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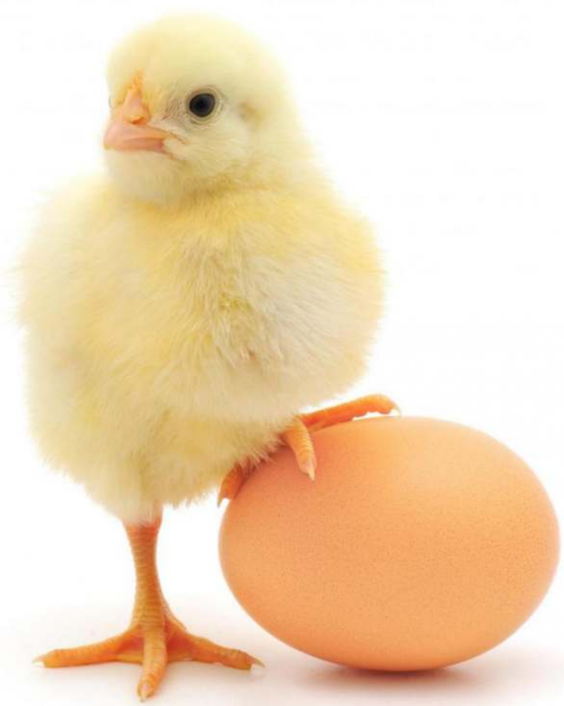

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Effect of Dietary Treatments of Ascorbic Acid on the Blood Parameters, Egg Production and Quality in Quail (*Coturnix coturnix japonica*) Subjected to Heat Stress

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Abstract: A study to determine the effects of dietary supplementation with ascorbic acid on blood parameters, egg production and quality in quail (*Coturnix coturnix japonica*) subjected to heat stress was conducted. Forty eight adult female quail were divided into 6 equal groups (A to F) and fed *ad-libitum* on either commercial layers mash ration (Groups A and D), layers mash supplemented with 200mg (Groups B and E) or 1000mg per kilogram ascorbic acid (Groups C and F). From the day treatment with Ascorbic acid started, Groups A, B and C were exposed to high temperature of about 38°C for about 8 hours a day for two weeks. Daily feed and water consumption, egg production and quality and blood parameters were measured. By the end of the second week, feed consumption was higher in groups that were not subjected to heat stress (Groups D, E and F) than in those subjected to heat stress (Groups A, B and C). There was however no significant difference in feed consumption between birds treated with the two levels of ascorbic acid (AA) and those that were not, in both heat stressed and non stressed groups. Birds in heat stressed groups given ascorbic acid supplementation consumed more water than those not given any ascorbic acid. There was also no significant difference in weight of birds, egg production and egg weight between the groups except in birds that were not heat stressed and treated with 1000g ascorbic acid whose eggs were significantly heavier ($P<0.05$). No significant difference was also observed in the mean egg weight between quail that were neither exposed to high temperatures nor treated with AA (Group D) and those that were exposed to high temperatures only (Group A), treated with AA only (Groups E and F) and exposed to high temperatures and treated with AA (Groups B and C). Following one week of the experiment, birds that were not exposed to heat stress but treated with 1000mg of AA (Group F) had heavier eggs ($P<0.05$) than those that were heat stressed and given no AA (Group A) ($P<0.05$) and those that were heat stressed and given 200mg AA (Group B) ($P<0.05$). After 2 weeks of experiment however, eggs from birds in Groups E and F were significantly heavier ($P<0.01$; $P<0.001$) than those from Group A. Birds exposed to heat stress and given 1000mg AA (Group C) had heavier eggs ($P<0.05$) than those also exposed to heat stress but were not given AA (Group A). The eggs of birds exposed to heat stress and not given AA (Group A) were also significantly lighter ($P<0.05$) than those also not given AA but not expose to heat stress (Group D). Birds on heat stress given 200mg AA similarly had lighter eggs($P<0.05$) than those given the same level of AA but not heat stressed (Group E). There were no significant differences in the length and width of quail eggs between any of the six groups. Mean shell weight and thickness of quail eggs were however higher in birds given AA than in those not given but the differences were not significant except between birds in Group F and those in Group A ($P<0.01$) and Group B ($P<0.01$), respectively. No significant difference in internal egg quality as measured by Haugh unit was observed between any of the groups of the six groups. There was also no significant difference between any of the six groups in RBC count, Hb concentration, PCV%, MCV MCH, MCHC and WBC count except for a higher WBC count in Group F compared to Group A ($P<0.05$). It was concluded that the quail appear to exhibit some level of heat resistance compared with other species of birds and also to benefit from ascorbic acid supplementation under hot environments; however, further studies would need to be undertaken.

