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Effects of dietary ascorbic acid and betaine on the serum levels of oxidative stress markers and some geometric properties of eggs laid by Japanese quail hens during the dry season

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Abstract

This study investigated the effects of dietary ascorbic acid (AA) and betaine (BET) on the serum levels of oxidative stress markers and some geometric properties of eggs laid by Japanese quail hens during the dry season. A total of 252 quail hens were used for the study. After seven days of acclimatization, the quail hens were randomly assigned to four groups of 63 per group. The four groups and their specific diets were as follows: Untreated Control, fed basal diet with no AA or BET; AA group, fed diet containing 200 mg/kg AA; BET group, fed diet containing 2 g/kg BET; AA + BET group, fed diets containing 200 mg/kg AA + 2 g/kg BET. The study was done at the peak of dry season, between January and March, and the quails were fed for 28 days. The dry-bulb temperature (DBT), relative humidity (RH), and temperature-humidity index (THI) were measured daily at 08:00 h, 13:00 h, and 17:00 h. Malondialdehyde (MDA), catalase, and reduced glutathione (rGSH) levels were assayed in serum obtained from blood samples collected from the quails on days 49 and 70 of age. The egg surface area (SA), volume, geometric mean diameter GMD), sphericity and shell index (SI) were measured on days 49, 56, 63, and 70 of age. Results showed that there were significant ($p < 0.05$) fluctuations in DBT, RH, and THI, which exceeded the recommended thermoneutral zone for Japanese quails. The AA, BET and AA + BET groups had significantly ($p < 0.05$) lower serum MDA levels when compared to the untreated control on days 49 and 70, but the serum levels of catalase and rGSH of the AA, BET and AA + BET groups were significantly ($p < 0.05$) higher than that of the untreated control on days 49 and 70. Eggs from the three treated groups (AA, BET and AA + BET) had significantly ($p < 0.05$) higher SA, volume, GMD, and sphericity on days 49, 56 and 70, when compared to eggs from the untreated control. It was concluded that dietary AA and BET given alone or in combination positively modulated serum levels of oxidative stress markers and significantly improved egg quality indices of the Japanese quails.

Keywords: Japanese quails; Dry season; Dietary supplementation; Ascorbic acid; Betaine; Oxidative stress; Egg quality.

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Introduction

Global changes in climate have the potential to adversely affect poultry production worldwide. The earth's changing climate significantly influences ambient temperature (AT) and relative humidity (RH) (Nawab *et al*., 2018). High AT and RH can cause heat stress in poultry birds such as Japanese quails (*Coturnix japonica*) (Batool *et al*., 2023). Heat stress can lead to a decrease in poultry production by reducing feed intake and egg production, ultimately causing economic losses (Wasti *et al*., 2020).

Japanese quail eggs are a source of affordable protein (Ojediran *et al*., 2022). Quails are cheaper to maintain and require less space for production. However, heat stress adversely affects them, especially in summer and tropical climates (Biswal *et al*., 2022). Heat stress can also affect the quality of their eggs, making them fragile and deformed (Zhang *et al*., 2022). It has been reported that heat stress can negatively affect processes involved in egg formation (Wasti *et al*., 2020).

Geometric parameters can be used to evaluate the market value of eggs produced by poultry birds (Nyalala *et al*., 2021). These parameters are also relevant to proper egg packaging to avoid breakage during transportation (Narushin *et al*., 2021). Egg mass and some geometrical attributes of the egg are related, and egg length and diameter can be used to determine the geometric characteristics of an egg (Wang *et al*., 2021). These characteristics also reflect the quality of eggs produced by poultry birds like Japanese quails (Fathi *et al*., 2021).

It has been shown that various environmental modifications such as early heat conditioning, open sheds, and cooling systems may not meet the special requirements of stressed poultry birds (Nawab *et al*., 2018). There is insufficient information on the effect of ascorbic acid and betaine supplementation on the geometric characteristics of eggs laid by Japanese quail hens reared under thermally stressful conditions. Ascorbic acid is a potent antioxidant that scavenges free radicals, while betaine has antioxidant properties. This study investigated the effects of dietary ascorbic acid and betaine on the serum levels of oxidative stress markers and some geometric properties of eggs laid by Japanese quail hens during the dry season.

Materials and Methods

Ethical Statement: The protocols for handling and management of birds used for the study adhered to guidelines stipulated by the Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 1996).

Location where the study was done: This study was conducted at the Animal House of the Department of Parasitology and Entomology, Faculty of Medicine, University of Nigeria, Nsukka, Nigeria. The study location is in the Derived Savannah ecological zone of Nigeria (Omaliko, 1981). The annual rainfall of the location ranges from 986 to 2098 mm (Momoh *et al*., 2010). The study was conducted during the dry season and peak heat period in that region, between January and March (Uguru *et al*., 2011).

