

Modulatory effects of ascorbic acid and/or betaine on responses of packed cell volume and some production traits of male Japanese quails during dry season.

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Abstract

The experiment investigated the modulatory effects of ascorbic acid and/or betaine on responses of packed cell volume and some productive traits of male Japanese quails during the dry season. A total of 240 (14 day-old) male Japanese quails (*Coturnix japonica*) were purchased. Birds were acclimatized and randomly assigned to 4 groups, each having 60 birds. Each group consisted of 3 replicate (20 birds per replicate). Birds were fed Control (basal); AA, BET and AA + BET diets. Ambient temperature (AT) and relative humidity (RH) recorded ranged from 25.0 – 37.0 °C and 48.0 – 92.0 %, respectively, and were mostly outside thermoneutral zone for Japanese quails. Production parameters were evaluated as average daily feed intake (AVDFI), average daily water consumption (AVDWC) and liveweight. Packed cell volume (PCV), serum malondialdehyde (MDA) and reduced glutathione (rGSH) were evaluated at 28, 49 and 70 day-old. Serum MDA, AVDFI and AVDWC were lower ($P < 0.05$) in birds fed diets enriched with either AA, BET or their combinations when compared with those in control group. However, dietary enrichment with AA ± BET enhances ($P < 0.05$) PCV and liveweight in male quails in comparison to the control birds. In conclusion, dietary ascorbic acid and betaine modulated packed cell volume and production parameters such as feed and water consumption, as well as, liveweight of male Japanese quails during the dry season.

Keywords: Antioxidants, heat stress, quails, production

Introduction

High ambient temperature (AT) and high relative humidity (RH) adversely affects poultry production in tropical regions of the world (Oke et al., 2021). These environmental conditions are recorded during the dry season in the Derived Guinea Savannah zone of Nigeria (Ani and Okpara, 2019). The combined effects of high AT and high RH, as measured by temperature – humidity index (THI) could result in heat stress in Japanese quails (Gouda et al., 2021). Heat stress induces lipid peroxidation and oxidative stress due to excessive generation of free radicals (Akbarian et al., 2016). Heat stress overwhelms the endogenous antioxidant defences increasing the susceptibility of quails to the deleterious effects of the thermal conditions (Surai et al., 2019). Heat-stressed quails show reduced packed cell volume (Abdelhady et al., 2017). The stressful environmental conditions, induced by high AT and high RH, negatively impacts production from high mortality and weight loss (El-Kholy et al., 2017). Heat stress decreases packed cell volume in poultry birds (Scanes, 2016). Thermoregulation is adversely affected during osmotic stress and consequently, blood volume constriction or prevention of volume expansion (Yahav, 2009).

Dietary antioxidants supplementations are considered cheaper alternatives to ameliorating the adverse effects of heat stress in the poultry industry (Shakeri et al., 2018). Betaine (BET) is utilized in the poultry industry for its antistress properties (Saeed et al., 2017). The methyl donor and osmolyte functions of betaine enhance thermoregulatory potentials of poultry birds exposed to heat stress (Al-Tamimi et al., 2019). Ascorbic acid (AA) ameliorate lipid peroxidation induced by excess generation of free radicals (Ali et al., 2020). However, there is paucity of information of the beneficial effects of AA and/or betaine on production traits of Japanese quails (*Coturnix japonica*) subjected to thermally stressful

environmental conditions. The aim of the study was to investigate the modulatory effects of ascorbic acid and/or betaine on responses of packed cell volume and some productive traits of male Japanese quails during the dry season.

Materials and Methods

Ethical Statement

The methods of handling protocol were in accordance of the Animal Use and Care Committee of University of Nigeria, Nsukka, Nigeria. All quails were managed in accordance with the Guide for the Care and Use of Laboratory Animals (Committee on Care and Use of Laboratory Animals, 1996).

Experimental Site

The experiment was performed at the Animal House of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, located in the Derived Savannah Ecological zone, Enugu State, South-Eastern Nigeria (Uguru et al., 2011). The town has an annual rainfall ranging from 986 – 2098 mm (Momoh et al., 2010). The study was carried out from January – March.

Animals and Management Conditions

A total of 240 male Japanese quail chicks; 14 day-old, with average weight of 56.4 ± 0.4 g were sourced commercially for the study. Following 7 days of acclimation, the quails were assigned by complete randomized design into 4 groups of 60 birds each. Each group was sub-divided into 3 replicates with 20 quails (Ciftci et al., 2016; Al-Salhi et al., 2017). Each male group had 60 quails, with 20 birds per replicate. Birds in every replicate were housed in cages measuring 91.44 cm × 76.2 cm × 91.4 cm and placed within the animal house. The birds were reared under natural environmental conditions. Birds in the control groups (Control) were fed basal diets with no supplementation.

