Original article

Haematology and hormonal assay of heat-stressed broiler chickens managed by feather clipping and ascorbic acid supplementation

^aJimoh A.A., ^{*a}Ibitoye E.B., ^aYusuf M.Y., ^aBuhari A. and ^bMungadi H.U.

^aDepartment of Theriogenology and Animal Production, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, City Campus, Sokoto, Nigeria.

^bDepartment of Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, City Campus, Sokoto, Nigeria.

ARTICLE INFO

Article history:

Received: January 20, 2021

Received in revised form: August 14, 2021

Accepted: September 10, 2021

Keywords:

Ascorbic acid
Feather clipping
Haematology
Heat stress
Hormone.

ABSTRACT

Introduction: High ambient temperature (HAT) induces stress in birds and affects their wellbeing. This study investigated the haematology and serum levels of corticosterone, triiodothyronine and thyroxine of heat-stressed broilers managed by feather clipping (FC) and ascorbic acid (AA) supplementation.

Methods: One hundred Anak broiler chicks were distributed into five treatments of 20 chicks each in 2 replicates and were all fed basal diets. The positive control (PC) birds were intact (not feather clipped) and managed under controlled (28 – 30°C) environment. Other groups were managed in open pen under HAT (43.0 ± 2.0°C). The negative control (NC) birds were intact. The treatment 1 (T1) birds were feather-clipped, the treatment 2 (T2) groups were intact, together with 200 mg AA/kg feed supplemented while the treatment 3 (T3) birds were feather-clipped and fed with 200mg AA supplemented feed. Data were analysed using ANOVA.

Results: The results showed that feather clipping and AA supplementation did not affect blood parameters of the birds except white blood cell count and heterophil-lymphocyte ratio. The levels of the three hormones differed significantly ($P < 0.05$) in the PC group compared to HAT-exposed groups. Corticosterone levels decreased in T1 (8.20 ng/mL) and T3 (7.99 ng/mL) significantly against 9.70 ng/mL in NC, the lowest value of 7.88 ng/mL was recorded in PC. Triiodothyronine (4.04 ng/mL) and thyroxine (11.29 ng/mL) levels were highest in PC, lowest triiodothyronine (2.78 ng/mL) and thyroxine (7.97 ng/mL) concentrations were recorded in NC and T1 groups respectively.

Significance: Therefore, feather clipping and/or AA supplementation ameliorated adverse effect of extreme heat in the broiler chickens.

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Introduction

The Sahel savannah vegetation of the north-western Nigeria is a relatively hot climate, with ambient temperature often measuring up to 43.0 ± 2.0°C in hot season. High ambient temperature (HAT) elicits detrimental effects on birds (Lara *et al.*, 2013). It disrupts homeostasis of metabolic and stress hormones thereby suppressing immunity and resistance of birds to diseases (Mack *et al.*, 2013). Heat stress is accompanied by a decrease in basal metabolic rate and plasma thyroid hormones. Poultry are highly reactive to heat stress (Lara *et al.*, 2013), and their high metabolic heat production, lack of sweat glands and presence of feathers which cover almost the entire body further aggravate impacts of heat on them. However, the exposed parts of their body such as comb and wattle aid in heat dissipation to the environment (Adeyemi and Robert, 2000). Feathers are good insulators responsible for thermoregulation in birds as they conserve heat during cold weather but may prevent free heat dissipation from birds' body during hot weather (Hughes *et al.*, 1986). In response to HAT, birds

exhibit panting and wing elevation thereby exposing their skin to air for heat dissipation (DEFRA, 2005). Feathers hinder expression of full genetic potential of birds for growth by preventing heat dissipation from their body (Cahaner *et al.*, 2008). Effectiveness of dietary ascorbic acid (AA) supplementation in heat-stressed chickens has been reported (Lin *et al.*, 2006). AA is essential in biosynthesis of corticosterone and thyroid hormone as well as in boosting immunity (Pandurang *et al.*, 2011). Keeping birds under temperature-controlled pen aimed at eliminating heat stress in birds is economically not affordable to small and medium scale broiler chicken farmers. Feather clipping and/or AA supplementation may be an alternative means of managing heat stress in broiler chickens. The study was conducted to investigate the efficacy of feather clipping and ascorbic acid supplementation in the control or management of heat stress with focus on the haematological responses and serum concentrations of the stress hormone (corticosterone) and some metabolic hormones (triiodothyronine and thyroxine) of heat-stressed broiler chickens.