Animals and Management Procedures: A total of 252 female Japanese quails (*Coturnix japonica*) weighing 198.8 ± 1.7 g at 35 days old were commercially sourced and used for the study. The quails were acclimatized for 7 days and fed commercial diets, with composition as presented in Table 1. From days 35 to 49 of age, they were fed a grower diet (with 15.5% crude protein (CP) and 2250 kcal/kg of metabolizable energy (ME)/kg of feed, and from days 49 to 70 of age, they were fed a layer diet (16.8% CP, 2680 kcal/kg ME). At day 42 of age, the birds were randomly assigned to four groups using a completely random method. Each group comprised 63 birds, which were then divided into three replicates,

Table 1. Composition and proximate analysis of the basal quail diets used for the study.

Feed Composition	Grower	Finisher
Ingredients (%)		
Maize	0	34
Sweet potato meal	60	26
Blood meal	5	5
Groundnut cake	29.7	29.7
Wheat offal	1	1
Bone meal	3.25	3.25
dl-Methionine	0.25	0.25
Lysine	0.25	0.25
Vitamin Premix*	0.3	0.25
Salt	0.25	0.25
Proximate Analysis		
Metabolizable energy (Kcal/kg)	2250	2680
Crude protein (%)	15.5	16.8
Crude fiber (%)	6.84	4.3
Calcium (%)	1.41	1.2
Phosphorus (%)	0.45	0.45
Lysine (%)	1.22	1.3
Methionine (%)	0.5	0.56
Cystine (%)	0.36	0.3
Dry matter (%)	93.73	94.14
Ether extract (%)	2.75	3
Ash (%)	6.95	7.4
Nitrogen-free extract (%)	67.97	69.21

* Vitamin premix supplied per kg diet: vitamin A – 10,000IU, vitamin D3 – 2,000 IU, vitamin E – 51 IU, vitamin K – 2.34 mg, riboflavin – 5.5 mg, calcium pantothenate – 10 mg, niacin – 25 mg, chlorine chloride – 250 mg, folic acid – 1 mg, manganese – 56 mg, zinc – 50 mg, copper – 10 mg, iron – 20 mg, and cobalt – 1.25 mg.

with 21 quails per replicate, as earlier described (Marchiori *et al*., 2019). Each replicate of the groups was kept in an enclosed space measuring 0.91 m × 0.76 m × 0.91 m. The enclosures were well ventilated and the birds were exposed to a light duration of 17 hours light and 8 hours of darkness (Elkomy *et al*., 2019). The quails were reared naturally under the late dry season environmental conditions with no simulation.

The four experimental groups and their different diets were as follows: Untreated Control group, which was fed the basal unsupplemented diet; AA group, that was fed diet containing ascorbic acid (AA) at 200 mg/kg; BET group, which was fed diet containing betaine (BET) at 2 g/kg; and AA + BET group, that was fed diet containing a combination of 200 mg/kg ascorbic acid + 2 g/kg betaine. Ascorbic acid (Kempex Holland BV, Volkel, The Netherlands) was included in the diets of the AA and AA + BET groups at 200 mg/kg of feed (Sahin *et al*., 2002), while betaine hydrochloride (Sigma-Aldrich, St. Louis, Missouri, USA) was incorporated into the diets of the BET and AA + BET groups at 2 g betaine/kg of feed (de Jong *et al*., 2012). The combination diets included AA and betaine at 200 mg/kg and 2 g/kg of feed, respectively. The dietary inclusion of ascorbic acid and/or betaine was performed daily before feeding, and the birds had access to their group-specific diets and drinking water *ad libitum*. The quails were fed with the experimental diets for 28 days.

Microenvironmental conditions: The micro-environmental conditions measured were dry-bulb temperature (DBT, °C); relative humidity (RH, %) and temperature-humidity index (THI). The DBT was measured using a Mason's type Wet and Dry Bulb Hygrometer (Zeal, London, England). The thermometer used to measure DBT had a measurement range of -8 °C to 50 °C and an accuracy of +/-1 °C [or +/-5%]. The RH was obtained using hygrometric tables for computation of relative humidity (Zeal, London, England); and THI was derived with a formula as shown below (Zulovich and DeShazer, 1990). The THI indicates the combined effects of temperature and humidity on the quails: THI = $0.6T_{db} + 0.4T_{wb;}$ where T_{db} = dry-bulb temperature (°C); and T_{wb} = wet-bulb temperature (°C). The recording of micro-environmental parameters was done at 08:00 h, 13:00 h and 17:00 h on each day of the study period.

