

## ANTIOXIDANT POTENTIAL OF WATERMELON JUICE AND ASCORBIC ACID IN THE MANAGEMENT OF HEAT-INDUCED OXIDATIVE STRESS IN LAYER CHICKENS

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### ABSTRACT

High ambient temperature ( $\geq 30^{\circ}\text{C}$ ) is an important environmental stress factor confronting poultry industry in the tropical regions worldwide. It generates excess reactive oxygen species (ROS) to overwhelm endogenous antioxidant enzymes activities and induces oxidative stress which causes lipid peroxidation and cell damage. This study investigated the potentials of watermelon juice (WJ) and ascorbic acid (AA) in boosting antioxidant enzymes activities against oxidative stress and lipid peroxidation in heat-stressed layer chickens. A total of ninety-six ISA brown layers of 34 weeks old were randomly allotted into four groups each containing 24 birds with three replicates. The control group (C) was given water (non-supplemented), the test groups (T1 and T2) were supplemented with 20 and 40% WJ in water respectively and the reference group (R) was supplemented with 200mg AA/litre of water. Data generated were subjected to one-way ANOVA. Results revealed that supplementation with WJ and AA significantly ( $p < 0.05$ ) boosted activities of superoxide dismutase (C: 0.73U/ml, T1: 3.82U/ml, T2: 10.00U/ml and R: 9.27U/ml), catalase (C: 7.51nmol/min/ml, T1: 11.09nmol/min/ml, T2: 26.70nmol/min/ml and R: 18.20nmol/min/ml) and glutathione peroxidase (C: 6.11nmol/min/ml, T1: 47.88nmol/min/ml, T2: 93.53nmol/min/ml and R: 55.01nmol/min/ml) and significantly decreased malondialdehyde plasma concentrations (C: 0.52 $\mu\text{M}$ , T1: 0.33 $\mu\text{M}$ , T2: 0.01 $\mu\text{M}$  and R: 0.19 $\mu\text{M}$ ). The effects were generally distinctive at 40% WJ supplementation level. Due to antioxidant effectiveness of WJ and AA recorded in this study. It was concluded that WJ be supplemented in water for combating heat stress in laying chickens.

**Key words:** Antioxidant, Ascorbic acid, Lipid peroxidation, Oxidative stress, Watermelon juice.

### INTRODUCTION

High ambient temperature (HAT) is an environmental stress factor confronting poultry production in the tropical regions of the world (Lara and Rostagno, 2013). Poultry perform optimally within the range of 12°C and 26°C ambient temperature described as thermo-neutral zone (Rozenboim *et al.*,

2007). However, at 30°C and above, thermal stress occurs in birds which can induce oxidative stress by stimulating generation of excess reactive oxygen species beyond the normal physiological demands of the body cells (Daghir, 2008; Hassan *et al.*, 2016). Free radicals are highly reactive compounds mostly generated during cellular respiration

and normal metabolic activities (Halliwell and Guetteridge, 2007). Reactive oxygen species including free and non-free radicals play mediatory roles in the development of several pathological conditions such as lipid peroxidation, protein oxidation, DNA damage and cellular degeneration (Ma *et al.*, 2008; Chen *et al.*, 2008). Heat stress increases lipid peroxidation by producing free radicals in excess, capable of initiating peroxidation of polyunsaturated fatty acids (Altan *et al.*, 2003). However, antioxidants are capable of attenuating negative effects such as lipid peroxidation and oxidative damage to membranes caused by free radicals (Halliwell and Guetteridge, 2007) by scavenging for radicals or their precursors.

The endogenous antioxidant system consists of enzymatic (superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic (ascorbic acid, alpha-tocopherol, pyruvate and glutathione) components (Halliwell and Guetteridge, 2007). When the endogenous antioxidant system is overwhelmed or depleted by oxidative stress, supplementation from exogenous sources (vitamin C, E,  $\beta$ -carotene) boosts the activities of the enzymatic antioxidants or replenishes the depot of the non-enzymatic antioxidants to provide defense against oxidative stress (Halliwell and Guetteridge, 2007). As advocacy for alternative medicine shift towards nutraceuticals, certain fruits like watermelon, pink guava, pawpaw, cucumber and pumpkin (Ambreen, *et al.*, 2013) and vegetables like *Moringa oleifera* (Hassan *et al.*, 2016) which are good sources of natural antioxidants of great nutritional and therapeutic values should be investigated.

