

Performance and physiological response of heat stressed growing pullets fed diets supplemented with ascorbic acid

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Abstract

Effects of dietary supplementation of Ascorbic Acid (AA) on performance and physiological response of growing pullets were investigated in a 7-week trial. In a completely randomized design, Bovans Brown pullets ($n=96$) aged 12 weeks weighing 0.81 ± 0.04 kg were randomly allotted to three treatments of four replicate and each replicate comprised eight birds. The pullets were initially fed basal diet for three weeks followed by dietary supplementation with AA at 0 (T1), 300 (T2) and 600 mg/kg (T3). Feed and water were provided to the pullets ad libitum. Performance and physiological parameters were monitored. Data were subjected to ANOVA and polynomial regression and means separated at $_{0.05}$. Ambient temperature, relative humidity and temperature humidity index were 29.0 ± 1.5 °C, 71.9 ± 0.3 % and 26.9 ± 1.3 , respectively. Feed intake (kg) (0.89, 0.87, 0.88) and livability (93.8, 96.9, 96.9%) of pullets on T1, T2 and T3, respectively were not significantly affected ($P > 0.05$) by supplemental AA. Weight gain (kg) of pullets on T1 (0.36) and T3 (0.42) were similar ($P > 0.05$) but were significantly lower ($P < 0.05$) than T2 (0.47). Feed conversion ratio of pullets on T2 (1.89) and T3 (2.09) were significantly lower ($P < 0.05$) than T1 (2.48). Panting rate (Breath/min) of pullets on T1 (231.8) and T2 (225.0) were similar ($P > 0.05$) but significantly higher ($P < 0.05$) than in T3 (207.8). Rectal temperature of pullets on T3 (41.9) was lower than T1 (41.5). In conclusion, dietary supplementation of AA improved performance and physiological response of growing pullets in this study.

Keywords: Point of cage pullets, Rectal temperature, Supplemental ascorbic acid, Temperature humidity index

Introduction

Heat stress remains a serious impediment to increased poultry production in the tropics. Heat stress in poultry is prompted by combinations of environmental temperature and humidity that prevent the bird's thermoregulatory process from effectively dissipating heat produced during metabolism (Webster, 1983). There is a range of temperature outside which all animals become stressed. Lower temperatures below the requirement of the animal may constitute cold stress while a temperature higher than what the animal can cope with may lead to heat stress.

The thermoneutrality of chickens raised in the tropics used to be between 18-22 °C

(Charles, 2002) and now 18-26 °C (Holik, 2009). Outside the thermo neutral zone, chickens become uncomfortable and as such a limitation is placed on the attainment of their intrinsic genetic potential thereby resulting to reduction in feed intake, feed efficiency, live weight gain and quality of eggs coupled with increased mortality (Demir *et al.*, 1995; Bollengier *et al.*, 1999). However, the temperature in the hot humid tropical environment of Ibadan, Nigeria, often times exceeds this (Majekodunmi *et al.*, 2013).

There are various strategies to minimize heat stress in poultry birds either by changing their environmental condition or by modification of their diets (Ajakaiye *et*

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al., 2010). The viable nutritional strategy during heat period is based on diet balancing in order to cover the needs of stressed birds for amino acids (protein), energy and electrolytes (Balnave, 2004; Daghir, 2009). Nutritional intervention by the use of supplemental vitamins may be the necessary solution to address this menace because their supply in diets does not alter the energy or protein requirement of the animals.

The AA has been implicated in the management of stress related issues in livestock production. Ordinarily, birds will naturally synthesis AA in adequate amount but during stress related activities, the requirement of AA is greater than the amount synthesized by normal tissues (Balogun *et al.*, 1996) thus the need to be included in diets of chicken. Reports (Abioja *et al.*, 2005; Ogunwole *et al.*, 2012; Majekodunmi *et al.*, 2013) have demonstrated the role of supplemental AA in the alleviation of stress in poultry. Similarly, there has been reported increase in feed intake with AA supplementation (Sahin and Kuck, 2001) while there was reported improvement in the feed conversion ratio by dietary inclusion of AA in poultry diets (Onu, 2009 and Blaha *et al.*, 2010).

Despite avalanche of reports on importance of AA for stress mitigation in poultry production, there has been dearth of information on the appropriate levels of dietary AA supplement to be fed to growing pullets. Also, most reports on stress alleviation in poultry (Sahin and Kuck, 2001; Abioja *et al.*, 2005; Blaha *et al.*, 2010; Majekodunmi *et al.*, 2015) were devoted to broilers (Blaha *et al.*, 2010; Majekodunmi *et al.*, 2013) and laying hens (Genedi, 2000; Cifti *et al.*, 2005; Ajakaiye *et al.*, 2010) with relative dearth of little information on its effect on growing pullets. This study was

therefore aimed at investigating the performance and physiological response of growing pullets given diets supplemented with graded levels of AA.