Blood collection and Serum harvest: Birds in each group were properly identified using number tags affixed to one leg. Six quails were randomly selected from each group (at two birds per replicate) for blood collection. A volume of 3 ml of blood was collected from each bird and quickly dispensed into labelled plain sample bottles without anti-coagulants, after slaughter and decapitation (Erol *et al*., 2009). Blood samples were collected from quails at day 49 and 70 of age. The blood samples were kept at room temperature for one hour to clot properly. Thereafter, the blood samples were centrifuged at 3000 *x g* for 10 minutes at room temperature. Sera were harvested and immediately analyzed (Lee *et al*., 2017).

Assay of Malondialdehyde level in serum: The determination of serum concentration of malondialdehyde (MDA) was performed using the method described by Stocks and Dormandy (1971). The technique is based on the principle of the quantification of thiobarbituric acid reactive substances (TBARS) formed from the reaction of thiobarbituric acid with MDA. Briefly, 0.5 mL serum was mixed with 20% trichloroacetic acid in a ratio of 1:1, incubated at room temperature (25°C) for 20 minutes, centrifuged at 2500 × *g* for 10 minutes and the supernatant harvested. Following the addition of 1% thiobarbituric acid to the supernatant, samples were heated in a water bath (100 °C) for 15 minutes. Contents were then cooled and centrifuged at 2500 × g for 15 minutes. The optical density of the supernatant was read at 532 nm against a blank using a spectrophotometer (Jenway 6305; Jenway, Essex, UK), and MDA levels were deduced from a standard curve constructed using various MDA concentrations of 0 – 20 nM/ml (Sigma, St. Louis, MO, USA).

Assay of Catalase concentration in serum: The technique described by Goth (1991) was used to evaluate serum catalase activity. Briefly, serum (0.2 mL) was incubated in 1 mL substrate (consisting of 65 µmol per ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) for 60 seconds at 37°C. Catalase (1U) breaks down 1 µmol of hydrogen peroxide per minute. A spectrophotometer (Jenway 6305; Jenway, Essex, UK) was used to determine the optical density of the stable yellow complex formed, against a blank at 405 nm. Catalase activity was expressed as kU/L.

Assay of reduced Glutathione (rGSH) concentration in serum: Serum levels of reduced glutathione (rGSH) were evaluated by the technique described by Moron *et al.,* (1979), which was based on the principle of complex formation from the reaction of dithionitrobenzene (DTNB) and acid sulfhydryl groups (non-protein thiols containing 93 % GSH-Rd). Briefly, 0.5 mL of serum was mixed with 0.1 mL of 25 % TCA and kept for some minutes on ice. The mixture was then centrifuged for 10 minutes at 3000 × *g*. A 0.2 M sodium phosphate buffer (0.7 mL, pH 8) and 0.6 mM DTNB (2 mL; Sigma, St. Louis, MO, USA) were mixed with the 0.3 mL of supernatant for 10 minutes. A spectrophotometer (Jenway 6305; Jenway, Essex, UK) was then used to measure the optical density of the product at 412 nm. The serum concentration of rGSH was determined from a graph of standards (Sigma, St. Louis, Missouri, United States) of various GSH concentrations and expressed as nM/ml**.**

Measurements on the Eggs and Calculation of Geometric Properties of Eggs: The geometric properties of the eggs calculated were the surface area (cm^2), the volume of eggs (cm^3), geometric mean diameter (cm), sphericity (%) and eggshell index (%). The egg geometric properties were calculated using formulas based on the measurements of egg length (*L*) and width (*W*) that was done using a vernier caliper (Kales Tool Industry and Trade Co., Ltd., Jinhua, China). The formula described by Baryeh and Mangope (2003) was used to calculate the surface area of eggs (*S*). $S = \pi * Dg^2$, while that by Kumbar *et al*. (2016) was used to derive the volume of eggs $(V) = (\pi/6) * L * W^2$.) The geometric mean diameter of eggs (*Dg*) was calculated with the formula described by Mohsenin (1970) $Dg = (L*W^2)^{1/3}$, the degree of sphericity of eggs $(\Phi) = (Dg/L) * 100$ (Severa *et al*., 2013); and the egg shape index (*SI)* was calculated from the formula described by Sarica and Erensayin (2004) as $SI = (W/L)*100$.

Statistical analysis: Data obtained were subjected to statistical analysis and compared using analysis of variance (ANOVA), followed by Tukey's *post-hoc* test. Values of $p < 0.05$ were considered significant (Snedecor and Cochran, 1994). GraphPad Prism (GraphPad Software, Incorporated, San Diego, California, USA) version 6.0 was used for data analysis. The results were presented as mean ± standard error of the mean (SEM).