Those in treatment groups consumed basal diets supplemented with ascorbic acid at 200 mg/Kg (AA group); betaine at 2 g/Kg (BET) and combination of AA; 200 mg/Kg and betaine; 2 g/Kg (AA + BET) of diets. Betaine hydrochloride (Sigma-Aldrich, St. Louis, Missouri, USA; $\geq 99.0\%$ purity) and ascorbic acid (Kempex Holland BV, Volkel, Netherlands) were used for the study. Betaine was included in diets for the experimental groups at rate of 2 g/kg (de Jong et al., 2012), while ascorbic acid (AA), at 200 mg/kg of feed (Sahin et al., 2002). Daily dietary supplementation with ascorbic acid and/or betaine lasted 49 days. The following feed types were provided for the birds in their feeders: commercial starter diet (22 % CP; 3100 kcal/kg of metabolizable energy; ME/kg of feed), grower mash (15.50 % CP; 2550 kcal/kg ME) and layer mash (16.80 %; 2680 kcal/kg ME). Quails were given access to water and feed ad-libitum throughout the study period.

Thermal Environmental Conditions

The thermal environmental conditions were measured as ambient temperature (AT), relative humidity (RH) and temperature – humidity index (THI). The AT was recorded using Mason's Type Wet and Dry Bulb Hygrometer (Zeal, London, England; accuracy of $\pm 1^\circ\text{C}$ (or $\pm 5\%$) and RH, obtained using Hygrometric Tables for computation of relative humidity (Zeal, London, England). The THI was calculated according to the formula described by (Zulovich and DeShazer (1990): $\text{THI} = 0.6\text{Tdb} + 0.4\text{Twb}$.

Where Tdb = dry-bulb temperature ($^\circ\text{C}$) and Twb = wet-bulb temperature ($^\circ\text{C}$). The THI describes the combined effects of temperature and humidity on the quails. All environmental conditions were recorded at 08:00 h, 13:00 h and 17:00 h on daily basis throughout the duration of the study (49 days).

Evaluation of Production Parameters

Parameters relevant to production of quails were evaluated as average daily feed intake (AVDFI), average daily water consumption (AVDWC) and liveweight. Daily feed and water consumed were measured throughout the study period. Method described by Hassan et al. (2010) was used to obtain AVDFI and AVDWC. Daily feed intake and water consumed were measured by subtracting final from initial amounts of feed and water given the birds; and subsequently, divided by the number of quails in each group. Liveweight of 15 males, tagged for identification, were individually weighed using digital scale to the nearest 0.1 g (Asia Techno weigh India, Haryana, India).

Blood and Serum Collection

Blood collection from quails were performed in the morning periods (between 09:00 h – 11:00 h) (Makeri et al., 2017) at ages 21, 28, 49 and 70 day-old. A total of six birds from each group (2 birds per replicate) were randomly selected, identified with number leg tags. Each quail was weighed using digital scale to the nearest 1g (Asia Technoweigh India, Haryana, India) and slaughtered by decapitation (Erol et al., 2009), and its blood (about 3 mL) quickly collected into properly labelled sample bottles without anticoagulant (sodium ethylenediaminetetraacetate). Blood samples were kept at room temperature for 3 hours to clot. Thereafter, they were centrifuged at $3000 \times g$ for 10 minutes under room

temperature (El-Tarabany, 2016). Sera were harvested and immediately used for analysis.

Biomarkers of Oxidative Stress

Serum malondialdehyde (MDA) concentration was determined 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 ratio 1:1, incubated at room temperature (25°C) and centrifuged at $2500 \times g$ for 10 minutes. Following the addition of 1 % thiobarbituric acid to the supernatant, samples were warmed in water bath (100°C) for 15 minutes. The content were then cooled, centrifuged at $2500 \times g$ for 15 minutes. The optical density of supernatant was determined at 532 nm against blank, using spectrophotometer (Jenway 6305; Jenway, Essex, UK) and a standard curve constructed using various MDA concentrations of 0 – 20 nMoles. Concentration of serum MDA was expressed as nMol/mL

Serum reduced glutathione (GSH) was evaluated using the technique described by Moron et al. (1979). This is based on principle of complex formation from reaction of dithionitobenzene (DTNB) and acid sulfhydryl groups (non-protein thiols containing 93 % GSH-Rd). Briefly, a mixture of serum (0.5 mL) and 0.1 mL of 25 % tricarboxylic acid (TCA) was centrifuged for 10 minutes at $3000 \times g$. In addition, 0.2 M of sodium phosphate buffer (0.7 mL, pH 8) and 0.6 mM of DTNB (2 mL; Sigma, St. Louis, MO, USA) were mixed with the supernatant (0.3 mL) for 10 minutes. Thereafter, spectrophotometer (Jenway 6305; Jenway, Essex, UK) was used to measure optical density of product at 412 nm. Reduced glutathione concentration was determined from a standard graph (Sigma, St. Louis, Missouri, United States) of various GSH concentrations and expressed as nMoles/mL.