Materials and methods

Bovan Brown pullets (n=96) aged 12 weeks weighing 0.81 ± 0.04 kg with standard husbandry records were purchased from a reputable farm and allotted to three dietary treatments of four replicates and eight birds per replicate in a completely randomised design. Pullets were initially raised on an isocaloric and isonitrogenous basal diet for the first 3 weeks after which the diets were supplemented with ascorbic acid. Details of the basal experimental diet composition have been documented (Adedeji *et al.*, 2017) and are shown in Table 1. Treatment 1 (T1) is the control with no supplemental AA. Treatment 2 (T2) had 300 mg/Kg AA while Treatment 3 (T3) had 600 mg/kg AA. Feed was offered daily and left over weighed weekly to calculate feed intake. Weight gain was measured on a weekly basis while feed conversion ratio was calculated as the ratio of feed intake to weight gain. Mortality was recorded against each replicate; Panting rate (PR) was determined by counting the number of breaths per minute; Rectal temperature (RT) was measured by inserting the probe of a digital thermometer into the rectum of selected pullets; ambient temperature and relative humidity were monitored using digital thermo-hygrometers mounted within the pen house while Temperature Humidity Index (THI) was calculated according to Zulovich and DeShazer (1990) using the formula:

$$\text{THI layers} = 0.6 \text{Tdb} + 0.4 \text{Twb}.$$

Where:

THI= Temperature Humidity Index

Tdb= Dry bulb Temperature

Twb= Wet Bulb Temperature

Table 1: Gross composition (%) of basal diet fed to the growing pullets

Ingredient	% (kg)
Maize	50.80
Soybean meal	18.00
Wheat offal	27.85
Oyster shell	1.00
Bone meal	1.6
*Vitamin-mineral premix	0.25
Table salt	0.30
DL-Methionine	0.10
L-lysine	0.10
Calculated Nutrients	
ME (Kcal/kg)	2751.27
Crude Protein (%)	16.87
Crude Fibre (%)	4.55
Methionine (%)	0.37
Lysine (%)	0.98
Calcium (%)	1.07
Available Phosphorus (%)	0.42

*Vitamin-mineral premix - Vitamin A –10,000IU, Vitamin D₃–1800IU, Vitamin E –40mg, Vitamin K –1.43 mg, Vitamin B1 –0.7mg, Vitamin B2 –4mg, Vitamin B6 –2.5mg, Vitamin B12 –0.2mg, Niacin –10mg, Panthothenic –10,000mg, Folic acid –0.25mg, Biotin –100mg, Choline Chloride –300mg, Manganese–80mg, Zinc –60mg, Iron –40mg, Copper – 80mg, Iodine –0.8mg, Selenium –0.2mg, Cobalt –0.3mg, Antioxidant–100mg

Statistical analysis

Data were subjected to ANOVA and polynomial regression of SAS (2003) and significant means were separated using Duncan's Multiple Range Test of the same software at $p < 0.05$

Results and discussion

The ambient temperature and relative humidity of the pen house for the study period is shown in Figure 1. During heat stress, environmental parameters of AT, RH and Temperature humidity Index (THI) are invaluable tools in the presumptive diagnosis of an animal's state of health, as well as adaptability (Tao and Xin, 2003; Karaman *et al.*, 2007). The AT (29 ± 1.49 °C) and RH ($71.93 \pm 0.27\%$) were perpetually high during this trial. High temperature and low humidity (32 °C and 40 % RH) have been reported to be well tolerated by birds

while high temperature and high humidity (32 °C and 90% RH) are problematic (Lesson and Summers, 2005).

The response of chickens at high temperatures differs with different relative humidity. High temperature when accompanied with high humidity is more detrimental to layer performance than high temperature with low humidity (Ajakaiye *et al.*, 2010). Thermo-neutral zone (TNZ) is the range of ambient temperature which does not affect regulatory changes in metabolic heat production or evaporative heat loss in birds (Kingma *et al.*, 2012). Dei and Bumbie (2011), reported that diurnal ambient temperature fluctuations usually exceed the TNZ of chickens thus resulting in heat stress. The THI (26.90 ± 1.28) in this study as shown in Figure 2 were outside the TNZ of chickens thus further suggesting that the chickens were thermally stressed in the rearing period.