Results

Micro-environmental conditions: The mean, minimum and maximum values for the micro-environmental conditions at various quail ages during the study period are presented in Table 2. During the study period, the extreme minimum DBT, RH and THI were 25.0 °C, 62.0 % and 75.6, respectively. The extreme maximum value for DBT was 37.0 °C; while that of RH was 96.0 % and that of THI was 90.1. The lowest mean DBT value of 31.4 ± 0.8 °C was recorded during the first week, while the highest mean value of 32.1 ± 0.8 °C was obtained in the fourth week of the study. Similarly, the values of 84.4 ± 1.0 and 85.5 ± 1.1 were recorded as the lowest and highest mean values of THI during the first week and fourth week, respectively. The mean RH of 78.1 ± 1.5 % was the lowest recorded in the third week, while the highest value of 79.6 ± 1.3 % was obtained on the second week of the study.

Table 2. Micro-environmental conditions that prevailed in Nsukka Nigeria during the study period.

Age of bird (days)	Experimental period (days)	Micro-environmental conditions (means ± SEM with minimum and maximum values in brackets)		
		DBT (°C)	RH (%)	THI
49	7	31.4 ± 0.82 (25.0 – 35.0)	78.9 ± 2.1 (68.0 – 96.0)	84.4 ± 1.0 (75.6 – 87.8)
56	14	31.6 ± 0.70 (25.0 – 34.0)	79.6 ± 1.3 (62.0 – 96.0)	84.9 ± 1.0 (75.6 – 87.4)
63	21	32.0 ± 0.63 (26.0 – 35.0)	78.1 ± 1.5 (72.0 – 91.0)	85.4 ± 0.8 (77.4 – 89.2)
70	28	32.1 ± 0.80 (26.0 – 37.0)	78.6 ± 1.8 (71.0 – 95.0)	85.5 ± 1.1 (75.9 – 90.1)

DBT – Dry bulb temperature; RH – Relative humidity; THI – Temperature-humidity index.

The diurnal variations in the micro-environmental conditions are presented in Table 3. The highest mean maximum DBT and THI were recorded at 13:00 h, while the lowest RH was recorded at the same time. On the other hand, the lowest DBT and THI and the highest RH were recorded at 08:00 h. At 08:00 h, the extreme minimum DBT of 25.0 °C and THI of 75.6 were observed, whereas the extreme minimum RH of 62.0 was recorded at 13:00 h. Conversely, the extreme maximum of DBT and THI of 37.0 and 90.1, respectively, were obtained at 13:00 h, while the extreme maximum RH was recorded at 08:00 h.

Oxidative Stress biomarkers: The mean serum MDA levels of the AA, BET and AA + BET groups were significantly ($p < 0.05$) lower than that of the untreated control group on days 49 and 70 of age, with the AA + BET group having the lowest mean MDA value (Figure 1). In contrast, the mean serum catalase activities of the AA, Bet and AA + BET groups were significantly ($p < 0.05$) higher than that of the untreated control group at days 49 and 70 of age, with the AA + BET group having the highest mean catalase activity level each time (Figure 2). The serum activity of rGSH also followed the pattern of the catalase on days 49 and 70 of age, but the rGSH activity of the AA group and that of the AA +BET group was outstandingly higher than those of the untreated control and BET groups on day 70 (Figure 3).

Geometric properties of eggs: Quails in the AA, BET and AA + BET groups laid eggs with significantly ($p < 0.05$) higher surface area, volume, geometric mean diameter and sphericity when compared to the untreated controls on days 49, 56 and 70 of age (Tables 4, 5, 6 and 7), but there was no significant ($p > 0.05$) variations between the four groups in their egg shell index all through the study (Table 8). As the birds aged, the surface area, volume, geometric mean diameter and sphericity of the eggs continued to increase compared to the values at day 49 of age, especially in the treated groups (Tables 4, 5, 6 and 7).

Table 3. Diurnal fluctuation of micro-environmental conditions recorded at Nsukka Nigeria during the study period.

Meteorological variables	**Hour of the Day**		
	08:00 h	**13:00 h**	**17:00 h**
Dry-bulb temperature (°C)	28.0 ± 0.3 (25.0 – 29.0)	34.1 ± 0.5 (28.0 – 37.0)	33.1 ± 0.3 (28.0 – 34.0)
Relative humidity (%)	86.7 ± 0.7 (82.0 – 96.0)	73.2 ± 1.2 (62.0 – 87.0)	76.5 ± 0.9 (68.0 – 88.0)
Temperature-humidity index	80.1 ± 0.5 (75.6 – 82.4)	88.1 ± 0.6 (79.5 – 90.1)	87.0 ± 0.4 (79.5 – 88.2)

