

Ameliorative effects of *Citrullus lanatus* (watermelon) juice on survival, performance and lipid peroxidation status of heat-stressed broiler chickens

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Abstract

Heat stress is highly detrimental to the wellbeing of broiler chickens especially at the finisher stage (4-8 weeks) of production. Ameliorative effects of watermelon juice (WJ) supplement were investigated over three weeks period to monitor survival, performance and lipid peroxidation of finisher broiler chickens raised during hot season. Proximate and phytochemical analyses of the WJ were conducted. Then, one hundred five-week-old broiler chickens of 0.9kg average body weight were allotted into 4 groups- (Control, Test 1, Test 2 and Reference) each in 3 replicates in a completely randomised design. The control group received non-supplemented water, the test groups (T1 and T2) received 20% and 40% WJ respectively, and the reference group (RG) received 200mg of ascorbic acid (AA) /litre of water. Data were analysed using one way ANOVA. The results showed that relatively high survival rates were recorded in the test (T1: 84.0%, T2:100.0%) and reference (RG: 80.0%) groups compared to the control group (CG: 36.0%). Feed intake and weight gain improved significantly ($p < 0.05$) in all the supplemented groups. The best ameliorative effects of the WJ on weight gain (1.60kg/b), Feed Conversion Ratio (0.90), survival rate (100%) and malodialdehyde (MDA) concentration (3.25) were obtained at 40% supplementation level (T2) compared to other treatments. In conclusion, WJ effectively alleviated negative impacts of heat stress in the finisher broilers at 40% supplementation level. Its supplementation is therefore recommended for broiler chickens to minimise economic loss commonly encountered in the tropical regions where extreme ambient temperature is a major constraint to poultry production.

Keywords: *Citrullus lanatus* juice, heat stress, lipid peroxidation, broilers

Introduction

The tropical regions of the world are characterised by high ambient temperature (HAT) often combined with high relative humidity which can induce heat stress. Poultry perform optimally within thermo-neutral ambient temperature ranges of 12°C and 26°C (Holik, 2009; Hassan *et al.*, 2016), but at 30°C and above, heat stress occur in birds (Hassan *et al.*, 2016). Extremely high ambient temperature is the most important environmental stress factor confronting poultry production in the

tropical regions of the world (Lara and Rostagno, 2013). It causes several negative effects which are more pronounced and often fatal in broilers of market age than the young birds, and thus leading to great economic loss to the farmers (Hassan and Reddy, 2012). Physical responses of birds to extremely high ambient temperature include; reduced feed intake, increased water intake, constant panting, restricted movement, wings elevation and spending of more time at rest (Mack *et al.*, 2013). Thermal stress also leads to poor

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performance, immune-suppression and high mortality rate in broilers (Star *et al.*, 2008; Quinteiro-Filho *et al.*, 2010). Stress increases generation of reactive oxygen species (ROS) which in excess cause lipid peroxidation and cell damage (Altan, *et al.*, 2003). Antioxidants remove free radicals, activate endogenous antioxidant enzymes and inhibit oxidative stress (Luqmans, *et al.*, 2012). As powerful scavengers for free radicals, vitamins A, C and E are constantly being used in the management of stress (Atta, 2002; Ajakaiye *et al.*, 2010). The use of certain herbs, fruits and functional foods (nutraceuticals), which had been proven to contain antioxidant bioactive components, in the management of stress in poultry, is gaining research focus.

Citrullus lanatus, commonly known as Watermelon, belongs to the family *Cucurbitaceae* (Edwards *et al.*, 2003). It contains almost 92% water and 7.55% carbohydrates, out of which 6.20% are sugars and 0.40% dietary fiber (Quek *et al.*, 2007). It is rich in lycopene, a strong antioxidant which gives the pink-red colour to the fruit (Perkins-Veazie *et al.*, 2001), as well as beta-carotene, vitamins C, E and specific amino acids –arginine and citrulline (Collins *et al.*, 2007; Charoensiri *et al.*, 2009; Ambreen, *et al.*, 2013). These pools of antioxidants protect cells, DNA and other tissues in the body from oxidative damage (Altas *et al.*, 2011; Chaturvedi, *et al.*, 2014). The antioxidant potentials of watermelon juice are yet to be investigated in heat-stressed broiler chickens and other poultry species. It is on the basis of these potentials that watermelon is being investigated in this study, as an antioxidant supplement for ameliorating negative impacts of heat stress in broiler chickens.