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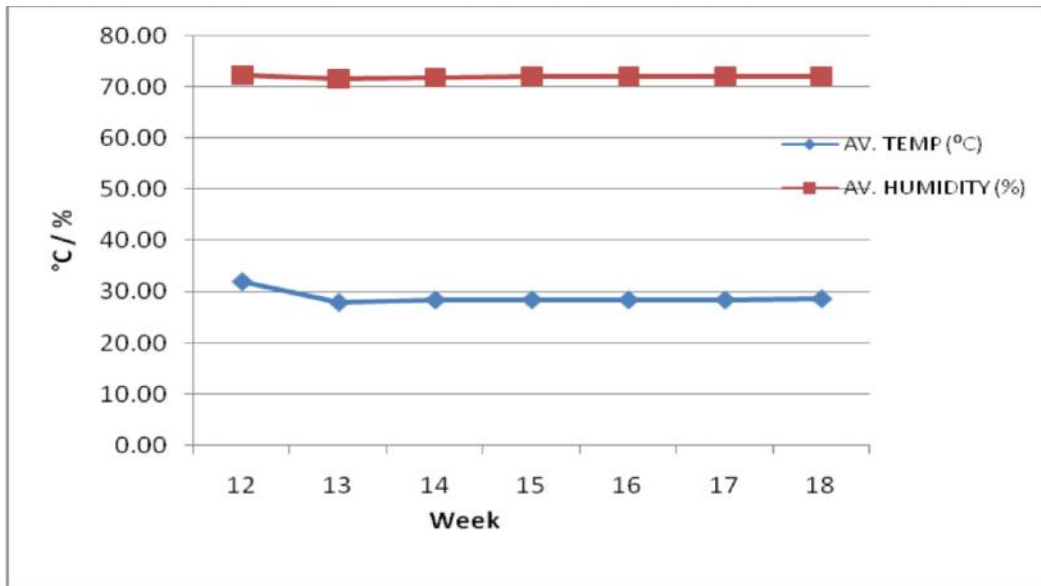


Figure 1: Ambient temperature and relative humidity of the pen house during the trial period

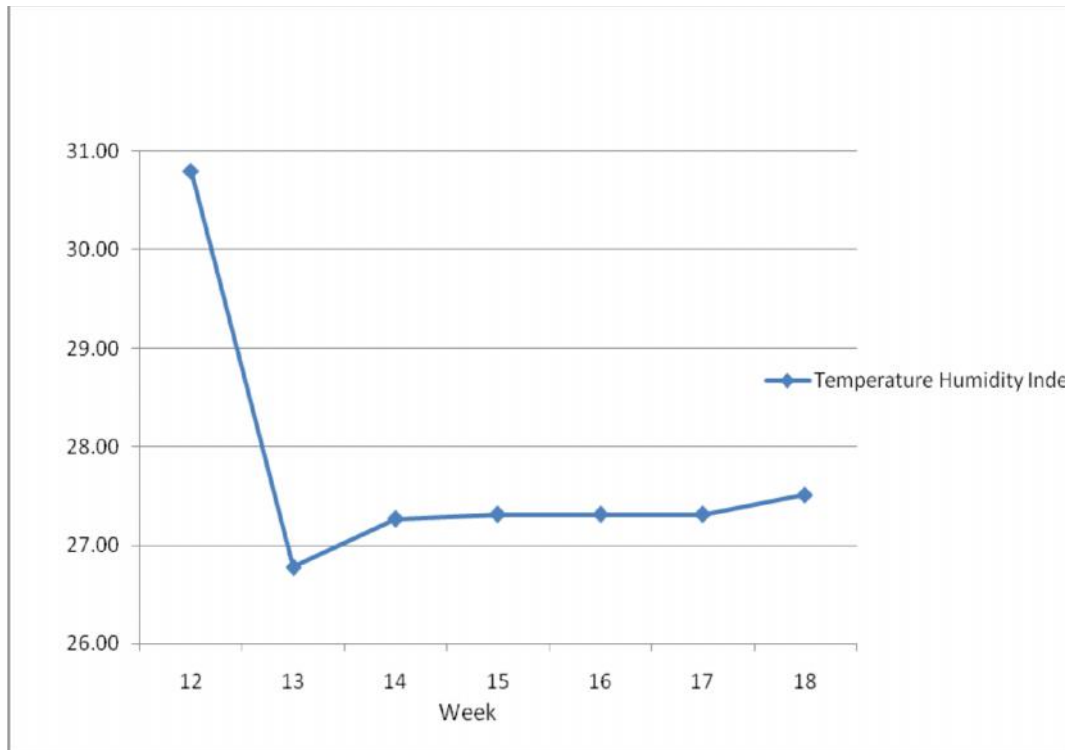


Figure 2: Temperature humidity index of pen house at the growing phase

Performance of growing pullets fed diets supplemented with AA is shown in Table 2. Final weight, weight changes, feed intake and livability values were within the range of 1.19 (T1) to 1.26 (T2); 0.36 (T1) to 0.41 (T3); 0.87 (T2) to 0.89 (T1) and 93.8 (T1) to

