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Effect of dietary ascorbic acid supplementation on the performance of Japanese (*Coturnix coturnix japonica*) quails in a tropical environment

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1 SUMMARY

A study was conducted to determine the effect of dietary ascorbic acid (AA) supplementation on performance of growing Japanese quails (*Coturnix coturnix japonica*) reared under high environmental temperatures (heat stress). Three hundred and fifteen (315) two-week old male and female Japanese quails were randomly divided into five groups of equal numbers and fed *ad libitum* on a maize/soyabean based diet meal. The meal were supplemented with AA at graded levels of 0, 50,100,150 and 200mg/kg diet and labeled groups 1,2,3,4 and 5 respectively. The birds were reared under maximum environmental temperature of 36.33^{0} C and maximum relative humidity of 64 %. The results of the study demonstrated significant ($P < 0.05$) increase in feed intake and protein intake of all quail on AA supplemented diets compared with the non-supplemented group. However, there was no corresponding increase in feed and protein conversion ratios of quails fed AA supplemented diet. Birds exposed to heat stress and given AA diets had similar body weight gain with birds on AA free diet. The carcass yield was not significantly different between any of the five groups. There was also no significant different between any of the groups in red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total protein. It was concluded that the quails appeared to exhibit some level of resistance to heat stress, dietary supplementation of quail diets with up to 200 mg AA per kg was not necessary and not economically advantageous.

2 INTRODUCTION

In the tropics heat stress is one of the major stressors in poultry production and it produces a wide range of behavioral and physiological responses (Pardue *et al*., 1985; Njoku, 1986; Minka *et al*., 2007). It has been shown that during stress the generation of free radicals in the body is often elevated to a level that overwhelms tissue antioxidant defence systems which results in oxidative stress and impairment of the activity of the antioxidant, Vitamin C or Ascorbic acid (AA) in vivo (Halliwell, 1996; Sahin *et al.,* 2001). In situations of stress border-line deficiency of AA has been demonstrated (Tuleun and Njoku, 2000). Under such situations exogenous AA supplementation has been shown to be beneficial in attenuating the adverse effect of environmental stress in broiler chickens (Kafri and Cherry, 1984; Ayo *et al*., 2007) and stress-induced tissue damage (Sen, 2001). Commercial chickens are increasingly been subjected to harsh environmental temperatures in the tropics that are unsuitable for optimum production. It is not economical for most producers to modify the environment within the poultry house to account for these problems and so it would be useful to search inward for nutritional strategies that could be used to ameliorate the adverse effects of heat on chicken in order to improve their productivity.

A lot of information has been documented on the beneficial effects of AA on domestic fowls exposed to thermally stressful environments in many parts of the world (Mckee and Harrison, 1995; Tuleun and Njoku, 2000; Adenkola and Ayo, 2009). In recent times, a new genus of poultry, Japanese quail (*Coturnix coturnix japonica*) was introduced into Nigeria by the National Veterinary Research Institute (NVRI) Vom to expand the poultry subsector and help supplement the domestic chicken production through meat and eggs (Edache *et al*., 2007; Anie *et al.,* 2009). The quails have unique characteristics and advantages over other species of poultry which include early attainment of sexual maturity, short generation interval making it possible to have many generations in a year (Anon, 1991), high rate of egg production between 200-300 eggs in 360 days and are very resistant to common epidemics of poultry (NRC, 1991). The quail are hardy birds that can adapt to many different environments (Haruna *et al.,* 1997a). Their meat and eggs are renowned for their high quality protein, high biological value and low caloric content, making it a choice product for hypertensive patient (Haruna *et al.,* 1997b, Olubamiwa *et al.,* 1999). For quail farming to be commercially profitable in this hot (tropical) environment measures to alleviate the effects of high ambient temperatures on production of the birds must be found.

The aim of this study therefore was to determine the effect of ascorbic acid on growth performance, carcass and hematological parameters of Japanese quails raised during the hot months (March – April) of the year in Makurdi, Nigeria.

3 MATERIALS AND METHODS

The experiment was conducted at the poultry unit of the livestock Teaching and Research Farm, University of Agriculture, Makurdi (07° 4' N, 08 ° 37'E), Benue State located in the Southern Guinea Savannah Zone of Nigeria. Makurdi is situated along the River Benue, which is very warm, with the daily temperature ranging from 26.5 to 42^0 C. The area has an annual rainfall of 1,317 – 1,323 mm which spans 6 - 7 months (Adenkola *et al*., 2004).