Materials and methods

Preparation and analyses of watermelon juice

Fresh watermelon fruits were purchased

from a selected farmer from Sabon Birni Local Government area of Sokoto State. The variety of watermelon fruit (i.e. Icebox) used for the study was presented to the Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, for identification. The watermelon was washed with clean water and then the flesh was separated from the rind. The seeds were removed and the flesh was blended using electric blender and then sieved with double layered cheese cloth to obtain the juice. Proximate analysis of the watermelon for moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract contents was assayed in accordance with the methods described by AACC (2000) while assays for minerals and vitamins contents were conducted using methods described by AOAC (2006). **The analyses** were conducted **at** the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria, using **Atomic Absorption Spectrophotometer** (Varian AA 20, Austria). Isolation and quantification of lycopene was carried out using the method described by **Aghel *et al.* (2011)**.

Experimental design

One hundred and fifty white *hubbard* strain of broiler chicks were purchased from Arewa Chicks Nigeria Limited, Kaduna. The birds were managed on deep litter system, fed on commercial diet and watered *ad libitum* for the first five weeks. During this period, they were vaccinated against Newcastle disease and Infectious bursa disease accordingly. At the end of 5th week, 100 finisher broilers of 0.9kg average weight were randomly selected and allotted into four treatment groups- Control group (CG), Test groups (T1 and T2) and reference group (RG). The Control group (CG) was given 1litre of plain water, test group 1 (T1) was given 20% WJ (200ml of WJ + 800ml of water), test group 2 (T2) was given 40% WJ (400ml of WJ + 600ml of

water) and the reference Group (RG) was given 200mg of AA/litre of water).

Some 200 and 400mL of water melon juice were respectively introduced into 1Litre cylinder, and were made up to the mark with drinking water. The juice was prepared twice daily for immediate use throughout the 3 weeks of the study. All the groups were served with equal volumes of water (supplemented with WJ and AA and non-supplemented) *ad libitum* but feeding was restricted to early morning (7:00-9:00am) and night (7:00-10:00pm) to minimize metabolic heat generation that may aggravate the stress.

The study area

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, located within Sokoto metropolis, Sokoto State during the months of May and June. The study area lies between longitude 5° and 6° E and latitude 13° and 14°. Daily ambient temperature (AT) and relative humidity (RH) were monitored in the morning (07:00-10:00 h) and afternoon (13:00-16:00 h) throughout the period and were measured using digital temperature hygrometer, BIOBASE® China. The temperature humidity index (THI) was calculated according to Ravagnolo *et al.* (2000) using the equation below.

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

where T = ambient temperature (°C) and RH = relative humidity (%)

Data collection

Performance parameters

Feed intake of the birds was measured weekly and calculated by subtracting the left over from the served feed using the following formula

Feed intake = amount of feed served (in kilogram) - amount of feed leftover (in kilogram)

Birds in each group were weighed weekly and the weight gains (WG) were calculated by using the following formula-

Weight Gain = Final weight (at the 8th week) - Initial weights (at the 5th week)

Feed conversion rate (FCR) in each group was calculated using the formula below-

Feed Conversion Rate (FCR) =

Feed intake (in kilogram)

Weight gain (in Kilogram)

Mortality rates in all the groups were recorded and then the percentage survival rates (PSR) in all the groups were calculated using the formula below-

Percentage survival rate (%) =

1 - (number of dead bird in a group / total number of bird in a group) x 100 (Mortality rate)

Estimation of lipid peroxidation, blood samples were collected from 5 birds per group through the wing vein for estimation of Malondialdehyde (MDA) concentration. Lipid peroxidation as evidenced by the formation of Thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehans and Samuelson (1968).

