investigations are indispensable to elucidate the influence of antioxidant vitamins upon the incubation results and quail chick performance.

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