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Original Research

Benefits of watermelon juice and ascorbic acid supplementation on *Institut de Sélection Animale* brown layers managed under hot climate

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ABSTRACT

Loss of homeostasis and poor egg production are common in laying hens during hot season. These negative effects of extreme ambient temperature have resulted in great economic losses to poultry farmers in the tropical regions. This study investigated benefits of supplementing watermelon juice (WJ) and ascorbic acid (AA) to *ISA Brown* (IB) hens managed under hot climate on productive performance and some hormonal responses. Ninety-six IB pullets aged 34 weeks were randomly allotted to four groups (C, T1, T2, and T3), each containing 24 in triplicates. The control group (C) was given water with neither WJ nor AA. Water given to groups T1, T2 was supplemented with 20% and 40% WJ, respectively, while T3 was supplemented with 200mg AA/litre of water. The results show a significant ($P \leq 0.05$) increase in water intake in T3 compared to other groups. Total egg production, daily egg production and hen-day egg production were significantly ($P \leq 0.05$) improved by the supplements, as total egg weights improved in T1:(272.2 ± 35.16g) and T2:(272.1 ± 35.06g) compared to C:(212.2 ± 25.06g) and T3:(238.5 ± 19.23g). The supplements caused 24.46%, 22.70% and 9.43% rise in percentage egg production in groups T1, T2, and T3 respectively. Serum corticosterone (C:10.88 ± 1.12; T1:10.20 ± 1.06; T2:9.61 ± 0.792; T3:7.23 ± 2.36ng/mL) and thyroxine levels (C:1.45 ± 0.562; T1:0.41 ± 0.109; T2:0.39 ± 0.367; T3:0.60 ± 0.489µg/dL) decreased significantly ($P \leq 0.05$) in supplemented layers, while serum prolactin and triiodothyronine differed non-statistically. In conclusion, WJ and AA supplementation ameliorated heat stress in IB layers managed under hot climate as it significantly lowered corticosterone and improved egg production. Therefore, supplementing 20%, 40%WJ or 200mg AA/litre in water is recommended for managing heat stress in IB layer chickens.

Keywords: Ascorbic acid, egg laying, watermelon juice, hot climate

INTRODUCTION

Heat or thermal stress is an environmental factor challenging poultry production globally (Lara & Rostagno, 2013). All animals respond differently to stressors depending on intensity or concentration of the stress factor, duration of exposure, genetic differences and idiosyncrasies (Soleimani *et al.*, 2011; Mack *et al.*, 2013), or existence of multiple stressors (Hemsworth, 2003; Boissy *et al.*, 2007). Among production animals, poultry are more sensitive and susceptible to heat stress (Lara & Rostagno, 2013). Severity of thermal stress depends on environmental temperature and relative humidity (Ayo *et al.*, 2011). Poultry perform optimally, in the tropical region within environmental temperature range of 12°C and 26°C. Rozenboim *et al.* (2007) have described the ambient temperature range of 24°C to 26°C as thermo-neutral. However, at 30°C and above, thermal stress occurs in birds (Daghir, 2008; Hassan *et al.*, 2016). The body temperature of poultry (41°C) is closer to the point of 'heat death' than 'cold death' (Jag, 2006). At 4°C above the normal body temperature, the lethal temperature may occur and result in mortality of birds (Jag, 2006). Poultry lack sweat glands, so their response to heat stress is further aggravated on account of feathers and high metabolic heat production during high growth rate (especially in broilers) and egg production in laying breeds (Jag, 2006). Birds under thermal stress reduce feed intake but drink more frequently, elevate their wings and pant constantly, restrict movement and spend more time resting (Mack *et al.*, 2013). Elnagar *et al.* (2010) have reported possibility of poor reproductive performance in layers under heat stress due to involvement of thyroid hormone at the onset of puberty and reproductive functions. Heat stress suppresses immune system, disrupts reproductive hormones profiles, and consequently diminishes reproductive performance, egg production and quality (Bozkurt *et al.*, 2012; Deng *et al.*, 2012). Different approaches have been used in the

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management of stress. The roles of vitamins A, C and E in the management of thermal stresses have been attributed to their antioxidant properties (Hoehler & Marquardt, 1996; Ajakaiye *et al.* 2010). However, functional foods or nutraceuticals are good sources of natural antioxidants and other bioactive components that are capable of preventing life threatening ailments. The use of such natural products for the prevention and treatment of different diseases is a growing area of research globally (Mota *et al*., 2009).