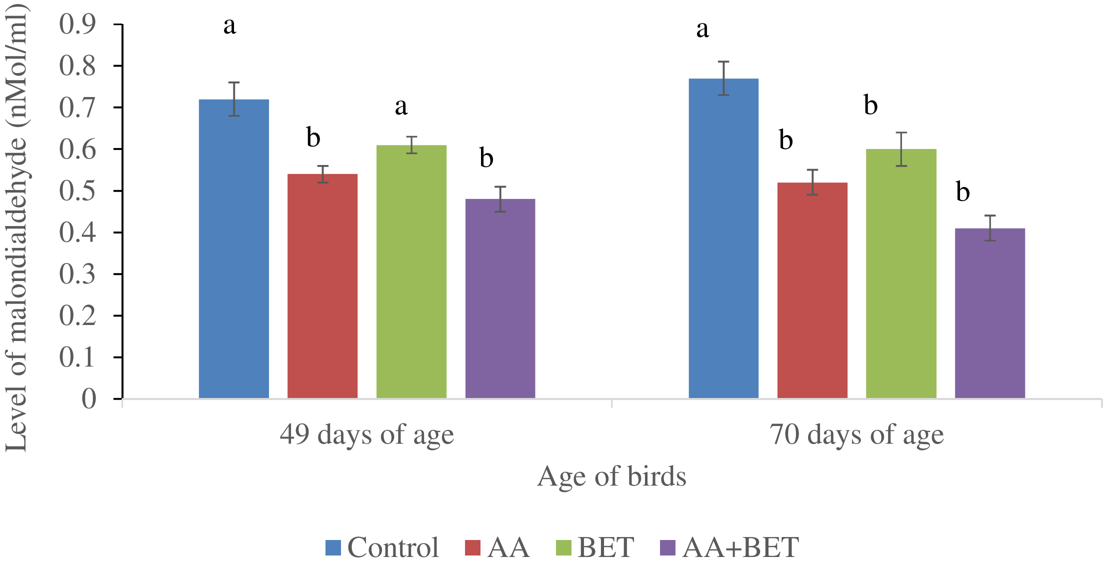

Figure 1. Serum malondialdehyde levels of Japanese quail hens fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control. [Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.] $^{a,\,b}$ Mean values with different superscript letters indicate significant difference ($p < 0.05$) between the groups.

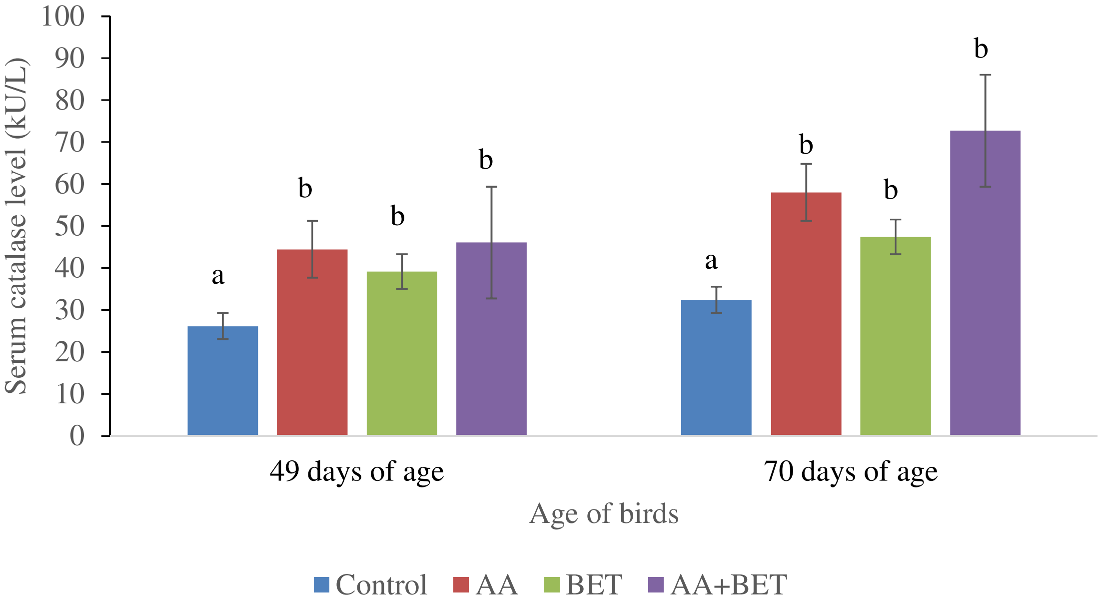

Figure 2. Catalase concentration in the serum of Japanese quail hens fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control. [Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.] $^{a,\,b}$ Mean values with different superscript letters indicate significant difference ($p < 0.05$) between the groups.