Key words: Pathology, quail, ascorbic acid, high ambient temperature, Newcastle disease, immune response

Introduction

The quail (*Coturnix coturnix japonica*), a native of Asia and Europe and farmed since ancient times in the Far East was introduced into Nigeria by the National Veterinary Research Institute (NVRI) Vom. The quail are hardy birds that can adapt to many different environments (Phillips, 1948). They mature early (as early as 5 to 6 weeks), reproduce rapidly (hens will lay up to 300 eggs in 360 days and are very resistant to common epidemics of poultry (Phillips, 1948; Banks, 1979; NRC, 1991). Because the birds are also cheap to acquire and easy to keep, requiring only simple cages and equipment and little space (Ruskin, 1991), they are rapidly becoming popular in all regions of the country.

In the semi-arid zone of Nigeria, with long dry and hot period that lasts from 8 to 9 months (Alaku, 1982) poultry production has known to be hampered by high ambient temperatures. For quail farming to be profitable in this region measures to alleviate the effects of high ambient temperatures on production of the birds must be found. There had been varying reports of the usefulness of ascorbic acid in improving heat resistance and reducing mortality associated with high ambient temperatures in chicken (Perek and Kendler, 1962, 1963; Ahmed *et al.*, 1967; Lyle and Moreng, 1968). In a recent study (Ubosi and Gandu, 1993), the use of supplementary ascorbic acid in diets of caged laying chickens in the semi arid zone of Nigeria has demonstrated significant improvement in hen-day egg production, feed efficiency and egg weight. The aim of this study therefore is to determine the effect of ascorbic acid on egg production and quality parameters of quail subjected to artificially high temperatures. The result of this study will improve the knowledge on the use of ascorbic acid in alleviating the detrimental effects of high ambient temperatures, which may be beneficial to quail farmers in the semi-arid region of Nigeria.

Materials and Methods

Forty eight female Japanese quail (*Coturnix coturnix japonica*) that have been in lay for about 4 months were randomly divided into 6 equal groups (A to F) in compartments partitioned with cardboard. All birds were fed *ad-libitum* on commercial layers mash ration (ECWA, Nigeria Ltd.) and water. The feed was however supplemented with 200mg per kilogram ascorbic acid for birds in Groups A and D and 1000mg for those in Groups B and E. The amount of feed and water remaining in the feeders and drinkers were recorded daily to determining feed and water consumption, respectively. A thermometer was placed at the level of the top of the birds in each of the compartments and thermostatically controlled flexible plastic-insulated heating elements with a sensor (Eco - Element - ECoSTAT, Redruth, UK.) was suspended over the top of each of 3 groups (A, B and C) and adjusted to provide steady heat at about 38^{o}C. From the day ascorbic acid supplementation begun the heating elements in Groups A, B and C were switched on daily for about 8 hours during the daytime. Temperature in each of the compartments was recorded 3 times daily (morning, afternoon and evening). Feed and water consumption were recorded daily. Eggs were collected from each compartment at the same time every morning. From all the eggs collected in a week, ten eggs were selected randomly for weighing, measurement of length and width using a Vernier caliper, Egg shell thickness using a micrometer gauge, individual albumen height and diameter using calipers and shell weight. Haugh unit and albumen index were calculated. Two weeks after the commencement of the experiment all birds were weighed to the nearest 0.1g. Blood samples were collected for determination of blood parameters (Red Blood Cell (RBC) count, Haemoglobin (HG) concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell (WBC) count) at the beginning and at the end of the treatment period using standard methods. On the day the experiment commenced ad two weeks after all birds were weighed to the nearest 0.1g.

Statistics: One way analysis of variance (Steel and Torrie, 1980) was used to establish significant differences between means. The Bonferroni ‘post test’ method of GraphPAD Software (1990) computer program was used to determine differences between means of specific groups. All results were regarded as significant when $P < 0.05$. All means were presented as ± SD.