Watermelon (*Citrullus lanatus*) is rich in lycopene, a very powerful antioxidant which gives the pink-red colour to the fruit (Bruton *et al*., 2009). The fruit belongs to the family *Cucurbitaceae* (Edwards *et al*., 2003) and originated from the Kalahari Desert in Southern part of Africa, occupying much of Botswana and parts of Namibia and South Africa. But nowadays, the fruit is cultivated abundantly in the tropical regions of the world including many drier parts of West Africa where it has become naturalized (Koocheki *et al.,* 2007). In addition to lycopene, it also contains β-carotene and specific amino acids; arginine and citrulline. Depending on the variety, the lycopene content in red fleshed watermelons ranges from 35 – 112 mg/kg fresh weight and the carotenoid content varies from 37 – 121 mg/kg fresh weight (HonCode, 2008). Aghel *et al.* (2011) have reported lycopene content ranging from 4.50 to 4.81 mg/100g of watermelon paste. Its nutritional profile showed that, watermelon contains almost 92% water and 7.55% of carbohydrates, out of which 6.20% are sugars and 0.40% dietary fiber (Quek *et al*., 2007). Its fat content is cholesterol-free and it is considered as a low caloric fruit (Bruton *et al*., 2009). It is rich in vitamins like thiamine, riboflavin, niacin and folate, as well as minerals like potassium, magnesium, calcium, phosphorus and iron (Quek *et al*., 2007). The rich antioxidant properties of *Citrullus lanatus,* would be effective at ameliorating negative impacts of stress on the health of layer chickens and boost egg production.

MATERIALS AND METHODS

THE STUDY LOCATION

The study was conducted at the Teaching and Research Poultry pen of the Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, City Campus complex, Usmanu Danfodiyo University Sokoto, located within Sokoto metropolis, Sokoto State within the months of March and May. The hot dry season in the state falls between March and June. The study area lies between longitude 5′ and 6′ E and latitude 13′ and 14′ (Mamman *et al*., 2000).

EXTRACTION AND ANALYSIS OF WATERMELON JUICE

The juice was prepared as described by Jimoh *et al.* (2018). The variety of watermelon identified as '*Icebox'* at the Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto was used for the study. It was sourced from Sabon Birni Local Government Area, Sokoto State. The watermelon flesh, after being separated from the seeds and the rind, was blended with electric blender, sieved to extract the juice. The proximate analysis of watermelon was according to the recommended method of Association of Official Analytical Chemists (AOAC, 1990), 18th Edition was used to assess the quantities of moisture content, crude protein, ash, and fat. All analysis was done in triplicate and the mean calculated and reported. And the vitamins content of the watermelon fruit was assayed at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria, using Atomic Absorption Spectrophotometer (Varian AA 20, Austria, 1997). Below are the detailed procedures for both proximate and vitamin analyses of the watermelon fruit;

DETERMINATION OF MOISTURE CONTENT:

Ten grams (10g) of watermelon fruit samples were taken and placed in a petri-dish and dried in previously heated laboratory oven at 105℃ to a constant weight. Moisture content (wet basis) was calculated using Equation

$$\%MC_{wb} = \frac{M0-M1}{M0} \times 100$$

where, MC_{wb} = Moisture content (wet basis); M0 = mass of sample before drying; M1= mass of sample after drying.

DETERMINATION OF CRUDE PROTEIN CONTENT:

The micro Kjeldahl method was used for the nitrogen (N) determination. Crude protein was calculated by multiplying nitrogen (N) with a protein factor 6.25.