Watermelon (*Citrullus lanatus*) is a common, natural and safe fruit belonging to the family *Cucurbitaceae* (Edwards *et al.*, 2003). Its pool of antioxidant constituents such as lycopene (Perkins-Veazie *et al.*, 2001), beta-carotene, vitamins C, E and specific amino acids –arginine and citrulline (Charoensiri *et al.*, 2009; Ambreen, *et al.*, 2013) has been reported to protect cells, deoxyribonucleic acid (DNA) and other tissues in the body from oxidative damage (Altan *et al.*, 2011). Since the essence of the body's antioxidant defense system is to prevent oxidative stress and maintain redox balance between pro-oxidants and antioxidants (Srinivasan *et al.*, 2009), returning animal's body to its physiological equilibrium is an expected outcome if any antioxidant supplement is said to be effective. Therefore, investigating watermelon juice as an antioxidant supplement in ameliorating oxidative stress in layer chickens will provide useful information necessary for further studies in the area of nutraceuticals.

## **MATERIALS AND METHODS**

### **Preparation and analyses of watermelon juice**

The watermelon juice was prepared according to methods described by Jimoh *et al.* (2018). 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 the flesh was separated from the rind. The seeds were removed, 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).

### **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 Sokoto, located within Sokoto metropolis, Sokoto State from 16<sup>th</sup> of March to 10<sup>th</sup> of May, 2017. 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° N.

### **Experimental protocol**

One hundred and fifty (150) ISA (i.e. *Institut de Sélection Animale*) brown chicks were raised on deep litter system from day-old to fourteen weeks of age. The birds were fed on Hybrid<sup>®</sup> feed and watered *ad libitum* to the point of cage and were vaccinated against Newcastle disease, infectious bursa disease, fowl pox and fowl typhoid as at when due. At

14 weeks of age, a total of ninety-six (96) chickens were selected and randomly divided into four groups each containing 24 chickens in three replicates in cages.

The management of the birds continued until March 16, 2017, when environmental temperature was as high as 39°C and relative humidity was 13% as measured by the digital thermo-hygrometer, BIOBASE<sup>®</sup> China, placed in the poultry pen. The birds responded to the changes in the environment by panting, abduction of wings, increased water intake, reduced feed intake and severe anorexia to the extent that some of the birds were resuscitated by dipping into cold water. At this point, the real study commenced and the birds were at 34 weeks of age and 60% weekly egg production. Then the control group (C) was given water (non-supplemented), the test groups (T1 and T2) were supplemented with 20% WJ (200 ml of WJ + 800 ml of water, i.e. 1:4v/v) and 40% WJ (400 ml of WJ + 600 ml of water, i.e. 2:3v/v) respectively, and the reference group (R) was supplemented with 200 mg AA/litre of water.

The watermelon juice was prepared twice daily for immediate use throughout the eight weeks of the study. Equal volumes of water were given *ad libitum* both in the supplemented and non-supplemented groups with improvised plastic containers (each per cell of the cage) but feeding was restricted to early morning (7:00-9:00 am) and night (7:00-10:00 pm) to minimize metabolic heat generation that may aggravate the stress. The daily ambient temperature (AT) and relative humidity (RH) were monitored throughout the period using a digital thermo-hygrometer,

BIOBASE® China. The average temperature humidity index (THI) was calculated weekly using the equation below according to Ravagnolo *et al.* (2000) and was analyzed using:  $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$ .

$$\text{Percentage Egg Yield} = \frac{\text{Total number of eggs laid by birds per a treatment group for 8 weeks}}{\text{Total number of birds in a group}} \times 56 \text{ days of the study}$$

### **Serum extraction and estimation of antioxidant enzymes**

At the end of the 8 weeks study, about 3 ml of blood were directly collected from the heart and dispensed into plain labeled sample bottles. The blood was allowed to clot and centrifuged at 4000 rpm for 15 minutes to harvest serum. The serum obtained was pipetted into 3 labelled aliquots/tubes for estimation of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). The analysis was conducted at the Chemical Pathology Laboratory, Usmanu Danfodiyo Teaching Hospital, Sokoto.