Results

Feed consumption: Mean daily feed consumption of quail for one week prior to and the two weeks after the commencement of the experiment is shown in Table 1. Even though by the first week of the experiment feed consumption was higher in groups that were not subjected to heat stress (Groups D, E and F) than in those subjected to heat stress (Groups A, B and C) the differences were not significant. The differences were however significantly higher ($P<0.05$; $P<0.05$; $P<0.05$, respectively) by the end of the second week. There was however no significant difference in feed consumption between birds treated with the two levels of ascorbic acid (AA) and those that were not treated in both the groups exposed to heat stress (Groups A, B and C) and those not exposed to heat stress (Groups D, E and F).

Water consumption: The effects of exposure to heat stress and treatment of quail with two levels of AA on water consumption are present in Table 2. By the end of the first week of the experiment birds subjected to heat stress and given 1000mg AA (group C) consumed more water than those not subjected to heat stress and given no ascorbic acid (Group D), 200mg (Group E) or 1000mg AA (Group F) ($P<0.01$; $P<0.01$; $P<0.01$). By the end of the second week water consumption continued to be significantly higher in Group C than in Groups D, E and F ($P<0.05$; $P<0.01$; $P<0.01$). Birds on heat stress

Table 1: Mean weekly feed consumption (g) of quail one week before and one and two weeks after exposure to heat stress and treatment with ascorbic acid

Group no.	1 week pre-exposure and treatment	1 weeks post-exposure and treatment	2 weeks post-exposure and treatment
1	214.3±37.80	182.9±23.60	117.9±34.50
2	231.4±20.35	157.1±18.59	150.0±41.82
3	204.3±28.78	167.9±40.09	139.3±34.93
4	204.3±16.18	189.3±31.51	178.6±39.33
5	190.0±20.00	186.4±19.30	182.1±27.81
6	208.0±34.36	193.6±30.91	178.6±36.59

Table 2: Mean weekly water intake (ml) of quail one week before and one and two weeks after exposure to heat stress and treatment with ascorbic acid

Group no.	1 week pre-exposure and treatment	1 weeks post-exposure and treatment	2 weeks post-exposure and treatment
1	337.1±54.68	382.2±62.44	389.3±28.34
2	378.6±74.03	392.9±53.45	450.0±63.69
3	344.3±78.70	500.0±91.28	412.9±63.69
4	327.9±58.15	339.3±77.53	321.4±68.35
5	311.4±15.73	325.7±53.80	307.1±70.28
6	288.6±60.67	321.4±143.92	285.7±47.55

treated with 200mg AA (Group B) also consumed significant more water than those in Groups D, E and F (P<0.001; P<0.001; P<0.001).

Body weight: There was no significant difference between any of the groups in the weight of birds (Table 3).

Egg production: Table 4 shows the effects of heat stress and/or treatment with AA on mean daily egg production of quail. Mean egg production was higher in the non-heat stressed groups treated with AA compared with those that are heat stressed and/or not treated with AA, the differences were however, not significant.

Egg external parameters: Mean weight, length, width, shell thickness, shell weight and percent shell weight of eggs of quail subjected to heat stress and/or treated with AA are shown in Table 5. Following both one week and two weeks of the experiment there was no significant difference in the mean egg weight between quail that were neither exposed to high temperatures nor treated with AA (Group D) and those that were exposed to high temperatures only (Group A), treated with AA only (Groups E and F) and exposed to high temperatures and treated with AA (Groups B and C). Following one week of the experiment, birds that were not exposed to heat stress but treated with 1000mg of AA (Group F) had heavier eggs (P<0.05) than those that were heat stressed and given no AA (Group A) (P<0.05) and those that were heat stressed and given 200mg AA (Group B) (P<0.05). After 2 weeks of experiment however, eggs from birds in Groups E and F were significantly heavier (P < 0.01; P < 0.001) than those from Group A. Birds

Table 3: Mean weight of quail (g) on Day one and two weeks after exposure to heat stress and treatment with ascorbic acid