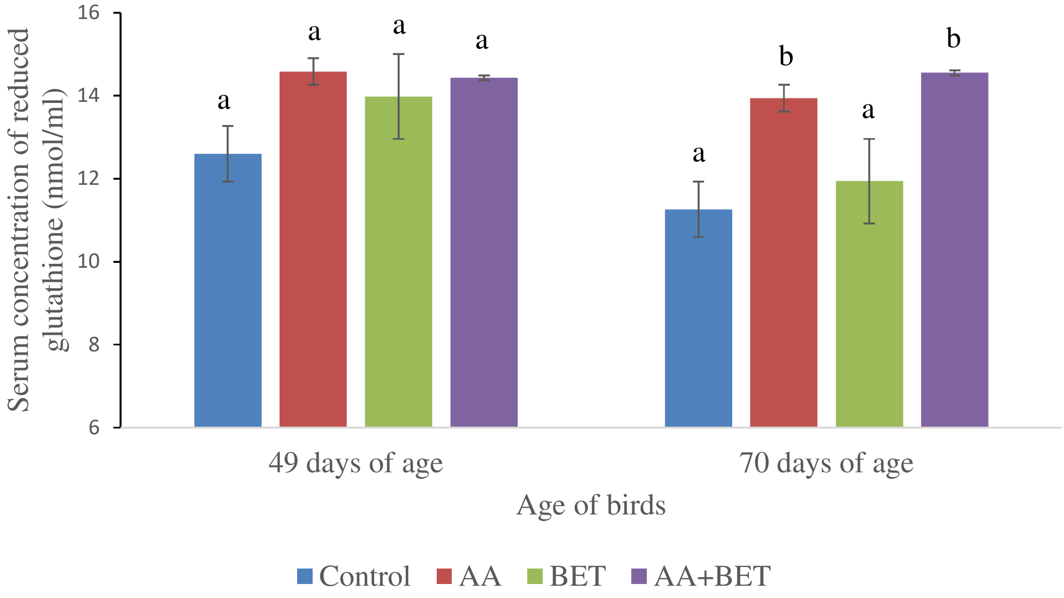

Figure 3: Serum levels of reduced glutathione concentration in Japanese quail hens fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control. [Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.] $^{a, b}$ Mean values with different superscript letters indicate significant difference ($p < 0.05$) between the groups.

Table 4. Surface area (cm^2) of eggs laid by Japanese quails fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control.

Age of birds (Days)	**Experimental Groups**			
	Control	**AA**	**BET**	**AA + BET**
49 (n = 29)	$77.53 \pm 2.26^{a,1}$	$90.52 \pm 2.56^{b,1}$	$92.22 \pm 2.37^{b,1}$	$89.75 \pm 2.80^{b,1}$
56 (n = 150)	$99.3 \pm 2.31^{a,2}$	$104.4 \pm 1.82^{a,2}$	$111.7 \pm 1.96^{b,2}$	$107.9 \pm 1.92^{b,2}$
63 (n = 156)	$110.4 \pm 1.45^{a,2}$	$112.5 \pm 1.81^{a,2}$	$114.9 \pm 1.46^{a,2}$	$113.20 \pm 1.91^{a,2}$
70 (n = 213)	$91.59 \pm 3.29^{a,2}$	$116.8 \pm 1.26^{b,2}$	$114.0 \pm 1.48^{b,2}$	$116.20 \pm 1.57^{b,2}$

[Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.]

^{a,b} Mean values with different superscript letters indicate significant different ($p < 0.05$) between the groups. n – number of eggs measured per group at the corresponding age of the birds.

$^{1, 2}$ Different number superscripts in each column indicate significant ($p < 0.05$) difference from day 49 result across time.

Table 5. Geometric volume (cm^3) of eggs laid by quails fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control.

Age of birds (Days)	Experimental Groups			
	Control	**AA**	**BET**	**AA + BET**
49 (n = 29)	$7.779 \pm 0.12^{a,1}$	$8.385 \pm 0.12^{b,1}$	$8.455 \pm 0.011^{b,1}$	$8.346 \pm 0.12^{b,1}$
56 (n = 150)	$8.74 \pm 0.10^{a,2}$	$8.983 \pm 0.09^{a,2}$	$9.301 \pm 0.07^{b,2}$	$9.147 \pm 0.08^{b,2}$
63 (n = 156)	$9.266 \pm 0.06^{a,2}$	$9.361 \pm 0.07^{a,2}$	$9.449 \pm 0.06^{a,2}$	$9.377 \pm 0.07^{a,2}$
70 (n = 213)	$8.135 \pm 0.16^{a,2}$	$9.543 \pm 0.05^{b,2}$	$9.409 \pm 0.06^{b,2}$	$9.497 \pm 0.06^{b,2}$

[Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.]