Principle and Procedure: The assay is based on the reaction of Malondialdehyde with Thiobarbituric acid, forming an MDA-TBA₂ adduct that absorbs strongly at 535nm.

Using micro pipette, 0.1ml of serum was put into a test tube and 2 cm³ of TBA-TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) in ratio 1:1:1 was added. The tube was placed in water-bath for 15 minutes, cooled and centrifuged at room temperature for 10 minutes at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm. The concentration of TBARS was calculated using the molar extinction coefficient of Malondialdehyde (1.5 x 10⁵ mol/l/cm).

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Statistical analysis

Data were subjected to one-way ANOVA at 5% probability using the parametric analytical tools of InStat Version 3.0 statistical software (GraphPad, Software, Inc., San Diego, CA, USA). The data were presented in tables and bar charts.

Results

The results of the proximate, mineral and phytochemical analyses of the watermelon are presented in Table 1. The values obtained were; moisture (91.02), Ash (0.26%), crude protein (0.48%), crude fat (0.13%), crude fibre (0.34%) and Nitrogen

Free Extract (7.77%). The results showed that the watermelon contains considerably high concentration of potassium (260mg/100g) but low calcium (0.70mg/100g) and moderate sodium (23.0mg/100g) concentrations. The results also showed that the watermelon contains about 4.0mg lycopene/100g of the paste, moderate concentrations of vitamins A (4.15mg/100g) and B1 (4.77mg/100g), but low concentration of vitamin C (0.38mg/100g).

Table 1: Proximate and phytochemical compositions of the watermelon

Parameters	Concentrations
Proximate composition	(%)
Moisture	91.02
Crude protein	0.48
Crude fat	0.13
Crude fibre	0.34
Ash	0.26
NFE (CHO)	7.77
Mineral Content	(mg/100g)
Sodium (Na)	23.00
Potassium (K)	260.00
Calcium (Ca)	0.70
Magnesium (Mg)	1.20
Phosphorus (Ph)	1.65
Phytochemical	(mg/100g)
Lycopene	4.00
Vitamin A	4.15
Vitamin B1	4.77
Vitamin C	0.38

The average daily ambient temperatures and relative humidity recorded during the period of the study were 39.0°C and 23% respectively and the temperature humidity index (THI) was estimated to be 84.2. The results of supplementing watermelon juice and ascorbic acid on performance indices of the finisher broilers are presented in

Table 2. Feed intake significantly ($p < 0.05$) improved in all the supplemented groups (T1: 1.41kg/b, T2: 1.44kg/b and RG: 1.40kg/b) compared to the control group (CG: 0.85kg/b). It was however not statistically different among the groups supplemented with WJ and AA. In term of weight gain, supplementation with WJ and

AA also caused significant ($p < 0.05$) difference among the treatment groups. The best ameliorative effects of the supplement

on weight gain (1.60kg/b) and FCR (0.9) were observed in the group which received 40% WJ (T2).

Table 2: Performance indices of heat-stressed broilers supplemented with Watermelon Juice (WJ) and Ascorbic Acid (AA)

Parameters	CG	T1	T2	RG	SEM
Body weight (kg/b) at week 5	0.90	0.90	0.90	0.90	0.01
Final body weight (kg/b) at week 8	1.40 ^a	1.90 ^b	2.50 ^c	2.00 ^b	1.13
Weight gain (kg/b) within 3 weeks	0.50 ^a	1.00 ^b	1.60 ^c	1.10 ^b	0.72
Total feed intake (kg/b) for 3 weeks	0.85 ^a	1.41 ^b	1.44 ^b	1.40 ^b	0.45
Feed Conversion Ratio (FCR)	1.70	1.41	0.90	1.27	---

abc: means within rows with different superscripts are significantly different ($p < 0.05$)

KEY: CG, control group (plain water), T1 (20% WJ), T2 (40% WJ) and RG (200mg of AA/1litre of water).