96.9 (T3), respectively. These parameters were however not influenced by dietary inclusion of AA, while weight changes and FCR were significantly different across the treatments with values ranging from 0.36 (T1) to 0.47 (T2) and 1.89 (T2) to 2.48 (T1), respectively.

Table 2: Performance of growing pullets fed diets supplemented with ascorbic acid

Parameters	T1	T2	T3	SEM
Feed intake (kg)	0.89	0.87	0.88	0.004
Initial weight (kg)	0.83	0.81	0.80	0.01
Final weight (kg)	1.19	1.28	1.23	0.03
Weight gain (kg)	0.36 ^b	0.47 ^a	0.42 ^{ab}	0.01
FCR	2.48 ^a	1.89 ^b	2.09 ^b	0.09
Livability (%)	93.8	96.9	96.9	1.78

^{a,b} : means in the same row with different superscripts are significantly different (p<0.05).

T1: 0mg ascorbic acid; T2: 300mg ascorbic acid; T3: 600mg ascorbic acid; FCR: Feed Conversion Ratio

The variations in the final weight observed in this study were not significantly different (P>0.05). This observation conformed to the report of Tuleun *et al.* (2011) and Cifti *et al.* (2015) in Japanese quail and laying chickens but was however, contrary to the observation of Oso *et al.* (2013) in turkey and Ogunwole *et al.* (2015) in broiler chickens fed supplemental AA. Weight changes of pullets on T2 (0.47) and T3 (0.42) were similar (P>0.05) but were significantly higher (P<0.05) than 0.36 in birds on T1. The similarity observed in the weight changes of pullets on T1 and T3 could be that the supplementation of AA beyond 300mg was in excess of dietary need of the pullets thus interfering with weight gain. Contrary to earlier reports (Nwoku and Nwazota, 1989; Demir *et al.* 1995; Sahin and Kuck, 2001) on increased feed intake with supplemental AA in poultry. Observation from this study conformed to reports (Oniseibo and Alu, 2006; Onu, 2009) that dietary supplementation of vitamin C had no effect on feed consumption. The relationship between dietary AA supplementation and weight gain in pullets is shown in Figure 1.

Supplemental AA correlated positively with the weight gain of pullets (R²=93.5). Supplemental AA beyond the maximum level used in this study would further improve weight gained by the pullets. The relationship is represented with the equation:

$$Y=0.000X + 0.328 (R^2=93.5) \dots \text{Equation 1}$$

The FCR of pullets on T2 (1.89) and T3 (2.09) were similar (P>0.05) but was significantly lower (P<0.05) than T1 (2.48). This observation corroborated earlier documentations (Onu 2009; Blaha *et al.*, 2010) on improved FCR with supplemental AA in poultry diets contrary to other earlier reports (Ogunwole *et al.*, 2013; Lohakhare *et al.*, 2015; Majekodunmi *et al.*, 2015) which did not observe any improvement. The relationship between supplemental AA and FCR in Figure 2 is represented by the equation:

$$Y = -0.001X + 2.691 (R^2=82.9) \dots \dots \dots \text{Equation 2}$$

This linear relationship also showed that 82.9% of the observed changes in FCR was due to supplemental AA in the diets of the pullets.