*Corresponding author Tel: +2347038228200; email address: eb.ibtioye@gmail.com
<https://doi.org/10.36759/svj.2021.126>

Materials and Methods

Study design

One hundred (100) day-old broiler (Anak strain) chicks of both sexes were divided into 5 treatments; PC: (Positive control), NC: (Negative control), T1, T2 and T3: (Treatments 1, 2 and 3) and were all fed the basal diets. The PC birds were intact (not feather clipped) and kept in a temperature-controlled experimental animal room with 1.5 horse power air-condition for 9 hours daily. Other groups were raised in an open poultry pen of the Usmanu Danfodiyo University, Sokoto under HAT ($43.0 \pm 2.0^{\circ}\text{C}$). The NC birds were intact (not feather clipped), the T1 birds were feather-clipped, the T2 birds were intact and were fed with diet supplemented with 200 mg AA/kg feed while the T3 group were feather-clipped and supplemented with 200 mg AA/kg feed. Feathers were clipped on the third and the fifth week with a new, sterile pair of scissors as described by Adeyemo *et al.* (2011). Clipping was carefully done to avoid injury to the birds, water and feeds were served adequately for the 7 weeks study. Table 1 below is presentation of the experimental feed.

Sample collection

Terminally, 4 chickens per group were selected and 1ml of blood was collected from each of the birds into plain sample bottles. The blood samples were allowed to clot and were centrifuged to harvest serum for hormone analysis. The hormones were assayed using Accu--bind® Elisa Kits procured from California, USA. From each of the bird, another 2ml of blood was collected into EDTA-coated bottles for haematological analysis. Blood parameters determined were, red blood cell count, packed cell volume, haemoglobin and white blood cell count using the methods of Jelalu (2014). Calculated values for mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were obtained as expressed below:

$$\text{Mean corpuscular volume (MCV)} = \text{PCV} \times 10 / \text{RBC (fl)}$$

$$\begin{aligned} \text{Mean corpuscular haemoglobin (MCH)} \\ = \text{Hb} \times 10 / \text{RBC (pg)} \end{aligned}$$

$$\begin{aligned} \text{Mean corpuscular haemoglobin concentraion (MCHC)} \\ = \text{RBC} \times 100 / \text{PCV (g/dl)} \end{aligned}$$

Monitoring of ambient temperature, relative -humidity and determination of temperature-humidity index

Daily monitoring of ambient temperature and relative humidity was achieved with the use of using a tempo-hygrometer at 08:00-10:00h, morning and 13:00-16:00h) throughout the eight weeks of the study. The temperature humidity index (THI) was calculated according to Ravagnolo *et al.* (2000) using the equation below:

$$\begin{aligned} \text{THI} &= (1.8 \times T + 32) \\ &- [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T \\ &- 26)] \end{aligned}$$

Where T = ambient temperature ($^{\circ}\text{C}$) and RH = relative humidity (%)

Data analysis and presentation

Data were analysed using analysis of variance at 5% probability using the parametric analytical tools of InStat version 3.0 statistical software. Software, Inc., San Diego, CA USA). The data were presented in tables.

Results

Table 2 presents the result of the haematology of the experimental birds. The result showed that feather clipping and or AA supplementation had no significant effect on most of the blood parameters of the birds except for white blood cell (WBC) and heterophil-lymphocyte ratio. WBC differed significantly among the treatments with the highest value in the PC group ($13.47 \times 10^9/\text{l}$). The WBC values were lower and similar in HAT exposed groups with the lowest ($10.47 \times 10^9/\text{l}$) in T3. The highest lymphocyte count was also recorded in PC group and its values decreased in all the HAT-exposed groups. The lowest heterophil-lymphocyte ratio (0.35) was also recorded in PC group while it was statistically similar among the HAT-exposed groups.

Table 3 presents results of the hormonal assay of the experimental birds. The results showed that the broiler chickens in PC group differ significantly ($P < 0.05$) in serum levels of corticosterone, triiodothyronine and thyroxine compared to HAT-exposed groups. Corticosterone levels differed significantly among the treatments and the controls with the least value recorded in the PC group. Nonetheless, values observed in T1 and T3 were similar to that of the PC. Triiodothyronine levels also differed significantly among the treatments and the controls with the least value recorded in the NC group and the highest value recorded in PC group. However, values recorded in T1, T2 and T3 were statistically similar.

For thyroxine, the highest (11.29 ng/mL) was obtained in PC as compared to T2 (8.46 ng/mL), T3 (8.55 ng/mL) NC (8.15 ng/mL) and the lowest (7.97 ng/mL) in T1 showing decrease in serum thyroxine levels in all the HAT-exposed groups. Thyroxine levels differed significantly among the treatment groups and controls with the highest value obtained in PC group and the least value recorded in T1 group. Nevertheless, the values of this hormone recorded in T1, T2 and T3 were statistically similar to that of the NC.