Group no.	Day 1 of exposure to heat stress and treatment with ascorbic acid	two weeks after exposure to heat stress and treatment with ascorbic acid
1	224.1±28.10	242.9±48.93
2	221.4±46.70	207.6±23.45
3	210.2±15.4	210.3±24.22
4	211.6±38.12	224.9±28.80
5	221.3±38.93	222.1±22.30
6	236.6±24.21	239.2±28.59

Table 4: Mean daily egg production of quail one week before and one and two weeks after exposure to heat stress and treatment with ascorbic acid

Group no.	1 week pre-exposure and treatment	1 weeks post-exposure and treatment	2 weeks post-exposure and treatment
1	4.6±1.71	4.1±1.34	2.3±1.11
2	2.4±0.78	3.0±0.81	3.3±1.11
3	2.9±1.77	2.4±1.61	3.1±1.57
4	2.4±1.80	2.4±1.27	1.6±1.27
5	1.4±1.51	1.2±0.75	1.9±1.57
6	2.2±1.80	2.8±1.57	3.7±0.75

exposed to heat stress and given 1000mg AA (Group C) had heavier eggs (P<0.05) than those also exposed to heat stress but were not given AA (Group A). The eggs of birds exposed to heat stress and not given AA (Group A) were also significantly lighter (P<0.05) than those also not given AA but not expose to heat stress (Group D). Birds on heat stress given 200mg AA similarly had lighter eggs (P<0.05) than those given the same level of AA but not heat stressed (Group E).

There were no significant differences in the length and width of quail eggs between any of the six groups. Mean shell weight and thickness of quail eggs were higher in birds given AA than in those not given but the differences were not significant except between birds in Group F and those in Group A (P<0.01) and Group B (P<0.01), respectively.

Internal egg quality: Table 5 demonstrates the effect of exposure of quail to high temperature and treatment with AA on Haugh unit of the quail's eggs. No significant difference in Haugh unit was observed between any of the six groups.

Blood parameters: Blood samples collected on the day of the commencement of the experiment clotted and could therefore not be analyzed. The blood parameters of quail two weeks after the commencement of the experiment is shown in Table 7. There was no significant difference between any of the six groups in RBC count, Hb concentration, PCV%, MCV MCH, MCHC and WBC count except for a higher WBC count in Group F compared to Group A (P<0.05).

Table 5: Egg parameters of quail one and two weeks after exposure to heat stress and treatment with ascorbic acid

Grp	Egg weight (g)		Egg length (cm)		Egg width (cm)		Shell thickness (cm)		Shell weight (g)		% shell of egg	
no.	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
1	10.8±1.22	9.4±0.82	3.0±0.16	2.9±0.10	2.4±0.08	2.3±0.08	0.30±0.05	0.30±0.07	0.74±0.07	0.73±0.08	6.92±1.09	7.85±1.29
2	10.1±0.92	10.2±1.30	2.9±0.10	2.9±0.12	2.3±0.08	2.4±0.10	0.31±0.07	0.34±0.07	0.78±0.06	0.80±0.07	7.74±1.03	7.95±0.87
3	11.7±1.37	11.5±0.70	2.9±0.31	2.9±0.26	2.5±0.09	2.4±0.05	0.44±0.32	0.35±0.08	0.82±0.05	0.83±0.06	7.03±0.75	7.24±0.04
4	12.2±0.64	11.3±1.43	3.1±0.09	3.1±0.17	2.5±0.04	2.5±0.11	0.26±0.04	0.25±0.04	0.85±0.05	0.77±0.06	6.95±0.39	7.01±1.17
5	11.6±0.69	12.1±1.21	3.0±0.08	3.0±0.09	2.4±0.05	2.4±0.07	0.33±0.08	0.35±0.09	0.78±0.08	0.78±0.08	6.73±0.59	6.45±0.27
6	12.6±1.79	12.2±1.46	3.1±1.79	3.1±0.17	2.5±0.10	2.5±0.13	0.26±0.06	0.29±0.05	0.93±0.12	0.88±0.09	7.36±1.18	7.22±0.46

Table 6: Internal quality of quail egg one and two weeks after exposure to heat stress and treatment with ascorbic acid