Serum superoxide dismutase activity was estimated according to the method described by Marklund (1980) using Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, USA).

Serum glutathione peroxidase activity was estimated according to the method described by Paglia and Valentine (1967) using Glutathione Peroxidase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, USA).

Serum catalase activity was assayed by the method described by Johansson and Borg (1988) using Catalase Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, USA).

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

Eggs collected in each treatment group during the 8 weeks study were recorded and expressed below as percentage egg yield:

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehans and Samuelson (1968) based on the reaction of malondialdehyde with thiobarbituric acid, forming an MDA-TBA<sub>2</sub> adduct that adsorbs strongly at 535nm.

### **DATA ANALYSIS AND PRESENTATION**

Data collected on Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GHS-Px) and serum concentration of MDA were subjected to one-way Analysis of Variance (ANOVA) at 5% probability using the parametric analytical tools of InStat version 3.0 statistical software (d Software Inc., San Diego, CA USA). To test the association between average egg production and THI, a linear correlation and regression with a two-tail hypothesis was performed using SPSS version 22.0. The data were presented in bar charts and a table.

### **RESULTS**

In this study, the calculated THI ranged from 79.50 to 83.00, with an average of 81.56 (Table 1). There is no statistically significant ( $p > 0.05$ ) correlation between average egg production and THI. The THI study also revealed that a weak negative correlation existed between average egg production and

THI for treatments C, T1 and T2, while a weak positive correlation was observed in the R group.

The average egg production and data on egg yield although not significantly different

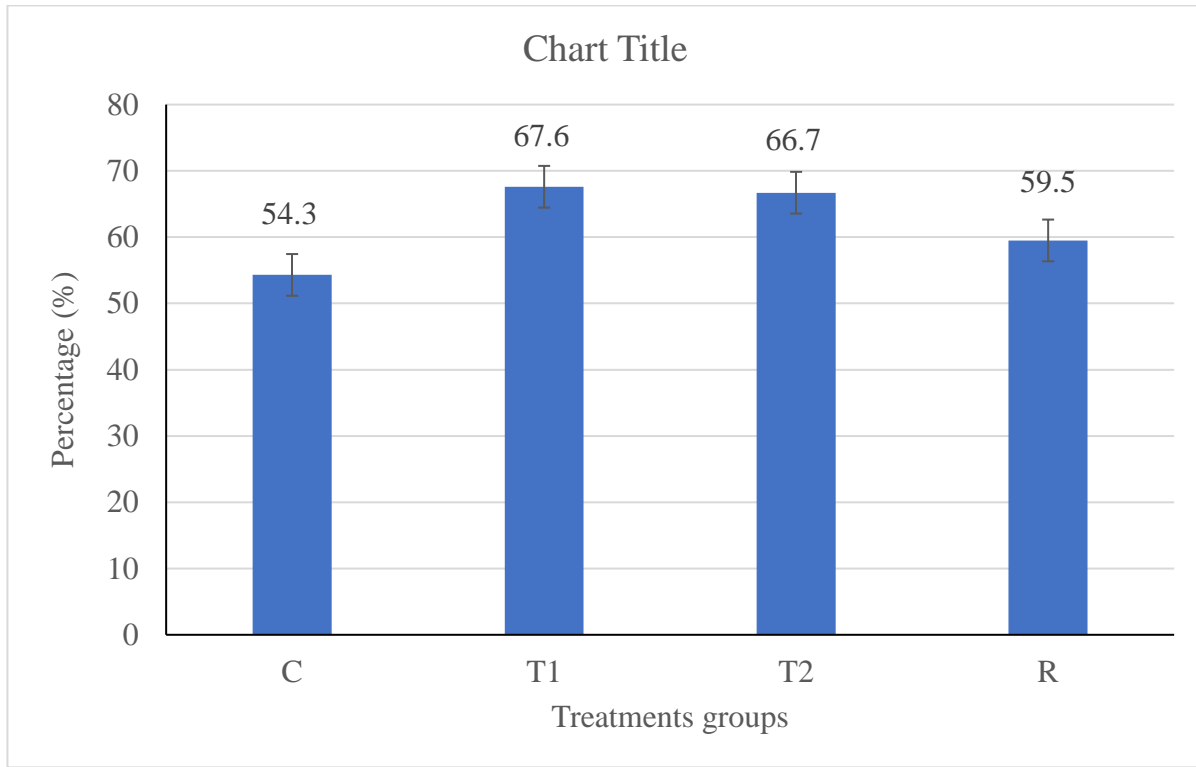
( $p > 0.05$ ) showed that intervention with WJ and AA in groups T1, T2 and R improved production and yields above the control groups(C) as shown in Table 1 and Figure 1.