Table 1. Composition of the experimental feeds

Ingredients (kg)	Starter feed (kg)		Finisher feed (kg)	
	Basal diet	Supplemented diet	Basal diet	Supplemented diet
Maize	42.03	42.03	50.34	50.34
Soybean	12.66	12.66	8.55	8.55
GNC	25.31	25.31	17.11	17.11
Wheat offal	15.00	15.00	19.00	19.00
Bone meal	2.50	2.50	2.50	2.50
Limestone	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
*Premix	0.30	0.30	0.30	0.30
Ascorbic acid	-	0.02	-	0.02
Total	100.00	100.02	100.00	100.02
Calculated values				
Crude protein	23.00	23.00	19.00	19.00
Energy	2753.73	2753.73	2780.21	2780.21

*Premix to supply Vitamin D (2,000mg), Vitamin K₃ (2,000mg), Vit. B₁₂ (10,000mg), pantothenic acid (10,000mg), niacin (26,000mg), folic acid (1,000mg), biotin (100,000mg), choline (150,000mg), manganese (10,000mg), zinc (50,000mg), cobalt (250mg), iron (40,000mg), copper (6,000mg), iodine (500mg), selenium (100mg).

Table 2. Mean values of haematological parameters of the heat-stressed broiler chickens managed by feather clipping and ascorbic acid supplementation

Parameters	(28 – 30°C)	Exposed to high ambient temperature (43.0 ± 2.0°C)				SEM
	PC	NC	T1	T2	T3	
PCV (%)	25.83	25.00	24.00	24.17	24.00	1.52
Hb (g/dl)	7.43	7.35	7.21	7.25	7.28	0.25
RBC (×10 ⁶ /μl)	2.38	2.32	2.24	2.29	2.25	0.37
MCV (fl)	108.53	107.76	107.14	105.55	106.66	5.13
MCH (pg)	31.21	31.68	32.19	31.66	32.36	0.97
MCHC (g/dl)	9.21	9.28	9.33	9.48	9.38	0.19
WBC (×10 ³ /μl)	13.47 ^a	11.58 ^b	11.53 ^b	11.88 ^b	10.47 ^b	0.42
Heterophil (×10 ³ /μl)	3.39	3.17	3.41	3.68	3.25	0.31
Lymphocyte (×10 ³ /μl)	9.77	7.07	8.09	8.36	7.52	0.18
Hete:Lymph(×10 ³ /μl)	0.35 ^a	0.45 ^b	0.42 ^b	0.44 ^b	0.43 ^b	0.01

a,b: Means in the same row with different superscripts differ significantly ($P < 0.05$)

Reference ranges: PCV: (22 – 25%), Hb: (7 – 13g/dl), RBC: (2.5 – 3.5 ×10⁶/μl), WBC: (12 – 30 ×10³/μl) MCV: (77.0 – 88.4fl), MCH: (26.0 – 28.83pg), MCHC: (31.02 – 33.88g/dl) adapted from (Bounous and Stedman, 2000) and (Al-Nedawi, 2018).

Keys: PC: positive control; NC: negative control; T1: clipped; T2: 200 mg/kg ascorbic acid + intact; T3: 200 mg/kg ascorbic acid + clipped Hete:Lymph: Heterophil-Lymphocyte ratio

Table 3. Mean values of selected hormones of the heat-stressed broilers managed by feather clipping and ascorbic acid supplementation

Parameters (ng/mL)	(28 – 30°C)	Exposed to high ambient temperature (43.0 ± 2.0°C)				SEM
	PC	NC	T1	T2	T3	
Corticosterone	7.88 ^a	9.70 ^b	8.20 ^a	9.46 ^b	7.99 ^a	0.79
Triiodothyronine	4.04 ^c	2.78 ^a	3.16 ^b	3.67 ^b	3.74 ^b	0.16
Thyroxine	11.29 ^b	8.15 ^a	7.97 ^a	8.46 ^a	8.55 ^a	0.21

a,b,c: Means on the same row with different superscripts differ significantly ($P < 0.05$)

Keys: PC: Positive Control; NC: Negative Control; T1: Clipped; T2: 200 mg/kg Ascorbic Acid + intact; T3: 200 mg/kg Ascorbic Acid + clipped