Group	Albumin height (cm)		Albumin length (cm)		Albumin width (cm)		Albumin index		Haugh unit	
no.	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
1	0.29±0.03	0.24±0.06	4.43±0.43	4.46±0.47	3.44±0.21	3.26±0.48	1.29±0.16	1.39±0.25	81.57±o.77	82.60±0.54
2	0.30±0.07	0.22±0.06	4.36±0.32	4.58±0.41	3.18±0.40	3.54±0.33	1.38±0.21	1.28±0.15	82.01±0.56	82.08±0.77
3	0.28±0.07	0.29±0.06	4.79±0.49	4.67±0.21	3.65±0.40	3.45±0.16	1.37±0.17	1.35±0.10	81.07±0.84	81.18±0.44
4	0.28±0.06	0.27±0.05	4.53±0.34	4.67±0.47	3.61±0.30	3.78±0.36	1.25±0.05	1.23±0.08	80.77±0.41	81.34±0.87
5	0.29±0.08	0.30±0.08	4.70±0.42	4.54±0.39	3.49±0.23	3.34±0.21	1.34±0.12	1.36±0.21	81.13±0.42	80.81±0.69
6	0.36±0.09	0.27±0.06	4.62±0.35	4.60±0.34	3.41±0.19	3.36±0.33	1.35±0.13	1.37±0.10	80.48±1.07	80.82±0.86

Table 7: Blood parameters of quail two weeks following exposure to heat stress and treatment with ascorbic acid

Group no.	RBC count$\times10^6$	HB conc.	PCV	MCV	MCH	MCHC	WBC$\times10^3$
1	3.42±0.39	12.35±0.50	38.00±0.80	112.1±12.42	36.4±4.11	32.5±0.64	8.3±2.10
2	4.59±0.34	12.95±0.53	40.3±1.50	88.7±5.35	28.5±2.96	32.2±0.28	11.8±1.49
3	5.12±0.34	13.88±0.63	43.3±0.95	84.6±4.53	27.1±1.74	32.1±0.87	14.7±1.25
4	4.71±0.47	13.85±0.58	41.3±1.50	88.1±6.19	29.6±1.88	33.5±0.33	13.7±1.88
5	4.31±0.98	13.35±1.30	40.8±4.11	96.9±14.28	31.7±4.61	32.8±0.75	12.8±3.09
6	4.96±0.69	13.48±0.69	40.8±2.50	83.2±11.08	27.6±4.06	33.1±0.78	17.0±1.29

Discussion

The reduction in feed intake in quail in the two weeks of exposure of quail to high temperatures in this study is in consistency with previous observations in chickens (Payne, 1966). Neither the addition of 200mg nor 1000mg AA per Kg of feed significantly affected feed intake. This is in agreement with observations by Cheng *et al.* (1990) in old laying chickens but in contrast to reports by Bell and Marion (1987) and Ubosi and Gandu (1993) who found that adding AA to diets of hens during the summer months reduced feed intake. It therefore appears that addition of AA to diets do not ameliorate the depressive effect of high ambient temperature on feed consumption probably because of the poor palatability of the vitamin given in feed. Water consumption, which increased with exposure to high temperatures, was on the other hand significantly improved in those birds subjected to high temperatures and given supplementary AA at both 200mg and 1000mg level. This agrees with reports that water consumption was increased when chickens were raised in ambient temperatures above 32°C (Boone and Huston, 1967).

The absence of any significant effect of both high environmental temperature and AA treatment on body weight of quail in this study tallies with similar observation in caged laying chickens (Ubosi and Gandu, 1993). The absence of significant effect of high environmental temperature on feed intake probably explained the absence of body weight loss in the birds. High ambient temperatures have however been reported to lead to reduced growth rate in other studies in chickens (Robinson and Lee, 1947; Sturkie, 1965).

The overall egg production in the study was low probably because the birds did not settle down well following the disturbance of movement to their respective groups by the time the treatments started. Even though heat stress has in general, been reported to result in decreased egg production in chicken (Warren and Schnepel, 1940; Robinson and Lee, 1947), a contrary observation was made in quail by Wilson *et al.* (1971) in which egg production was found to be greater in hot environment (32°C) than in cold temperatures. This effect was believed to result from change from long days (16hrs. of light) to short days (8hrs. of light). The absence of any significant effect of heat stress (approximately 38°C for 12 hours a day for 2 weeks) on egg production in quails in this study indicates the high resistance of this species to heat stress. The degree of heat stress (either or both temperature and duration of treatment), to which the quail in this study were subjected to, appear not to be sufficient enough to produce significant effect on egg production. Day length was not measured in this study, however, the period during which the experiment was conducted (August) has been known to have a longer day length in this region. Supplementation with AA also failed to significantly improve egg production in this study probably because depletion of the vitamin has not occurred in sufficient degree to depress egg production in the first place.