**Table 1.** Relationship between egg production and temperature humidity index (THI) of experimental birds

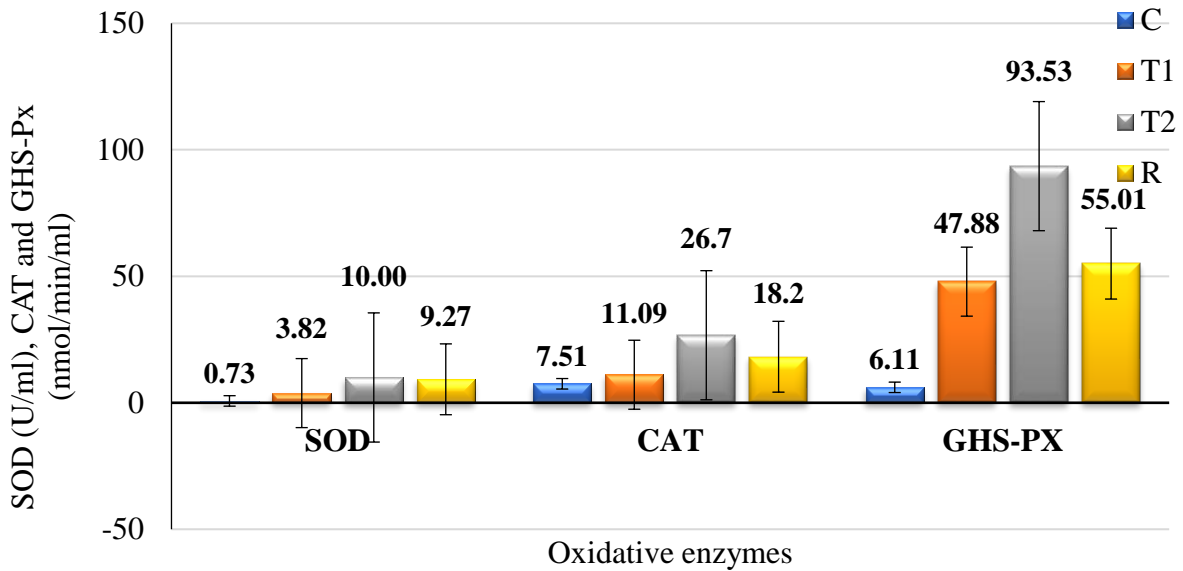
Week	T	RH (%)	THI	Average egg production per week			
				C	T1	T2	R
1	39	11.0	80.50	91	108	104	96
2	42	9.0	82.8	87	112	109	98
3	41	9.0	81.9	95	119	121	103
4	40	10.0	81.2	92	118	115	100
5	38	12.0	79.5	89	119	117	98
6	40	11.0	81.5	92	116	116	106
7	42	10.0	83.0	88	102	104	97
8	41	10.0	82.1	96	115	110	101
Total	323	82	652.5	730	909	896	799
Average	40.38	10.25	81.56	91.25	113.63	112	99.88
Pearson corr.				-0.058	-0.468	-0.322	0.101
Sig. (2 tailed)				0.891	0.242	0.436	0.812
Type of corr.				negative	negative	negative	Positive
Strength of corr.				weak	weak	Weak	Weak

The results of the oxidative enzyme activities in the heat-stressed chickens supplemented with watermelon juice (WJ) and ascorbic acid (AA) are presented in Figures 2 and 3. It was observed that supplementation with WJ and AA significantly ( $p < 0.05$ ) improved the activities of SOD, CAT and GHS-Px (Figure 2). At 40% supplementation level, WJ significantly ( $p < 0.05$ ) boosted the activities of the SOD (T2: 10.00U/ml), CAT (T2:

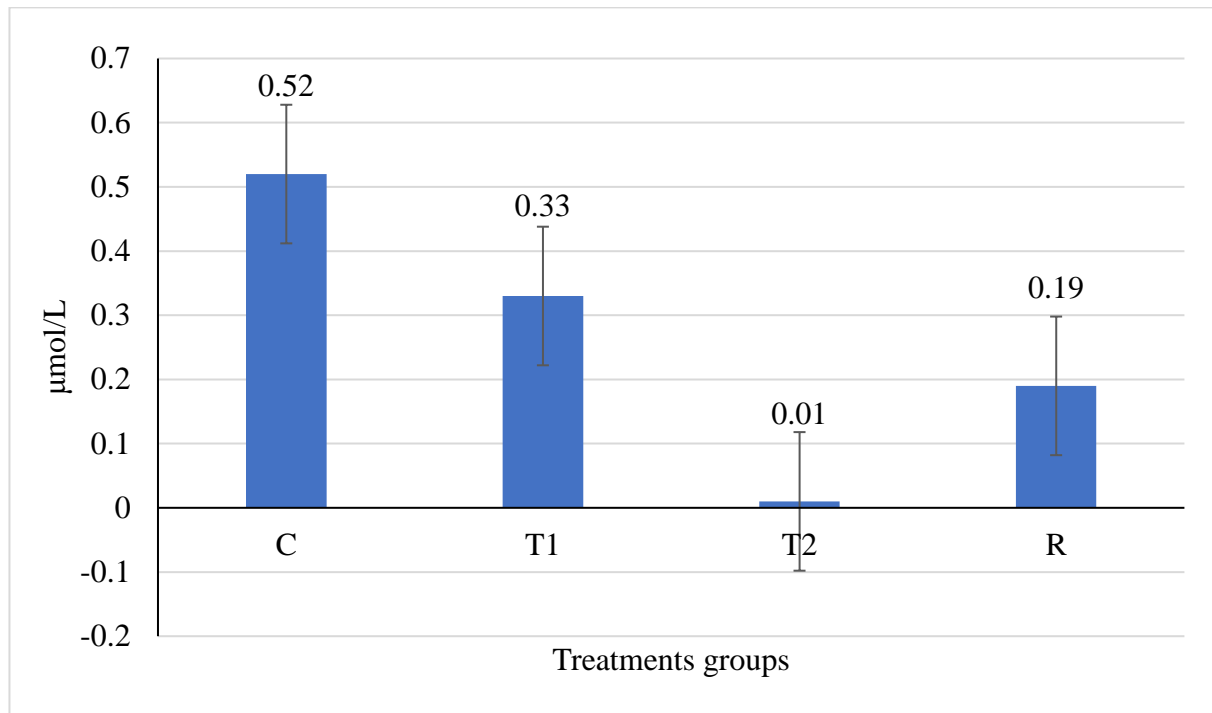
26.70nmol/min/ml) and GHS-Px (T2: 93.53nmol/min/ml) compared to 20% WJ and AA. However, the oxidative stress induced by the HAT significantly ( $p < 0.05$ ) suppressed the activities of these enzymes (SOD, CAT and GHS-Px) in the non-supplemented (C: 0.73U/ml, 7.51nmol/min/ml and 6.11nmol/min/ml) control groups respectively.



**Figure 1.** Percentage egg yield of experimental birds



**Figure 2.** Serum concentration of SOD, CAT and GHS-Px in heat-stressed layer chickens supplemented with watermelon juice and ascorbic acid



**Figure 3.** Serum concentration of MDA in heat-stressed layer chickens supplemented with watermelon juice and ascorbic acid.

The results also showed that the concentrations of MDA were significantly ( $p < 0.05$ ) lowered by supplementing 20% WJ (T1: 0.33), 40% WJ (T2: 0.01) and 200 mg AA (R: 0.19) compared to the corresponding control (C: 0.52) group (Figure 3). The lowest values (T2: 0.01) were obtained in chickens supplemented with 40% WJ. The concentration of MDA in the non-supplemented control group was highest.