Packed cell Volume (or hematocrit)

Blood samples (1 mL) were collected individually from 6 male and 6 female quails, into sample bottles containing anticoagulant (sodium ethylenediaminetetraacetic acid (EDTA) at a concentration of 1.5 mg/mL (Robertson and Maxwell, 1993). Thereafter, blood was drawn into capillary tubes (Fisher Scientific, Pittsburgh, Pa.) by capillary action and sealed at one end. Microhaematocrit centrifuge (Hawksley, England) was used to centrifuge the blood samples at $12,000 \times g$ for 5 minutes to obtain even packing of erythrocytes. Packed cell volume was obtained using microhaematocrit reader and expressed as percent (%).

Results

Environmental Conditions

The description of thermal environmental conditions that were recorded during the study period are shown in Table 1 and Figure 1. During the experiment, mean AT ($31.5 \pm 0.40^\circ\text{C}$), RH ($79.6 \pm 0.80\%$) and THI (84.8 ± 0.50) were obtained (Figure 1). The range values of AT were $25.0 - 37.0^\circ\text{C}$; RH, $63.0 - 91.0\%$ and THI, $75.6 - 91.0$. At 13:00 h, the highest mean AT ($33.7 \pm 0.32^\circ\text{C}$), THI (86.0 ± 0.55), and the lowest mean RH (67.9 ± 1.6) were observed (Figure 2). However, the lowest mean AT ($27.8 \pm 0.26^\circ\text{C}$), THI (78.5 ± 0.62), but highest mean RH ($79.6 \pm 1.80\%$) were obtained at 08:00 h (Figure 1).

Table 1: Environmental conditions prevailing during the study period and thermoneutral values for Japanese quails

	Environmental conditions		
	Ambient temperature (°C)	Relative humidity (%)	Temperature-humidity index
Mean ± SEM	31.5 ± 0.40	79.6 ± 0.80	84.8 ± 0.50
Range values	25.0 - 37.0	63.0 - 91.0	75.6 - 91.0
*Thermoneutral zone	23.8 ± 0.7	58.5 ± 5.7	76.0 - 80.0

*El-Tarabany (2016)

Table 2. Serum levels of oxidative markers in male Japanese quails fed dietary ascorbic acid and/or betaine during the study period (n = 6).

Oxidative markers	Age of birds (days)	Experimental groups			
		Control	AA	BET	AA+BET
Malondialdehyde concentration (nMol/mL)	28	0.67 ± 0.03	0.62 ± 0.05	0.56 ± 0.03	0.62 ± 0.05
	49	0.80 ± 0.03 ^a	0.61 ± 0.02 ^b	0.65 ± 0.02 ^b	0.62 ± 0.02 ^b
	70	0.85 ± 0.04 ^a	0.71 ± 0.01 ^{a,b}	0.66 ± 0.04 ^b	0.57 ± 0.03 ^b
Reduced glutathione (nMol/mL)	28	6.94 ± 0.76 ^a	8.85 ± 0.61 ^a	9.85 ± 0.57 ^b	8.93 ± 0.51 ^{a,b}
	49	6.27 ± 0.77	7.70 ± 0.64	7.98 ± 0.80	7.98 ± 0.80
	70	5.58 ± 0.42 ^a	8.09 ± 0.88 ^a	7.32 ± 0.81 ^a	8.76 ± 0.79 ^b

Control = basal diet; AA = Ascorbic acid, BET = betaine and AA+BET = Combination of ascorbic acid and betaine included in diets. ^{a,b} Mean values with different superscript letters within the same row are significantly different (P < 0.05).