[a,b] Mean values with different superscript letters indicate significant different ($p < 0.05$) between the groups. n – number of eggs measured per group at the corresponding age of the birds.

[1, 2] Different number superscripts in each column indicate significant ($p < 0.05$) difference from day 49 result across time.

Table 6. Geometric mean diameter (cm) of eggs laid by quails fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control.

Age of birds (Days)	Experimental Groups			
	Control	**AA**	**BET**	**AA + BET**
49 (n = 29)	$4.952 \pm 0.07^{a,1}$	$5.338 \pm 0.07^{b,1}$	$5.383 \pm 0.06^{b,1}$	$5.313 \pm 0.08^{b,1}$
56 (n = 150)	$5.564 \pm 0.06^{a,2}$	$5.719 \pm 0.05^{a,2}$	$5.921 \pm 0.05^{b,2}$	$5.823 \pm 0.04^{b,2}$
63 (n = 156)	$5.899 \pm 0.04^{a,2}$	$5.96 \pm 0.05^{a,2}$	$6.015 \pm 0.04^{a,2}$	$5.97 \pm 0.05^{a,2}$
70 (n = 213)	$5.179 \pm 0.10^{a,1}$	$6.075 \pm 0.03^{b,2}$	$5.99 \pm 0.04^{b,2}$	$6.046 \pm 0.04^{b,2}$

[Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.]

[a,b] Mean values with different superscript letters indicate significant different ($p < 0.05$) between the groups. n – number of eggs measured per group at the corresponding age of the birds.

[1, 2] Different number superscripts in each column indicate significant ($p < 0.05$) difference from day 49 result across time.

Table 7. Sphericity (%) of eggs laid by quails fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control.

Age of birds (Days)	Experimental Groups			
	Control	AA	BET	AA+BET
49 (n = 29)	17.29 ± 0.24^{a,1}	18.40 ± 0.19^{b,1}	18.29 ± 0.21^{b,1}	18.25 ± 0.22^{b,2}
56 (n = 150)	18.58 ± 0.30^{a,1}	18.98 ± 0.29^{a,2}	19.48 ± 0.13^{b,2}	19.35 ± 0.16^{b,2}
63 (n = 156)	19.55 ± 0.11^{a,2}	19.77 ± 0.16^{a,2}	19.72 ± 0.10^{a,2}	19.64 ± 0.15^{a,2}
70 (n = 213)	17.48 ± 0.35^{a,1}	19.92 ± 0.09^{b,2}	19.74 ± 0.11^{b,2}	19.94 ± 0.13^{b,2}

[Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.]

^{a,b} Mean values with different superscript letters indicate significant different ($p < 0.05$) between the groups. n – number of eggs measured per group at the corresponding age of the birds.

^{1,2} Different number superscripts in each column indicate significant ($p < 0.05$) difference from day 49 result across time.

Table 8: Eggshell index (%) of eggs laid by quails fed diets supplemented with ascorbic acid and/or betaine, compared with untreated control.

Age of birds (Days)	Experimental Groups			
	Control	AA	BET	AA+BET
49 (n = 29)	79.68 ± 0.63	79.93 ± 0.43	79.94 ± 0.39	80.68 ± 0.35
56 (n = 150)	79.50 ± 0.54	79.78 ± 0.52	79.87 ± 0.24	80.43 ± 0.37
63 (n = 156)	80.52 ±0.19	81.07 ± 0.41	80.05 ± 0.20	80.11 ± 0.23
70 (n = 213)	79.45 ± 0.26	80.39 ± 0.18	80.57 ± 0.25	81.09 ± 0.32

[Control – unsupplemented basal diet; AA – diet supplemented with Ascorbic acid; BET – diet supplemented with Betaine, and AA + BET – diet supplemented with Ascorbic acid + Betaine.]

No significant differences between the groups and across time ($p > 0.05$).

Discussion

The findings in this study showed that the thermal conditions during the study period fluctuated widely and that the DBT, RH, and THI values exceeded the thermoneutral zone of 23.8 ± 0.7 °C for DBT, 58.5 ± 5.7% for RH and 76.0 – 80.0 for THI recommended for Japanese quails (El-Tarabany, 2016). The hottest period of the day was in the afternoon, with the highest maximum and mean DBT values recorded at 13:00 h. Conversely, the coolest period was observed in the morning, with the highest maximum and mean RH values as well as the lowest DBT values obtained at 08:00 h. These findings suggest that quail hens were exposed to thermally stressful environmental conditions, which could adversely affect egg production and quality. The present findings agree with earlier reports that demonstrated that Japanese quails exposed to temperatures exceeding 30.0°C experienced heat stress (Mehaisen *et al*., 2017; 2019). Also, Vercese *et al*. (2012) reported that Japanese quails exposed to environmental temperatures beyond 27°C are at risk of heat stress. Additionally, the study found that during the dry season, the afternoon period poses a risk of thermal stress for mature Japanese quails due to the high ambient temperature and humidity (Egbuniwe *et al*., 2021). It is thought that these conditions constitute a stress that could negatively impact on egg production and quality by reducing feed intake and feed conversion efficiency in quails.