## DISCUSSION

According to Zulovich and DeShazer (1990), the result on THI in this study revealed that birds under this experiment were under heat stress, as the observed THI was 79.50 and above which indicates danger and emergency situations. Average egg production and egg

yield although not significant ( $p > 0.05$ ) was improved in the WJ and/or AA treated groups (T1, T2 and R), while it was lowered in the control group (C). A weak negative correlation was observed in groups C, T2 and T2, however the egg production in group C was quadratically lowered when compared with other treatments. This might be as a result of WJ and AA administered to other groups.

In this study, it was observed that chickens in the non-supplemented control group had significantly reduced SOD, CAT and GHS-Px concentrations compared to other groups. This indicated that the activities of these antioxidant enzymes (SOD, CAT and GHS-Px) in the control birds were suppressed by

oxidative stress. This may be due to overwhelming effects of the radicals generated by HAT which probably reduced the protective activities of the endogenous antioxidant enzymes or caused depletion of the non-enzymatic antioxidants (i.e., glutathione, pyruvate, alpha tocopherol and vitamin C) in the body. This finding agreed with the reports of Spurlock and Savage (1993) and Halliwell and Guetteridge (2007) that HAT induces generation of excess ROS and depletion of endogenous antioxidants.

However, supplementation of WJ and AA significantly ( $p < 0.05$ ) boosted the activities of SOD, CAT and GHS-Px in all the supplemented groups. The significantly best antioxidant effects of the supplement were recorded at 40% WJ supplementation level compared to 20% WJ and 200 mg/L AA supplementation. Watermelon juice is rich in antioxidant constituents such as lycopene (Perkins-Veazie *et al.*, 2001), beta-carotene, vitamins C, E and specific amino acids – arginine and citrulline (Charoensiri *et al.*, 2009; Ambreen, *et al.*, 2013) which are potentially active in modulating activities of the endogenous antioxidant enzymes against negative effects of radicals. 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). Increased potassium excretion and its low plasma level during heat period had been reported (Arit-Boulahsen *et al.* 1989). Ait-Boulahsen *et al.* (1989) had reported improved thermo-tolerance in chickens supplemented with potassium chloride when exposed to acute heat stress. Likewise, Jimoh *et al.* (2018), reported that watermelon

contains relatively high potassium (260 mg/100g) as well as other minerals and observed improved performance in the WJ-supplemented groups. Therefore, mineral replenishment especially potassium plays ameliorative role in chickens during heat stress.

Researchers have described MDA as a toxic carbonyl compound produced following some highly destructive chain of reactions involving lipids and free radicals which result in oxidative deterioration of lipids (Devasagayam *et al.*, 2003; Devasagayam *et al.*, 2004). Serum concentration of MDA has been used as a measure of cellular damage due to lipid peroxidation. It implies that the concentration of MDA become high when level of cellular damage is high and vice versa.

From the results of this study, the concentrations of MDA were significantly ( $p < 0.05$ ) reduced in all the groups supplemented with WJ (T1: 0.33 and T2: 0.01) and AA (0.19) compared to the control group where the concentration was statistically highest (0.52). The results thus indicated that supplementation of 20% and 40% WJ and 200 mgAA prevented or attenuated cellular damage due to lipid peroxidation. These results are in agreement with the reports of previous studies in which supplementation of lycopene-containing functional foods enhanced SOD, CAT and GHS-Px activities and reduced MDA concentrations (Bose and Agrawal, 2007; Alshatwi *et al.*, 2010; Mackinnon *et al.*, 2011). A work conducted in human subjects showed that supplementation of lycopene-enriched functional juice at 20.6 mg/day and

vitamin C at 435 mg/day caused significant decrease in MDA concentration (Jacob *et al.*, 2008). Absorption of lycopene and its ability to scavenge for singlet oxygen and free radicals and inhibit lipid peroxidation have been related to the action of its 11- conjugated double bonds (Srinivasan *et al.*, 2009).

## CONCLUSION AND RECOMMENDATIONS

It was observed that supplementation with 40% WJ elicited slightly better antioxidant potentials than 200 mg AA. However, both the WJ and AA significantly boosted antioxidant status of the birds by the increased activities of the endogenous antioxidant enzymes (SOD, CAT and GHS-Px) and reduced serum concentration of MDA thereby ameliorating negative impacts of oxidative stress in the layer chickens. It is therefore recommended that WJ and AA may be used to ameliorate the negative effects of oxidative stress in layer chickens. Future study may focus on synergistic potentials of WJ and AA.

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