Protein = %Nitrogen × 6.25.

DETERMINATION OF TOTAL ASH CONTENT:

Dried watermelon samples weighing 5g each were ground and put into porcelain crucible in triplicates and decarbonized in a Muffle furnace overnight at 550℃. The dry matter of the sample was used as the ash component of the watermelon.

Calculation: %Ash content= $\frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$

DETERMINATION OF FAT CONTENT

Twenty grams (20g) finely ground dried watermelon samples were placed in a cellulose thimble paper and fat extraction was carried out using hexane in a 250 mLSoxhlet extractor for 6 hours. The fat content was calculated using Equation:

$$\%Fats = \frac{Wf + U - (Wef \times 100)}{Ws}$$

where, Wf = weight of flask; U = fat extract; Wef = weight of empty flask; Ws = Weight of sample taken.

ESTIMATION OF ASCORBIC ACID IN WATERMELON

The principle of Atomic Absorption Spectrophotometry (AAS) is that free gaseous atoms absorb electromagnetic radiation at a very specific wavelength to produce a measurable signal. The absorbance is proportional to the concentration of the free atoms present in the optical path.

***Reagents*:** 10% Acetic acid, 10% Thiourea, 2,4-Dinitrophenyl Hydrazine, 85% Sulphuric acid and Bromine water, Standard vitamin C solution.

Procedure: Ten grams (10g) of blended watermelon sample was transferred into a 100ml volumetric flask, mixed with about 50ml of acetic acid solution, gently shaken to obtain a homogenous dispersion. Then the solution was topped up to mark with more acetic acid and was then filtered. To determine the level of vitamin C in the filtrate, a few drops of bromine water were added until the solution changed color. This confirms complete oxidation of ascorbic acid to dehydroascorbic acid. Then a few drops of thiourea solution were added to remove the excess bromine and thus the clear solution was obtained. Then 2, 4-dinitrophenyl hydrazine solution was added thoroughly with all standards and the oxidized ascorbic acid. Following complexing with 2,4 - dinitrophenyl hydrazine at 37°C temperature for about three hours, 85% H_2SO_4 was added to the solution and the resultant red color complex was measured under the Atomic Absorption Spectrophotometer at 280 nm.

EXPERIMENTAL BIRDS AND DESIGN

For this study, 150 *Institut de Sélection Animale Brown* (ISA Brown®) day-old chicks were purchased from Zartech Hatchery® and reared up till 14 weeks old on deep litter. The birds were fed Hybrid® feeds and watered *ad libitum*. The birds were vaccinated against common poultry diseases; Infectious bursa disease, Newcastle disease, fowl pox and fowl typhoid. Out of the flock of 150 pullets, 96 were randomly picked and grouped on cages into four treatment groups of 24 chickens in three replicates of eight birds. The control group (C) was given water with no supplement, while the test groups T1, T2 and T3 were given water supplemented with 20% watermelon juice (WJ) at 1:4 v/v that is 200ml of WJ plus 800ml of water, 40% WJ at 2:3 v/v (that is 400ml of WJ plus 600ml of water) and 200mg ascorbic acid (AA)/litre respectively. Throughout the eight weeks of the study, the birds were fed on formulated basal diet (Table I), and same measures of water, freshly prepared juice, and AA were served twice daily (early morning and at night). Average environmental temperature and the relative humidity within the pen (measured with an installed China-made digital thermo-hygrometer: BIOBASE®) were 40.38°C and 10.25% respectively located there.