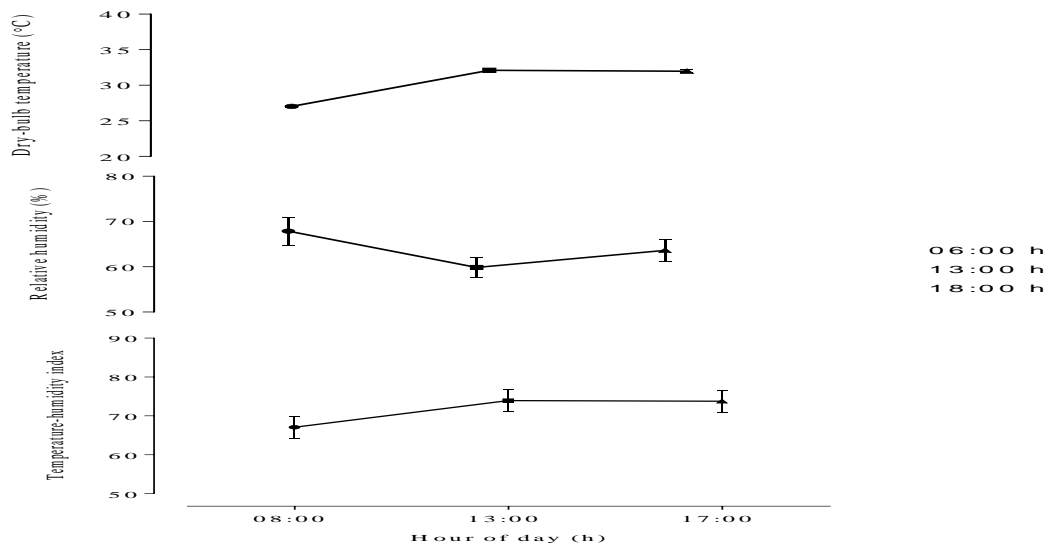


Figure 1. Hourly fluctuations in thermal environmental parameters during the study period.

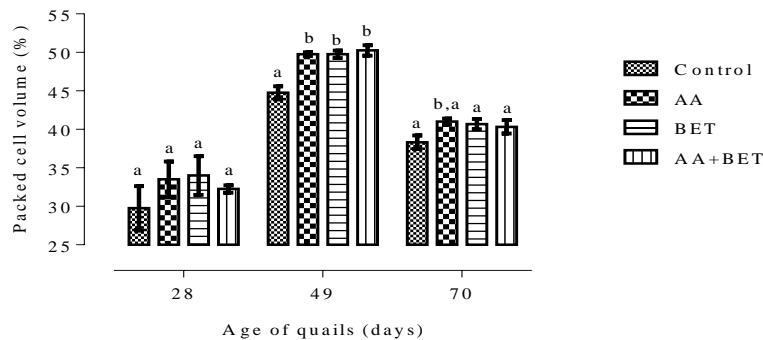


Figure 2. Packed cell volume of male Japanese quails at different day ages (n = 6).

Production Parameters

Average daily feed and water intake, and live weight for male Japanese quails are shown in Table 2. The AVDFI in male Japanese quails were lower ($P < 0.05$) in quails fed diets enriched with either AA or betaine when compared with those in control group. Similarly, AVDWC were lower ($P < 0.05$) in AA ± BET when compared with those of control birds. There were significantly higher ($P < 0.05$) values of liveweight in quails which consumed diets enriched with betaine either alone or its combination with AA during the study period.

Biomarkers of Oxidative Stress

Table 1 showed the levels of serum biomarkers of stress in male Japanese quails obtained during the study period. The MDA concentration of AA-fed quails was significantly lower when compared with the value assessed in control group at 49 days-old. Similarly, there were significantly ($P < 0.05$) lower levels of MDA concentration in quails fed diets containing either BET or AA+BET at 49 and 70 day-old. In addition, serum rGSH in 28 days old quails were significantly ($P < 0.05$) higher in quails fed BET-enriched diets when compared with that of control birds. Birds fed diets enriched with AA+BET recorded significantly ($P < 0.05$) more levels of rGSH activities when compared with birds in the control group at 28th and 70th days of age.

Packed Cell Volume

The PCV in male Japanese quails during the study period is shown in Figure 3. At 49 day – old, PCV values were significantly higher ($P < 0.001$) in male quails which consumed diets with either AA, betaine or AA+BET when compared with control value. At 70 days old, the birds fed AA diet recorded significantly higher PCV ($P < 0.05$), while those in either betaine- or AA+BET groups were similar.