The results of supplementing watermelon juice and ascorbic acid on survival rate are presented in the Figure 1. The survival rates (84.0%, 100.0%, and 80.0%) were better in the corresponding groups (T1, T2 and RG) supplemented with both WJ and AA compared to the control (CG; 36.0%), non-supplemented group. The highest survival

rate, 100% (equivalent to 0.0% mortality), was recorded in the treatment group T2, supplemented with 40% WJ. However, the lowest survival rate (36.0 %) was recorded in the control group (CG) and the groups supplemented with 20% WJ (T1) and 200mg AA (RG) had 84.0% and 80.0% between (RG) and respectively.

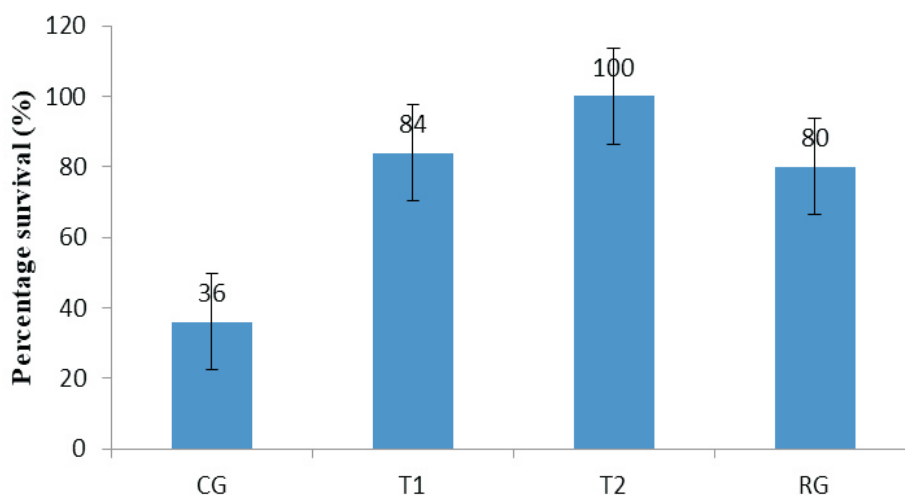


Figure 1. Percentage survival rate of the heat-stressed broiler chickens supplemented with watermelon juice and ascorbic acid

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The trend of the MDA concentration in the birds is presented in figure 2 below. MDA concentrations decreased significantly ($p>0.05$) from 7.07 in the control, non-supplemented group (CR) to the ranges of

3.25 to 3.87 in the supplemented groups. The lowest MDA concentration (3.25) was recorded group T2 supplemented with 40% WJ compared to the concentrations 3.87 and 3.63 recorded in groups T1 and RG respectively.