Even though heat stress in this experiment did not significantly affect the weight of quail egg, administration of AA significantly did. This indicates that AA probably had ameliorating effect on some stressors other than heat; such as the stress of change of group, handling etc, which allowed for improvement in the weight of eggs. Beneficial effect of AA supplementation upon egg weight, during hot summer months, has also been reported in eight-month-old White Leghorn by Perek and Kendler (1962 and 1963). They also reported a reduction in mortality.

The present study did not demonstrate a clear effect of heat stress and/or supplementation with AA on mean length, width, shell weight and thickness of quail eggs. Several studies elsewhere have however reported reduction in shell weight and thickness of chicken eggs as a result of heat stress, which have been significantly improved following supplementation with AA (Schnapel, 1940; Wilheim, 1940; Thornton and Moreng, 1959; El-Boushy, 1966). It has been suggested that this response to AA may be influenced by factors other than heat stress, such as dietary protein level, calcium level and the bird's metabolic rate.

The present study demonstrated neither significant effect of heat stress nor the beneficial effects of supplementation with AA on Haugh unit of quail eggs. Detrimental effects of heat stress on the internal quality of eggs as measured by Haugh unit and the ameliorating effect of supplementation with AA have been reported in chicken (Thornton and Moreng, 1959; Thornton, 1959; Thornton and Moreng, 1959; Thornton and Deeb, 1961). However some of these results were not repeated consistently. It has also been observed (Cunningham *et al.*, 1960) that the *per cent*of yolk per egg increased as the birds advance in age. A decline in Haugh units and total albumen solids were also noticed with aging (Cunningham *et al.*, 1960).

Figures for blood parameters of quail measured in this study agree closely with those measured elsewhere (Atwal *et al.*, 1964; Shellenberger *et al.,* 1965). Neither exposure to heat stress nor treatment with AA appears to have affected the figures significantly. This appears to conform to observed absence of significant effect of heat stress on the weight of the birds and egg production and reflects the seeming resistance of quail to heat stress compared to other bird species. In domestic chicken for example, dilution of plasma was one of the early responses to a sudden exposure to a hot environment. This was demonstrated by a decrease in blood solids (packed cell volume), haemoglobin and plasma protein concentration (Wakim, 1963). The haematocrit of birds have been shown to be significantly lowered in birds at an ambient temperature of 30°C compared with those of birds grown at cooler environments, indicating a higher erythrocyte concentration in the birds held at the cooler environments (Thornton and Deeb, 1961). Subaschandran and Balloon (1967) reported that the haemodilution caused by heat-stress, as indicated by decreased packed cell volume and haemoglobin, was not significantly alleviated by AA supplementation.

The seeming absence of clear effect of heat stress on production and other quality parameters in quail in the present study and the differences in observations between this study and some studies elsewhere either indicate some degree of heat resistance of this species or could have been as a result of differences in the degree of heat stress applied, age, breed and species of the birds used in the respective studies. These differences have been demonstrated to exist elsewhere (Ahmed *et al.*, 1967).

Conclusion: This study demonstrated the effects of supplementation of diet with ascorbic acid on egg production and quality parameters in Japanese quail subjected to heat stress. Heat stress did not affect feed consumption, body weight of quail and egg production. Ascorbic acid supplementation in diet appear to have some beneficial effect in improving egg weight in hot conditions but not other egg quality parameters. Even though shell thickness was higher in non-heat stressed birds supplemented with ascorbic acid than in heat stressed birds not supplemented, the difference was however not significant. There was also no significant effect of heat stress and/or supplementation with ascorbic acid on the blood parameters of quail. The absence of clear-cut effects of both heat stress and ascorbic acid supplementation on most of the other production and quality parameters suggest some level of heat resistance of this species which however needs to be fully elucidated by further in depth studies.

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