DATA COLLECTION

When the birds were at the 34th week of age, data collection started on egg production. Simultaneously, data on feed intake were recorded weekly and those of water intake were recorded daily. These were monitored and recorded for the following eight weeks. The mathematical expression of Huneau-Salaün *et al.* (2011) was adopted in calculating Hen-day egg production (HDEP) while percentage increase in egg production was calculated as follows:

Table I. Composition of the layers mash fed to ISA-brown layers supplemented with watermelon juice and ascorbic acid under hot climate

Ingredients	Basal Feed
Maize	40.00
Palm kernel cake	3.00
Soyabean	9.00
Groundnut cake	11.00
Wheat offal	15.00
Corn bran	10.00
Bone meal	2.50
Limestone	3.50
Salt	0.25
Lysine	2.50
Methionine	3.00
*Premix	0.25
Total	100.00
Calculated values	
Metabolizable energy	2468.90
Crude protein	16.45

*Premix to supply Vitamin D (2,000mg), Vitamin K_3 (2,000mg), Vit. B_{12} (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).

$$HDEP\ (\%) = \frac{Total\ number\ of\ eggs\ laid\ per\ day}{Total\ number\ of\ hens\ present\ on\ that\ day} \times 100$$

$$PREP\ (\%) = \frac{DEP(tg) - DEP(cg)}{DEP(cg)} \times 100$$

Where, PREP is percentage rise in egg production, *DEP (tg)* is daily egg production of the test groups, T1, T2 and T3, and *DEP (cg)* is daily egg production of the control group.

HORMONAL ASSAY

The serum was extracted from blood sampled terminally from five birds (n=5) from each group for assay of corticosterone, prolactin, triiodothyronine and thyroxine. The principle was based on competitive enzyme immunoassay technique and the procedure was carried out as described by

Burtis & Ashweed (1999) using Accu-bind Elisa Kits procured from California, USA.

The essential reagents used include antibody, enzyme antigen conjugate and a serum containing the native antigen. Competition reaction results between the native antigens, conjugate for a limited number of antibody binding sites.

The interaction is illustrated with equation below:

$$^{Enz}Ag + Ag + {}_{Btn} \rightleftharpoons AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)

$AgAb_{Btn}$ = Enzyme-antigen Complex

$^{enz}Ag\ Ab_{Btn}$ = Enzyme –antigen Conjugate-Antibody Complex

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

$K = k_a / k_{-a}$ = Equilibrium Constant

As precaution before proceeding with the assay, all reagents, serum references and controls were brought to room temperature (20-27^{0}C). The microplate wells for each serum reference, control and test sample in the assay were formatted in duplicate. Any unused microwell stripes were replaced back into the aluminum bag, sealed and stored at 2-8^{o}C.

PROCEDURES FOR ESTIMATION OF SERUM CORTICOSTERONE, PROLACTIN, THYROXINE AND TRIIODOTHYRONINE:

The procedures are similar for all the assayed hormones. Some 0.025ml (25µl) of the appropriate serum reference, calibrator, control and sample were pipetted into the assigned wells. Then 0.050ml (50µl) of Corticosterone (but 0.100ml of prolactin, thyroxine and total triiodothyronine) Enzyme Reagent was added to all wells. The microplates were gently swirled for 10-20 seconds to mix, and 0.050ml (50µl) of unknown hormone (corticosterone, prolactin, thyroxine and total triiodothyronine) Biotin Reagent was added to all wells. The microplates were swirled again for 10-20 seconds to mix. They were then covered and incubated for 60 minutes at room temperature. The contents of the microplates were afterward discarded by decantation, and the plates were blot-dried with absorbent paper. Then 0.350ml (350µl) of wash buffer was added and decanted. This procedure was repeated two additional times to make a total of three washes manually. Then, 0.100ml (100µl) of substrates solutions was added to all wells. Shaking of the plates was avoided after substrate had been added. The microwells were incubated at room temperature for 20 minutes. At the expiration of the time, 0.050ml (50ul) of stop solution was added to each well and was gently mixed for 15-20 seconds. Then the absorbance in each well was read at 450nm (using a reference wave length of 620-630nm to minimize well imperfections) in a microplate reader. The results were read within thirty minutes of adding the stop solution as instructed by the manufacturer. A dose response curve was used to determine the concentration of corticosterone, prolactin, thyroxine and triiodothyronine in the unknown (assayed) samples.