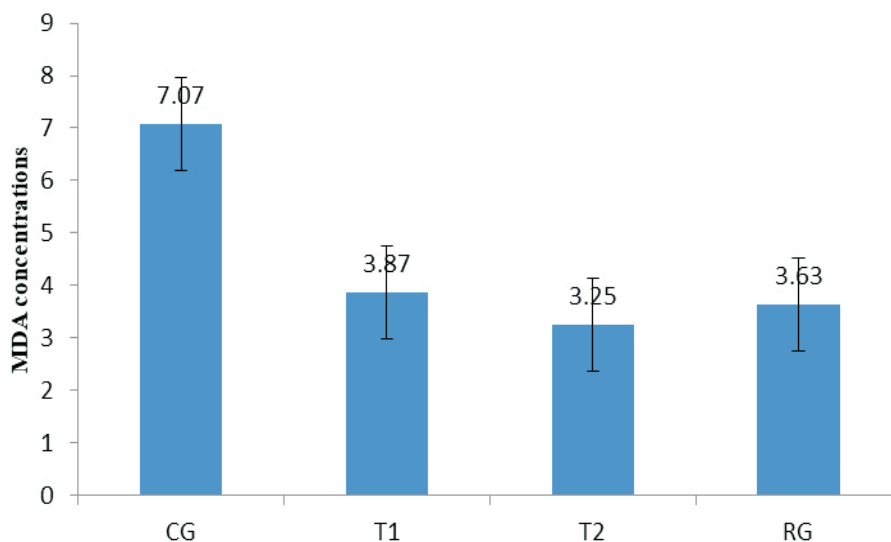


Figure 2. Concentrations of MDA in blood samples of heat-stressed broiler chickens supplemented with watermelon juice and ascorbic acid

Discussion

Natural products vary in their phytochemical compositions, partly due to soil nutrients or growing conditions. Assessment of a test material for availability and quantity of its micronutrients and bioactive components is a prerequisite step. From the results of proximate and phytochemical analyses of the watermelon juice presented in Table 1, it is shown that the watermelon used in this study is comparably similar in its physicochemical components to what have been reported in earlier studies (Quek *et al.*, 2007; Bruton *et al.*, 2009). The proximate analysis revealed moisture content (91.02%), Ash (0.26%), crude protein (0.48%), crude fat (0.13%), crude fibre (0.34%) as well as Nitrogen Free Extract (7.77%). The results agreed with the report

of Naz *et al.* (2013). Although watermelons vary in lycopene content with different varieties (Fish and Davis, 2003), the phytochemical analysis results showed that the lycopene yield (4.0mg/100mg) of the analysed watermelon in this study was relatively lower than the average range of 4.50 mg/100g and 4.81 mg/100g reported by Aghel *et al.* (2011) and Naz *et al.* (2013) respectively. However, it was within the range of 2.30 and 7.20 mg/100g fresh weight bases reported by Charoensiri *et al.* (2009). Analysis for mineral content of the watermelon showed that the concentrations of potassium (260mg/L) and sodium (23mg/L) were considerable high but that of calcium (0.7mg/L) was relatively low. The results were in variance with the report (K: 126mg/100g, Na: 5.6mg/100g: Ca: 0.8mg/100g) of Naz *et al.* (2013), that

analysed the “Sugar baby” variety of watermelon, indigenous to Pakistan. Varying concentrations of the mineral contents (Potassium; 107mg/100g, 114 mg/100g and 154 mg/100g; Sodium; 0.70 and Calcium; 6.40) of watermelon have also been reported (Proietti *et al.*, 2008; Colla *et al.*, 2006). The sharp variation in mineral contents of the tested watermelon in this study might be due to difference in soil mineral content, agronomic practices, ripening stage and harvesting season of the fruit, analytical method and reagents used as earlier suggested (Naz *et al.*, 2013).

In this study, significant improvement in performance, higher survival rate and lower MDA concentrations were obtained in the WJ and AA-supplemented broilers (T1, T2 and RG) compared to the non-supplemented control group (CG). Ascorbic acid is a tested and proven antioxidant and scavenger of free radicals and reactive oxygen species (Atta, 2002; Ajakaiye *et al.*, 2010). Likewise, watermelon contains micronutrients and bioactive components which are reportedly responsible for its antioxidant activities (Altas *et al.*, 2011; Ambreen, 2013). Mort *et al.*, (2008) had attributed higher ratio of lycopene to carotene (12:1) in watermelon to its remarkable antioxidant capacity. More so, the higher potassium content (260mg/100g) of the tested watermelon might have greatly influenced the improved performance recorded in the groups supplemented with WJ compared to the control groups and the AA supplemented groups. Arit-Boulahsen *et al.* (1995) had reported that supplementation of potassium chloride improves the thermo-tolerance of chickens exposed to acute heat stress. In an earlier study, Arit-Boulahsen *et al.* (1989) reported decreased plasma level of potassium and increased excretion of potassium through urine due to heat stress. The importance of