The findings in the present study that quail hens that were given ascorbic acid, betaine or a combination of ascorbic acid and betaine had significantly lower MDA concentrations and higher levels of catalase and reduced glutathione peroxidase in their serum, in comparison to the untreated control group suggest that the supplementation with ascorbic acid and betaine, either alone or in combination reduced lipid peroxidation and enhanced the activities of antioxidant enzymes, thereby improving the birds' antioxidant defenses. These findings are consistent with those of earlier reports by Egbuniwe *et al*. (2021), which showed that ascorbic acid and betaine improved antioxidant activities in heat-stressed quail. Additionally, the reports of Attia *et al*. (2018) that ascorbic acid supplementation decreased lipid peroxidation in poultry birds and increased antioxidant activities concur with the findings in this present study. Ascorbic acid scavenges free radicals, mitigating their adverse effects on physiological responses in quails (Egbuniwe *et al*., 2020). Betaine has antioxidant properties, as well as osmoregulatory and methylation properties in poultry birds (Ratriyanto and Mosenthin, 2018). It is believed that these capabilities of betaine may improve protein synthesis and antioxidant enzyme activity when quails are exposed to heat stress. It is also evident from our findings that the synergistic effect of ascorbic acid and betaine further improved antioxidant activities in the quail hens during thermally stressful environmental conditions.

Most of the geometric properties of eggs (surface area, volume, geometric mean diameter and sphericity) were significantly higher in the quails treated with ascorbic acid, betaine or ascorbic acid + betaine, and also increased with the age of the quails in the present study. This finding aligns with the reports of Konca *et al*. (2021) that showed that rosehip (*Rosa canina*) improved the geometric characteristics of quail eggs. The enhanced effects of ascorbic acid and/or betaine on these geometric parameters may be due to their antioxidant capabilities, which may have ameliorated the adverse effects of heat stress. Ascorbic acid has been reported to neutralize excess free radical production, and in that way reduce damage to enterocyte**s (**Gęgotek and Skrzydlewska, 2022**)**, while betaine enhances protein synthesis by methylating DNA (Arumugam *et al*., 2021). It is believed that these attributes of ascorbic acid and betaine helped improve feed conversion efficiency and utilization (Egbuniwe *et al*., 2020; Egbuniwe and Uchendu, 2022), and may underlie the observed improvement in egg geometry.

High ambient temperatures and relative humidity in tropical and subtropical regions cause heat stress in poultry birds (Jeelani *et al*., 2019). Heat stress can decrease egg production and quality, as well as feed intake and conversion efficiency (Kim *et al*., 2019). Though feed intake and conversion efficiency were not measured in the present study, it is believed based on the results that supplementation with ascorbic acid and/or betaine alleviated the adverse effects of thermal stress in laying quails during the thermally stressful dry season.

The geometric properties of eggs are critical for egg processing, manipulation, transportation, and for evaluating poultry production (Wang *et al*., 2021). The improvement recorded in the geometrical parameters of eggs laid by Japanese quail hens given dietary supplements of ascorbic acid and/or betaine in the present study could improve the marketability of the eggs. Eggs with better geometric properties can rest well inside crates, minimizing breakage during transportation. These properties can also be utilized to predict egg quality, breeding egg hatchability, and hatchling size (Severa *et al*., 2013).

A previous study by Egbuniwe *et al*. (2020) reported that supplementing betaine and/or ascorbic acid can decrease the cost of production by enhancing feed conversion efficiency and egg production in Japanese quails. These findings also agree with those of Desinguraja *et al*. (2020) who demonstrated that the lowest production cost per kilogramme of liveweight was found in broiler chickens fed diets with betaine, making its supplementation a cost-effective option. Betaine is particularly effective in protecting the integrity of the intestine, improving nutrient utilization and subsequently, promoting the growth of heat-stressed animals (Lakhani *et al*., 2020).

The present study demonstrated that ascorbic acid and betaine could improve responses to heat stress in Japanese quail hens by decreasing lipid peroxidation while enhancing the activities of antioxidant enzymes. The study also showed that quail farmers could improve profit from the sales of high-quality eggs from quail hens fed diets supplemented with ascorbic acid and betaine during stressful environmental conditions.

Conflict of Interest:

The authors declare no conflict of interests.

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