Discussion

Feather clipping alone or in combination with AA supplementation had no significant effect on blood parameters of the birds except for white blood cell and Heterophil-Lymphocyte ratio. Ajakaiye *et al.* (2010) reported that heat stress has no effect on most haematological parameters of chickens. However, reports have shown that immune system is mostly affected in chronic stress (Nazar and Marin 2011). This result implies that the best immune response is found in the broiler chickens managed under temperature-controlled environment (PC) when compared to the HAT exposed groups. Studies have shown that heat stress alter levels of circulating cells. In response to heat stress, circulating lymphocytes decrease in number against increased numbers of heterophils (Prieto and Campo, 2010; Felver-Gant *et al.*, 2012). Despite the intervention with feather clipping and ascorbic acid supplementation in this study, heat stress caused immunosuppression in birds as evident in the heterophil-lymphocyte ratio of the HAT-exposed groups. It may be inferred that keeping birds under a temperature-controlled environment improved immune system of the birds by lowering heterophil-lymphocyte ratio and it was the best among methods of managing heat stress experimented in this study. It has been well reported that the ratio of circulating heterophil and lymphocyte serve as an important indicator for evaluation of immune response of birds (Altan *et al.*, 2000; Felver-Gant *et al.*, 2012). Elevated value of heterophil-lymphocyte ratio indicates chronic stress and subsequently weak immune responses (Huff *et al.*, 2005; Prieto and Campo 2010; Nazaret. *al.*, 2012). Ajakaiye *et al.* (2010) reported marked increase in heterophil-lymphocyte ratio and decrease in egg production, especially in the control group of laying chickens transported under high ambient temperature, but the ratio decreased in the groups of layers treated with vitamin C and E. In all the groups, RBC counts were lower than the normal but PCV and Hb values were within the reference ranges reported by Bounous and Stedman, (2000). The MCV and MCH values were higher while MCHC values were markedly lower in all the groups compared to the reference ranges reported by (Al-Nedawi, 2018). The variations may be due to difference in strain and environment. The highest level of corticosterone (9.70 ng/mL) was recorded in NC group which indicated the negative effects of heat stress. This agrees with the report of Collier *et al.*, (2006) that highly stressed birds produce more corticosterone since the release of corticosterone is stimulated by stress conditions (Attia *et al.*, 2009). The PC group had the least concentration of corticosterone but similar to T1 and T3. This showed that feather clipping alone and feather clipping combined with AA supplementation achieved similar result to that of PC in the reduction of heat stress as shown by the significant ($P < 0.05$) decrease in levels of corticosterone in T1 (8.20 ng/mL) and T3 (7.99 ng/mL) respectively. It has been reported that ascorbic acid regulates biosynthesis of corticosterone in birds under stress (Daghir 2008; Attia *et al.*, 2009) by inhibiting essential enzymes (21-hydroxylase and 11- β hydroxylase) in the biosynthetic

pathway of corticosterone (Brake 1989). However, the corticosterone levels in T2 and NC groups are statistically similar. This may be due to higher rate of AA depletion than its restoration through supplementation (Bottje 2013), or owing to poor heat dissipation by the birds as their feathers were not clipped. These observations therefore emphasise the significance of feather clipping.

The low level of triiodothyronine observed in NC is an indication of the negative effect of heat stress on the birds in the group. This corroborates the reports of Star *et al.* (2008), Elnagar *et al.* (2010) and Mack *et al.* (2013) that high ambient temperature consistently decreases serum level of triiodothyronine. Kalmar *et al.* (2013) also reported low levels of metabolic hormones and poor growth rate in birds raised under hot environment (McKee and Harrison, 1995). However, birds raised under controlled environment had higher concentration of triiodothyronine. This observation substantiates the report of Yousef and Johnson (1990) that biosynthesis of triiodothyronine is relatively better in birds not exposed to high ambient temperature. More so, the ameliorative effects of feather clipping, AA supplementation and their combination were evident in the result as levels of triiodothyronine were raised in the groups managed with these interventions. This probably implies that heat might have been dissipated from the exposed skin surface of the birds to the environment due to feather clipping. This corroborate the findings of N'du *et al.*, (2007) which suggested that feather clipping may be an effective means of managing heat stress in broilers. There may also be a synergy between the effect of feather clipping and AA supplementation in elevating the levels of triiodothyronine in T3 group. This therefore explains why the T3 group treated with feather clipping and AA supplementation combination was second best to the PC group.

Thyroxine levels were significantly lower in NC, T1, T2 and T3 than that of PC group. The low levels observed in those groups may either be due to low thyroid hormones production or peripheral deiodination of thyroxine resulting from heat-induced functional hypothyroidism (Star *et al.*, 2008). It might also be due to rapid conversion of thyroxine to triiodothyronine by heat stress (Elnagaret *al.*, 2010; Mack *et al.*, 2013) in those groups. The highest level of thyroxine in PC group indicates that broilers managed under temperature-controlled environment performed better compared to those managed under HAT-exposed conditions.

Conclusion and Recommendation

The antioxidant effects of AA and the thermoregulatory effect of feather clipping are both ameliorative to negative effects of HAT. The results of this study showed the beneficial effects of combining feather clipping and AA supplementation at reducing heat stress and improving performance of the birds. Feather clipping and AA supplementation combination is therefore recommended for ameliorating heat stress in broiler chickens.

Acknowledgments

We recognised the assistance of non-academic staff of the department of Theriogenology and Animal Production, Faculty of Veterinary Medicine Usmanu Danfodiyo University Sokoto.

Competing Interests

There is no conflict of interest among the authors regarding this research.

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