DATA ANALYSIS

Data generated from the study were subjected to one way analysis of variance (ANOVA) using SPSS software version 20.0, expressed as means with standard deviation and the results presented on tables. Duncan post hoc test was used to test differences between means of the analyzed parameters and mean values were considered statistically significant at 5% confidence interval.

RESULTS

Mean initial body weight, mean final weights, feed intake and feed conversion ratio were statistically similar (P>0.05) across the four groups (Table II). There were reduced body weights from 1.53±0.22kg/bird average initial weights to 1.47±0.11kg/bird average final weights across the groups.

The mean water intake of the birds in group T3 differed

Table II. Mean ± SD performance indices of Isa Brown layer chickens in response to 8-week supplementation of watermelon juice (WJ) and ascorbic acid (AA) under hot climate

Parameters	C	T1	T2	T3	P-value
IBW (kg/bird)	1.54 ± 0.07	1.53 ± 0.22	1.55 ± 0.24	1.55 ± 0.20	0.281
FBW (kg/bird)	1.48 ± 0.31	1.49 ± 0.50	1.48 ± 0.08	1.47 ± 0.11	0.075
WD (kg/bird)	0.06 ± 0.35	0.04 ± 0.61	0.07 ± 0.22	0.08 ± 0.54	0.102
WI (litre/bird/day)	0.48 ± 0.01^b	0.49 ± 0.01^b	0.50 ± 0.02^b	0.56 ± 0.04^a	0.001
FI (kg/bird/week)	0.538 ± 0.09	0.548 ± 0.18	0.545 ± 0.13	0.521 ± 0.03	1.679
TEW(g/bird/week)	212.2± 25.06^c	272.2± 35.16^a	272.1± 35.06^a	238.5± 19.23^a	0.031
FCR	2.54 ± 0.36	2.01 ± 0.27	2.00 ± 0.32	2.19 ± 0.23	0.916

Means in the same row with different superscripts $^{(a,b,c)}$ are significantly (P≤0.05) different

Key: IBW: (initial body weight), FBW: (final body weight), WI: (water intake), FI: (feed intake), TEW: (total egg weight), FCR: (feed conversion ratio), SD: (standard deviation), WD: (weight difference).

significantly (P≤0.05) compared to other groups. Similarly, total egg weights (TEW) differed significantly (P≤0.05.05) in groups T1 (272.2 ± 35.16g) and T2 (272.1 ± 35.06g) compared to the control (212.2 ± 25.06g) and T3 (238.5 ± 19.23g). The values of feed conversion ratio in groups T1 (2.01 ± 0.27) and T2 (2.00 ± 0.32) ranked the best among the four experimental groups (Table II).

The result showed that birds under the control, non-supplemented group produced least number (730 ± 8.41) of eggs, while the groups supplemented with WJ and AA (T1, T2, and T3) produced more number (909 ± 7.91, 896 ± 4.55 and 799 ± 5.94 respectively) of eggs. The daily egg production of birds in groups T1 (16.23 ± 0.39 eggs) and T2 (16.00 ± 1.12 eggs) differed significantly (P<0.05) compared to the control (13.04 ± 0.67 eggs) and T3 (14.27 ± 0.71 eggs) groups. More so, the percentage hen-day egg production of all supplemented groups (T1, T2, and T3) also differed significantly compared to the control. The result also showed improved egg production as PREP of 24.46%, 22.70% and 9.43% were recorded in birds under groups T1, T2, and T3 respectively above the control, non-supplemented group (Table III).