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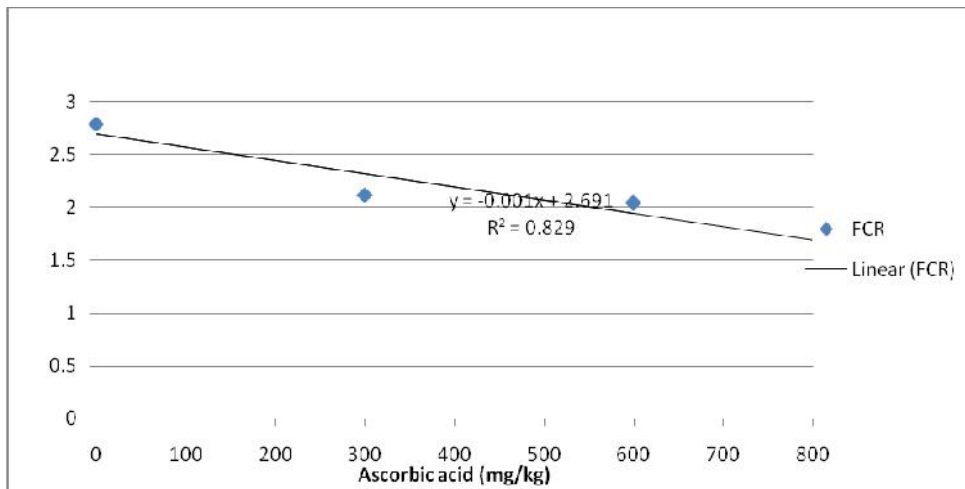


Figure 2: Relationship of supplemental ascorbic acid and FCR

Livability (%) of pullets on the different treatments was similar ($P>0.05$) though there was a numerical improvement with increasing levels of AA inclusion. However, authors (Skyles 1977; Kafri and Cherry, 1984; David and Brake, 1985) reported reduction in the incidences of mortality in chickens on diets with supplemental AA in hot environments. Free radicals generated during stress are harmful to cells in living organisms. Inability of the

organism to reduce or hinder the source of free radicals being generated would result in damaged organs, tissues and systems which in turn may lead to eventual death. The relative improvement in livability of birds on supplemental AA in this study could be adduced to the ability of vitamin C to mop excessive free radicals generated during heat stress from causing havoc to the body.

Table 3: Physiological response of growing pullets fed dietary ascorbic acid supplementation

Parameters	Ascorbic acid (mg/kg)			SEM
	T1	T2	T3	
Rectal Temperature (°C)	41.90 ^a	41.70 ^{ab}	41.52 ^b	0.07
Panting rate (Breath/min)	231.75 ^a	225.00 ^a	207.8 ^b	3.54

^{a, b} : means in the same row with different superscripts are significantly different (p<0.05).

T1: 0mg ascorbic acid; T2: 300mg ascorbic acid; T3: 600mg ascorbic acid

Physiological response of pullets to dietary AA supplementation is shown Table 3. Birds do not sweat but there is the need for them to release excess heat load to become comfortable. At temperatures beyond 27 °C chicken become intensely under stress and would resort to means of dissipating heat from the body. Some of the means adopted to naturally cool off the body of excessive heat load includes wing spreading and panting among others. Respiratory rate and cloacal temperature are variables widely used and considered the simplest ones for the evaluation of physiological conditions of animals (Bianca and Kunz, 1978).

Chickens present a significant increased respiratory rate under high temperatures (Silva *et al.*, 2007) with a value up to 165 breaths/min reported in broiler chickens. In this study, panting rate in pullets on T3 (207.8) was significantly lower than in T2 (225) and T1 (231), thus emphasising the importance of AA in the alleviation of heat stress in poultry. However, values obtained were higher than 100 to 151 breaths/min observed by Nascimento *et al.* (2012) in Avian and Cobb strains of broiler chicken exposed to heat.

The body temperature of domestic chickens is maintained within a relatively narrow range that is usually reflected by the upper 41.5 °C and lower 40.5 °C limits of a circadian rhythm (Daghir, 1995). This however fluctuates depending on the temperature of environment (Sottnik, 2002). Rectal temperature of pullets on T1 (41.90) in this trial was similar to those on

T2 (41.70) but significantly higher than RT of pullets on T3 (41.52). This showed that supplemental AA up to 600 mg/Kg (T3) in the pullet diets significantly lowered RT compared with the control. This observation supported the findings of Kutlu and Forbes (1993), though RT in this study were within the body temperature (41-42 °C) documented for normal chickens (Donkoh, 1989). Conversely, Pardue and Thaxton (1986); Genedi, (2000); Majekodunmi *et al.* (2015) observed no significant effect of supplemental AA on RT of broilers exposed to high temperature.

Conclusion

Environmental parameters revealed that birds in this study were heat stressed. The AA supplementation improved weight gain, feed conversion and physiological responses of pullets in this study.

Acknowledgement

Authors are highly appreciative of the cardinal roles of the Tertiary Education Trust Funds in the sponsorship of this research. We also thank and appreciate Folasade Jemiseye, Aderonke Mosuro, and Irete Oludoyi for assisting in proof-reading and editing of the manuscript.

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Received: 13th August, 2017

Accepted: 30th November, 2017