A total of three hundred and fifteen (315) two weeks unsexed Japanese quail birds with an average initial weight of 18.74 $\pm$ 0.16 g were purchased from National Veterinary Research Institutes (NVRI), Vom and used for an experiment which lasted for six weeks. The birds were weighed and randomly allotted into five treatments with three replicates of twenty one (21) quails each and housed in compartments of 164 cm x 83 cm x 183 cm in a Completely Randomized Design. The compartments were made of wooden pens, totally screened with wire mesh. The pens were fitted with spring doors to prevent escape of the quail chicks.

A practical maize-soy bean based diet providing 2828 Kcal/kg metabolizable energy (ME) and 23 % crude protein was used (Table 1) and graded levels of AA were added to equal portions of the basal mix to provide dietary treatments with 0, 50, 100, 150 and 200 mg AA/Kg diet. Feed and water were provided *ad libitum* throughout the study period. Feed intake and body weight changes were monitored on weekly basis. From the feed intake and weight gain data, feed conversion ratio and cost per unit gain in weight were calculated. Water intake was also monitored on daily basis.

During the study period (March-April) the meteorological parameters of dry-bulb temperature (DBT) and relative humidity were determined twice a day at 7.00 am and 1.00 pm at experimental site using a dry and wet bulb thermometers (Brannan®, England) calculated using the manufacturers' standard manual for the thermometer. The wind speed and direction was obtained from Nigeria Meteorological center, Makurdi, Nigeria.

Table 1: Gross Composition of the Experimental Diets for Growing Japanese quail Exposed to High Temperature

Ingredients	**Dietary Treatments**				
	1	2	3	4	5
Maize	47.31	47.31	47.31	47.31	47.31
Soyabean cake	26.86	26.86	26.86	26.86	26.86
Groundnut cake	13.43	13.43	13.43	13.43	13.43
Maize offal	8.00	8.00	8.00	8.00	8.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Salt	0.25	0.25	0.25	0.25	0.25
Oyster shell	1.00	1.00	1.00	1.00	1.00
Methionine	0.30	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10	0.10
Premix	0.25	0.25	0.25	0.25	0.25
Vitamin C (mg)	0 -	50 (+)	100 (++)	150 (+++)	200 (++++)
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrients					
Crude Protein	23.00	23.00	23.00	23.00	23.00
Metabolizable Energy (Kcal/Kg)	2828	2828	2828	2828	2828
Crude Fiber	4.33	4.33	4.33	4.33	4.33
Feed cost/ Kg (N)	78.76	79.16	79.56	79.96	80.36

At the end of the experiment, quails from each replicates were slaughtered by severing the jugular vein. Blood samples (2 ml) was collected as the quail bled into sampled bottles, containing the anticoagulant, sodium salt of ethylene diaminetetra-acetic acid at the rate of 2 mg/ml of blood (Oyewale, 1992). After collection, the samples were transferred to the Physiology Laboratory, Department of Veterinary Physiology and Pharmacology, University of Agriculture, Makurdi, for haematological analysis. Packed cell volume (PCV) was determined using microhaematocrit method, haemoglobin concentration (Hb) using the cyanomethaemoglobin method, total red blood cell (RBC) count using the haemocytometric method (Schalm *et al*., 1975) and total protein (TP) was determined using the routine laboratory procedure (Benjamin, 1985). Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of PCV, Hb and RBC count (Schalm *et al*., 1975). The bled quails were immersed in scalding water (95^{o}C) for five seconds and then defeathered according to the procedure of Oluyemi and Roberts (2000). This was followed by evisceration, the carcass cut and visceral organ measurements.

Statistical analysis: All data obtained were subjected to analysis of variance (ANOVA) based on completely randomized design (CRD) using the Minitab Statistical software (1991) and comparisons of treatment means were made by Duncan's Multiple Range Test (Steel and Torrie, 1980).

4 RESULTS

Table 2: Meteorological Data during the Study Period

Meteorological Parameters	
Ambient temperature (^{0}C)	
Maximum	36.33
Minimum	24.20
Relative Humidity	
Maximum	64.00
Minimum	37.50
Wind speed (Knot/sec)	2.47
Rainfall (mm)	91.55

The house environment was characterized by minimum and maximum temperature (AT) of 24.20 ±_1.4 ^{0}C and 36.33 ± 3.7 ^{0}C. The relative humidity was in the range of 39.5 ± 2.2 and 64.00 ± 4.8% and a mean wind speed of 2.47 Knot/second and the wind direction was North-East. The summary of the performance of the quails exposed to heat stress and fed graded levels of dietary AA is presented in Table 3.