The results showed that corticosterone and thyroxine differed significantly (P≤0.05) in all the supplemented groups compared to the control, whereas, prolactin and triiodothyronine were statistically similar (P>0.05) across all the experimental groups. Serum corticosterone level was highest (10.88 ± 1.12ng/mL) in the control group but its levels were relatively low (10.20 ± 1.06ng/mL, 9.61 ± 0.792ng/mL and 7.23 ± 2.36ng/mL) in all the supplemented groups (T1, T2 and T3) respectively. Serum levels of the thyroxine follow similar trend, the control group had the highest concentration (1.45 ± 0.562µg/dL) while reduced concentrations (0.41 ± 0.109µg/dL; 0.39 ± 0.367µg/dL and 0.60 ± 0.489µg/dL) were recorded in all the treatment groups (T1, T2 and T3) supplemented with both WJ and AA respectively (Table IV).

DISCUSSION

Increased voluntary water intake in laying hens has been considered as an important adaptive response to hot temperatures (Bessei, 2007; Ahmad *et al.*, 2008), and it is of great benefit to hyperthermic chickens (Balnave and Brake, 2005). High rate in water intake in chickens has been reported at the period of extremely high ambient temperature (Mack *et al.*, 2013). The estimated average water intake of a laying hen raised under thermo-neutral temperature is between 0.25 and 0.27 litres/bird/day (NRC, 1994). In this study, water intake increased greatly (Table 2) to values between 0.48 ± 0.01 litres/bird/day (control group) to 0.56 ± 0.04 litres/bird/day in birds fed on AA supplementation (T3). These are equivalent to between 92% and slightly >100% increase more than the recommended water intake across the groups. These findings agree with a range of 30% to 50% (PCRC, 2003), or even double to triple increase in water intake reported when environmental temperature is above 32°C (Oluyemi & Roberts, 2000). It has also been reported that increased water intake is beneficial to birds under heat-stress, as it facilitates heat dissipation through their respiratory tract (Belay & Teeter, 1993). Adequate water

Table III: Mean ± SD egg production efficiency of Isa Brown layer chickens in response to 8-week supplementation of watermelon juice (WJ) and ascorbic acid (AA) under hot climate

Parameters	C	T1	T2	T3	P-value
TEP (7weeks/group)	730.0 ± 8.41^{d}	909.0 ± 7.91^{a}	896.0 ± 4.55^{b}	799.0 ± 5.94^{c}	0.000
DEP (day/group)	13.04 ± 0.67^{c}	16.23 ± 0.39^{a}	16.00 ± 1.12^{a}	14.27 ± 0.71^{b}	0.000
HDEP (%)	54.32 ± 3.09^{c}	67.63 ± 1.76^{a}	66.67 ± 1.53^{a}	59.50 ± 2.02^{b}	0.000
PREP (%)	--	24.46	22.70	9.43	

Means in the same row with different superscripts $^{(a,b,c)}$ differ significantly at P≤0.05

Key: TEP: (total egg produced), DEP: (daily egg production), HDEP: (hen-day egg production), PREP: (percentage rise in egg production), SD: (standard deviation).

Table IV: Mean ±SD profile of some selected hormones on IB layer chickens in response to 8-week supplementation of WJ and AA under hot climate (n=6)

Parameters	C	T1	T2	T3	P-value
CORT (ng/mL)	10.88 ± 1.12^{c}	10.20 ± 1.06^{a}	9.61 ± 0.792^{b}	7.23 ± 2.36^{a}	0.010
PRL (ng/mL)	1.78 ± 0.722	1.18 ± 0.512	1.24 ± 0.287	1.55 ± 0.381	0.927
TIT (ng/mL)	0.63 ± 0.200	0.70 ± 0.582	0.74 ± 0.276	0.72 ± 0.267	0.495
THR (µg/dL)	1.45 ± 0.562^{a}	0.41 ± 0.109^{b}	0.39 ± 0.367^{b}	0.60 ± 0.489^{b}	0.046

Means in the same row with different superscripts $^{(a,b,c)}$ are significantly (P≤0.05) different