supplementing electrolytes and amino acids in the maintenance of physiological functions of chickens during hot weather has been documented (Brake *et al.*, 1994). It was also observed that the watermelon contained moderate vitamins A (4.15mg/100g) and B1 (4.77mg/100g) concentrations, but its vitamin C content (0.38mg/100g) was considerably low. There is strong indication that the improved performance recorded in the WJ-supplemented broilers might be hinged on the synergistic interactions among the micronutrient contents of the watermelon juice. Therefore, the improvement in weight gain and feed conversion ratio (Table 2) showed that both the watermelon juice and the AA are effective at improving performance in heat-stressed chickens. However, at 40% supplementation level, watermelon juice improved performance better than 200mg/1litre of water. Homidan (2000) had earlier supplemented 250mg Vit.C/litre of drinking water and reported improved performance in finisher broilers raised in hot climate. This showed that both the WJ and AA are effective at ameliorating the negative effects of heat stress.

The negative impact of heat stress was evident in the lowest survival rate (36.0%) recorded in the control, non-supplemented group (fig.1). Thermal stress causes poor performance, immune-suppression and high mortality in broilers (Mujahid *et al.*, 2009). Hassan and Reddy, (2012) also reported high mortality rate in finisher broiler chickens managed in open houses during hot season in tropical environment. The cause of high mortality in broilers during heat stress conditions may be related to disruption of the endogenous antioxidant system in the body and depletion, especially, of vitamin C depots. During extremely hot season, the intrinsic ability for vitamin C biosynthesis reduces in birds (Yigit *et al.*, 2002), leading to increase in

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demand for metabolic uptake of vitamin C (Whitehead and Keller, 2003). Heat stress disrupts oxidant-antioxidant equilibrium and induces excessive ROS generation leading to oxidative stress (Ma *et al.*, 2008). At this point, supplementation of exogenous antioxidants is a crucial means of boosting the endogenous antioxidant system and preventing lipid peroxidation. From the results, higher survival (T1; 84%, T2; 100% and RG; 80%) of the broilers were recorded in the groups supplemented with WJ and AA. This gives an indication of ameliorative effects of both WJ and AA on the negative impacts of heat stress.

Supplementation of WJ and AA significantly ($p < 0.05$) reduced plasma concentrations of MDA in the supplemented groups (T1, T2 and RG) compared to the relatively high concentration recorded in the non-supplemented control (CG) group. This might also be an indication of effectiveness of the supplements (WJ and AA) at alleviating lipid peroxidation in the heat-stressed finisher broilers. Extremely high ambient temperature is a stimulus for generation of excess free radicals which leads to lipid peroxidation. According to Devasagayan *et al.* (2003), malondialdehyde (MDA) is a toxic carbonyl compound produced following a destructive chain reaction involving lipids and free radicals which results in oxidative deterioration of lipids (Lipid peroxidation). Although no work was accessible to us where WJ was used to manage heat stress in broilers, a similar work conducted in human subjects by Jacob *et al.* (2008), where effects of supplementing lycopene-enriched functional juice (20.6 mg/day) and vitamin C (435mg/day) on the biomarkers of inflammation and oxidative stress were investigated. The result showed that the lycopene-enriched functional juice caused

significant decrease in MDA concentration. Watermelon is also a good source of lycopene, thus the antioxidant effectiveness of the WJ may be attributed to its lycopene content among other micronutrient constituents.

Conclusion and recommendations

From the findings of this study, WJ seem to have effectively ameliorated negative effects of heat stress as it enhanced survival rate, significantly improved performance and reduced MDA concentration in all the supplemented broilers. It compared favourably with AA which has been well investigated and reported to be an effective antioxidant. It is therefore recommended for ameliorating stress in poultry. Use of nutraceuticals (functional foods) as natural sources of antioxidant in the treatment of heat stress or other stress-related diseases is a growing area of research focus, so necessitating the need for further research in this area.

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