Table 3: Effects of different levels of ascorbic acid on performance of Japanese quail exposed to high temperature

Parameters	**Treatments**					
	1	2	3	4	5	SEM
Initial weight (gm)	18.76	18.74	18.79	18.68	18.68	0.04
Final weight (gm)	145.04	149.83	151.06	148.84	150.69	1.75
Average Daily weight gain (gm/day/bird)	3.60	3.75	3.78	3.72	3.77	0.05
Average Daily feed intake (gm/day/bird)	19.79^b	20.11^b	21.53^a	21.09^a	20.32^b	0.05**
Average Protein intake (gm/day/bird)	4.53^b	4.63^b	4.95^a	4.85^a	4.68^b	0.22**
Feed conversion ratio	5.66	5.37	5.69	5.66	5.41	0.12
Protein conversion ratio	1.27	1.23	1.31	1.30	1.26	0.03
Daily water intake (ml/day/bird	49.37	48.55	47.42	41.73	39.49	4.07
Feed/water ratio	0.44	0.42	0.46	0.51	0.51	0.04
Feed cost/gain	0.43	0.43	0.45	0.46	0.43	0.03
Mortality (%)	-	3.23	-	-	-	-

SEM = Standard Error Mean
Means in the same row with different superscript are significantly different (< 0.01)

There were no significant differences in mean daily weight gain, feed conversion ratio, protein conversion ratio, daily water intake between the control group (AA free diet) and the groups fed graded levels of dietary AA and batch exposed to heat stress. There were significant (P < 0.01) differences between values of feed intake and protein intake recorded by different treatment groups. There was no significant (P > 0.05) difference in the mean water intake between quails that were exposed to high temperature and fed AA free diet and those fed graded levels of dietary supplemental AA. The economic analysis revealed an upward increase in the cost of feed per unit weight gain of quails exposed to heat stress and fed ascorbic acid supplemented diets compared to the group on AA free diet. The data in Table 4 indicated that there were no significant (P >0.05) differences between treatment groups in carcass percentage and percentage relative cuts (shank, head, liver, heart; lungs and gizzard + proventriculus). However there was a significant (P<0.01) decrease in the percentage weight of pancreas of quails on AA supplement diets as compared to the control group.

Table 4: Effects of ascorbic acid on the carcass and internal organs of quails exposed to high temperature

Parameters	**Treatments**					
	1	**2**	**3**	**4**	**5**	**SEM**
Live weight (%)	150.00	143.75	143.75	143.75	143.75	1.97
Defeathered weight (%)	74.58	73.41	76.79	78.29	77.09	2.22
Dressed (%)	60.63	58.22	60.59	60.44	63.30	2.11
Shank (%)	1.82	1.72	1.79	1.89	1.89	0.28
Head (%)	4.42	4.36	4.80	4.87	4.89	0.33
Liver (%)	1.28	1.37	1.23	1.25	1.48	0.32
Pancreas (%)	0.41	0.22	0.15	0.20	0.22	0.23
Heart (%)	0.88	0.87	0.93	1.02	0.89	0.28
Lungs (%)	0.78	0.72	0.89	0.83	0.96	0.36
Proventriculus (%)	2.17	1.85	2.08	2.23	2.23	0.34

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The haematological indices of Japanese quails exposed to high ambient temperature and administered dietary AA is presented in Table 5. The result showed no significant ($P > \pm 0.05$) differences in PCV, Hb concentration, RBC count; TP, MCH and MCHC of quails reared under high ambient temperature, with or without AA supplemented diet.

Table 5: Ascorbic acid administration effect on haematological parameters of Japanese quail exposed to high ambient temperature

Parameters	Treatments					
	1	2	3	4	5	SEM
Packed Cell Volume (%)	40.75	38.75	42.75	40.50	38.25	1.97
Haemoglobin Concentration (gm/dl)	13.59	12.93	14.25	13.50	12.75	0.66
Total Red Blood Cell (x 10^6/µl)	5.33	5.00	5.99	4.49	5.65	0.51
Total Protein (gm/dl)	6.75	6.50	7.00	7.50	7.25	0.45
MCV (fl)	77.14	78.14	73.98	90.80	68.71	0.66
MCH (pg)	25.72	26.14	24.66	30.27	22.90	2.21
MCHC (gm/dl)	33.34	33.33	33.34	33.34	33.33	0.003