CORT: (corticosterone), PRL: (prolactin), TIT: (triiodothyronine), THR: (thyroxine), SD: (standard deviation)

intake is also essential for egg production as well as egg size and weight (Yi *et al.,* 2021). In this study, feed intake also reduced across the groups as values recorded were relatively lower to the recommended daily ration of 115g to 125g of feed/hen/day (Petek, 1999; Oluyemi & Roberts, 2000). Weight losses ranging from 0.04 kg to 0.08 kg observed (Table 2) in this study may be attributed to general decrease in feed intake recorded in all the groups. It could also be a form of physiological response in controlling heat load by reducing metabolic heat generated from the feed. This finding is in agreement with early reports that extreme ambient temperature causes reduced feed intake in birds (Attia *et al.,* 2009; Mack *et al.,* 2013). The detrimental effects of chronic heat stress on egg production have been documented (Mashaly *et al.,* 2004; Star *et al.,* 2008; Mack *et al.,* 2013). Farnell *et al.* (2001) have reported reduction of about 13.2%, 26.4% and 57% in egg production in laying hens subjected to heat stress during 8–14 days, 30–42 days and 43–56 days' studies respectively. Deng *et al.* (2012) have also reported 28.8% decrease in egg production in a 12-day heat-exposure study. These reports support and explain the reason for the poor feed conversion ratio, lighter egg weights, decreased daily egg production (DEP) and hen-day egg production (HDEP) recorded in the control group of this study. These findings are in agreement with earlier reports (Star *et al.,* 2008; Quinteiro-Filho *et al.,* 2010). However, heat stress and subsequent reduced feed intake have little or no effect on ISA Brown layers in the groups supplemented with WJ (T1 and T2) and AA (T3). Rather, egg production indices such as DEP, HDEP and weight of egg laid were improved. The improvement may be attributed to the improved feed conversion ratio in the birds contributed by the supplements which induced reduced stress in the birds, enhanced uptake of feed nutrients and their conversion to eggs. Ascorbic acid is a proven antioxidant which scavenges for free radicals and reactive oxygen species (Homidan, 2000; Ajakaiye *et al.,* 2010). Likewise, the role of electrolyte balancing especially, of potassium in ameliorating negative impacts of heat stress in birds has earlier been reported (Ahmad, *et. al.*, 2005; 2008). Findings have showed that watermelon juice has potentials for potassium restoration and antioxidant capacity (Jimoh *et. al.*, 2018; 2021). It also contains bioactive components such as phenolics, carotenoids, and flavonoids reportedly responsible for its antioxidant activities (Charoensiri *et al*., 2009; Ambreen *et al.,* 2013). Mort *et al.* (2008) have attributed remarkable antioxidant capacity of watermelon to its higher ratio of lycopene to carotene (12:1) content. Being functional food that is rich in such nutrients as vitamin C and a special amino acid, citrulline (Bruton *et al*., 2009), watermelon juice could have supplied necessary nutrients that are capable of boosting egg production in the stressed chickens.