Discussion

Environmental Conditions

The results of the present study showed that the environmental conditions prevailing during the dry season in the zone fluctuated widely and were predominantly outside the thermoneutral zone for Japanese quails as demonstrated by (El-Tarabany, 2016). These findings are evidenced by values of mean, minimum and maximum AT, RH and THI recorded during the study period. The diurnal fluctuations of environmental parameters observed in the present study is an indication of the wide fluctuation which occurred during the day time. Thus, the findings from the present study suggest that the thermal environmental conditions prevailing during the dry season could induce heat stress in Japanese quails. The results of the present study agree with those of Mehaisen et al. (2017) who showed that heat stress may occur in Japanese quails when AT exceeds 30 °C. The findings of the present study corroborates those of Sinkalu et al. (2015) who demonstrated that wide fluctuations in environmental conditions contribute in heat stress in poultry birds. High AT, RH and THI increase thermal load, and may consequently, impair the thermoregulatory mechanisms in the Japanese quails. Thermal environmental conditions exceeding the zone of comfort could also result in

excessive generation of free radicals, consequently, oxidative stress in quails (Sahin et al., 2008). Therefore, it is recommended that quail farmers enrich diets of Japanese quails as a management practice in order to ameliorate the negative effects of heat stress during the dry season.

Production Parameters

The findings of the current study show that AA and/or betaine enriched diets fed to male Japanese quails reduced AVFDI and AVWC, but increases liveweight in male Japanese quails during the dry season. The results of the present study is in agreement with those of Egbuniwe et al. (2018), who demonstrated that that liveweight increased despite decreases in average daily feed and water intake in male broiler chickens reared during the thermally stressful hot-dry season. The decreases in feed intake in the AA and/or betaine could be a thermoregulatory mechanism to reduce endogenous heat generation via metabolism (Herd and Arthur, 2009). The antioxidant property of AA and betaine may have exerted inhibitory effects of neuropeptide Y that is involved in regulating appetite (Halford and Blundell, 2000). In addition, AA fed the quails has been shown to exert a monoaminergic modulation to decrease feed intake (Rosa et al., 2016). Although, the activities of neuropeptide Y and leptin were not evaluated in the current study, further investigation may be required to determine the mechanism by which betaine influenced decreased appetite of male Japanese quails during the dry season.

From the results of the current study, it is shown that liveweight improved in quail fed diets enriched with AA and/or betaine despite the high AT and high RH. These findings from the current study suggest that AA and/or betaine could have enhanced the utilization of nutrient utilization as shown by improved liveweight of the birds during the dry season. Betaine is known to improve nutrient utilization, and consequently, enhances body weight in birds (Ratriyanto and Mosenthin, 2018). From the results of the present study, it is suggested that betaine could be utilized to improve liveweight during the thermally stressful dry season.

Biomarkers of Stress

The results of the current study demonstrated that AA and/or betaine enriched diets fed to male Japanese quails decreased MDA concentration, but increased reduced glutathione, an antioxidant enzyme, during the dry season. The findings of the present study show that AA and/or betaine exerted antioxidant properties and improved thermotolerance during the dry season. The findings of the current study agree with those of Dalkilic et al. (2015) who showed orange peel oil exerted antioxidant property and thermotolerance by decreasing MDA concentrations in heat stressed Japanese quails. The results of the present study collaborates those of Mehaisen et al. (2017) who showed that propolis attenuated lipid peroxidation by decreasing serum MDA levels in heat stressed laying Japanese quails. From the results of the present study it is demonstrated that AA and/or betaine reduced lipid peroxidation and enhanced activities of antioxidant enzyme, consequently improving the antioxidant defenses of male Japanese quails reared under thermally stressful environmental conditions.

Packed Cell Volume

The results of the present study show that AA and/betaine supplemented in diets of male Japanese quails improved PCV in quails exposed to thermally stressful conditions during the dry season. These findings are evidenced by higher values of PCV recorded in the treated quails when compared with that of control birds at all ages. The results of the present study is in agreement with those of Attia et al. (2019) who showed the antioxidant properties of propolis by improving PCV in heat stress roosters.

The finding of the present study indicate that the PCV obtained in the quails during study period were predominantly within the normal range of 30.0 – 45.1 % for Japanese quails, except for values obtained in birds at 49th day of age fed either AA, betaine or AA+betaine-enriched diets. The reason for the wide variation at 49 days old is uncertain, thus, may require further investigations. The PCV was observed to return to normal range at 70th day of age. Heat stress lowers PVC in poultry birds (Scanes, 2016), as well as results in lipid peroxidation and depression of erythropoietic organs (Surai et al., 2019). However, from the results of the present study, it is suggested that betaine may have improved red cell tolerance to heat stress and lowered hemolysis. Betaine is known to create osmotic balance and heat stress decrease lipid peroxidation in cells (Attia et al., 2019). Thus, the adverse effects of heat stress on PCV may be attenuated by dietary supplementation with antioxidants like AA and/or betaine to male quails.

Conclusion

In conclusion, dietary ascorbic acid and betaine improves production parameters, namely average daily feed and water intake, liveweight as well as packed cell volume of male Japanese quails during the dry season.

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