5 DISCUSSION

The meteorological data obtained during the study period indicated that the AT fell outside the established thermo neutral zone of 18 - 26 ^{0}C for poultry species in the tropics (Oluyemi and Roberts, 2000). Studies have shown that the optimum ambient temperature requirement for optimum performance of chickens falls between 15.55 ^{0}C - 23.88 ^{0}C (Jacqueline and Mattier, 2009). Heat stress in poultry begins to occur when ambient temperature rises above 27 ^{0}C (Kutlu, 2001). Temperatures above 27 ^{0}C has been reported to result in decrease feed consumption, increase water intake, decrease feed efficiency, live weight and growth rate in chickens (May *et al.,* 1998; Celik and Ozturkcan, 2003). Heat stress causes increase generation in free radicals (Halliwell and Gutteridge, 1989) and lowers the concentration of antioxidant vitamins such as vitamins C, A and E as well as minerals such as Zinc and chromium in the plasma and tissue leading to damage of cell membranes (Sahin and Kucuk, 2003).

The improved feed consumption in the quails exposed to high ambient temperature and fed dietary ascorbic acid supplementation may be associated with suppressed stress responses initiated by the presence of AA at the tissue and plasma level. This result supported the finding of Ali *et al.* (2010) that the addition of antioxidant, AA to the diet may scavenge the free radicals generated by heat stress, leading to improved feed consumption. Mckee *et al.* (1997) and Adenkola and Ayo (2009) reported that at high temperatures, antioxidants nutrient supplementation, especially vitamin AA can be used to attenuate the negative effects of high environmental temperature on performance of poultry. Kutlu and Forbes (1993) also reported that chickens under heat stress will choose a feed supplemented with AA if the feed is identifiable through the use of colour and will reverse their selection when the environmental temperature reverts to normal.

In this study dietary AA supplementation marginally improved the quail body weight gain and feed conversion compared to the control (AA – unsupplemented) group. This appears to conform to observed absence of significant effect of heat stress on the weight of the birds and feed utilization and also reflects the seeming resistance of quail to heat stress compared to other bird species. The non-significant difference in feed conversion and protein conversion ratio is in concordance with the findings of Konca *et al.* (2009) who reported that AA did not affect body weight gain and feed conversion ratio in chickens under conditions of natural summer temperature. These differences have also been demonstrated to exist elsewhere, where no clear effect of heat stress on production and other quality parameters in quail were fully observed (Ahmad *et al.,* 1969).

Earlier research findings of Boone and Auston (1967) showed that water consumption increased with increase in environmental temperature. The non-significant differences in water intake in this study varies with the report of Usman *et al.*(2008) who found a significant improvement in consuming water treated with supplemental ascorbic acid at both 200 mg and 1000 mg/kg. The result also disagrees with that of Sahota *et al*., (1993), Jones *et al*. (1996) and Adenkola and Ayo (2009) that AA supplementation during stressful conditions is beneficial to poultry.

The non-significant effect of AA supplementation in feed cost/gain among the treatment groups showed that AA did not depict any economic value in the heat stressed quails. The result however is in disagreement with the report of Njoku (1986) that supplementation of broiler chicken diet with an adequate level of AA during a period of heat stress will result in a substantial financial advantage. The absence of any significant effect of AA supplementation on carcass composition in quails in this study probably indicates that depletion of the vitamin has not occurred in sufficient degree to cause a beneficial effect on the measured traits. This result is not consistent with the report of Sahin and Kucuk (2003) who observed that AA supplementation had increased performance and yield better carcass traits in broiler quails reared under heat stress (32 ^{0}C). However similar differences have been demonstrated by Konca *et al.* (2009) with broilers reared under natural summer temperature.

The no significant difference on values of haematology of quails in MCV MCHC is in concordance with the findings of Usman *et al.* (2008) who reported no significant difference in RBC, HB, PCV, MCV, MCHC and white blood cell count in female Japanese quails reared under heat stress of 38 ^{0}C and supplemented with AA. In domestic chicken for example, dilution of plasma was one of the easily responses to a sudden exposure to a hot environment. Subaschandran and Ballon (1967) reported that the haemodilution caused by heat stress, as indicated by decreased PCV and Hb concentration, was not significantly alleviated by AA supplementation. This appears to conform to observed absence of clear-cut effect of both the control and AA supplemented group, thus suggesting some level of heat resistance in this species of birds.

6 CONCLUSION:

This study demonstrated that generally heat stress did not affect quail performance. However, AA supplementations in diet appeared to have some beneficial effect in improving feed and protein intake but not in other performance and blood parameters. The absence of significant effect of both heat stress and AA supplementation in most measured parameters in quails suggest some level of heat resistance. Further investigations are required to elucidate the thermoregulatory mechanism in quails and AA supplementation during the hot season.

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