The pathogenesis of low egg production in heat-stressed layer chickens is complex, and could involve the hypothalamic–pituitary–adrenal (HPA) axis (Akinyemi and Adewole, 2021). Stress stimulates HPA axis causing change in the concentration of the circulating metabolic and reproductive hormones (Deviche *et al.,* 2016). Stress conditions stimulate the release of corticosterone and lipid peroxidation in the cell membranes (Attia *et al.,* 2009). The multifunctional roles of corticosterone in stressed birds are thought to be mediated through alteration of the neuro-endocrine system (Cheng & Muir, 2004). These points may explain the elevated serum level of corticosterone recorded in the non-supplemented, control group. In this study, the chicken groups supplemented with AA had the lowest serum concentration of corticosterone indicating the positive role of AA in ameliorating heat stress. Reports have shown that AA regulates the biosynthesis of corticosterone in birds under stress when supplemented in feed or water (Daghir, 2008; Attia *et al.,* 2009). Brake (1989) had earlier reported that AA inhibits essential enzymes (21-hydroxylase and 11-βeta hydroxylase) in the biosynthetic pathway of corticosterone. The anti-oxidant and protective activities of AA in animals under stress are well reported (Homidan 2000; Atta, 2002; Ajakaiye *et al.,* 2010). The supplementation suppressed serum concentration of corticosterone in the other two groups of chickens supplemented with WJ (T2 and T3). The phytochemical analysis of watermelon juice showed that, apart from its lycopene content, it also contains some levels of vitamin C (Aghel *et al.,* 2011; Jimoh *et al.,* 2021). Availability of these antioxidant components in watermelon might be responsible for its suppressive effects on serum concentration of corticosterone. The two hormones (triiodothyronine and thyroxine) of the thyroid gland are responsible for regulation of body temperature and metabolic activities in mammals and birds (Frare *et al.,* 2021). Gendy *et al.* (1995) reported that high ambient temperature affects the weight of thyroid gland and its secretion of thyroxine and triiodothyronine. Likewise, reports are consistent about decrease in triiodothyronine concentration due to high ambient temperature (Star, *et al.*, 2008; Mack, *et al.*, 2013). Therefore, the slight increase in serum concentration of thyroxine observed in this study might have occurred due to less conversion of thyroxine into triiodothyronine probably due to stress. It may be inferred that both supplements, the WJ and the AA, affected the metabolism of thyroxine to triiodothyronine, and thus explained the reason for the slight decrease in concentrations of triiodothyronine in all the groups. The finding disagrees with Heninger *et al*. (1960) who reported a drop in thyroxine secretion in cockerels which were kept at a high ambient temperature. Also, other studies have reported inconsistent increase (Elnagar *et al*., 2010) or no alteration (Mack *et al.,* 2013) in the serum levels

of thyroxine. These variations in these reports might be attributed to difference species of birds used. The results of this study also showed that slight elevated levels of triiodothyronine were recorded in the supplemented groups indicating that the supplements (WJ and AA) improved production of triiodothyronine. Although, the supplements had no significant effect on serum concentration of prolactin, there was improved egg production in the supplemented chickens than those in the control group. It has been reported that egg production in birds declines with increasing concentration of prolactin (Reddy *et al*., 2006). Prolactin has been described as a hormone of broodiness in birds (Sharp *et al*., 1989; Oluyemi & Robert, 2000). It has also been documented that serum prolactin level increases during stress conditions (Bolarin, 2013). In this study, the serum concentrations of prolactin decreased by 33.7% from 1.78 ± 0.722 ng/mL in the control group to 1.18 ± 0.512 ng/mL in the 40% WJ supplemented groups (T2) of chickens. From this result, it may be suggested that supplementation of WJ has either ameliorated some negative effects of stress or seemed to have reduced prolactin secretion. Therefore, this may be a reason, among others, for the improvement in egg production recorded in the groups supplemented with WJ and AA above the control. Report of Reddy *et al*. (2006) who supplemented bromocriptine (an anti-prolactin chemical) in feed of Girirari birds and observed decrease in serum concentration of prolactin, increase in estradiol and progesterone and approximately 5.12% increase in egg production is also in support of findings of this study. It has also been reported that prolactin inhibits gonadotrophin-stimulated ovulation and estrogen production at ovarian level in chickens (Reddy *et al*., 2001), and a decrease in prolactin has been reported before and during the preovulatory LH surge (Zadworny *et al*., 1985). Therefore, change in concentrations and activities of these hormones by stress had negatively affected the reproductive and productive performances of the untreated experimental *ISA* brown layer chickens.

CONCLUSION AND RECOMMENDATIONS

In conclusion, supplementation of watermelon juice and ascorbic acid in drinking water had contributed positively to ameliorating negative effects induced by heat stress in the ISA brown layer chickens by lowering serum levels of corticosterone and prolactin, leading to improved egg production and mean egg weights. The two best results were obtained at both levels (20% and 40%) of watermelon juice supplementation, and then followed by AA supplementation. It could be recommended that watermelon juice and ascorbic acid be supplemented in drinking water to ameliorate negative impacts of heat stress in ISA brown layer chickens reared under hot climate.

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