Effects of Supplemental Vitamin C and E on Serum Antioxidant Enzymes and Malondialdehyde Concentration in Improved Fulani Ecotype Chickens

***Omodewu I. A.,[1*] Daramola J. O., [2] Abiona. J. A.,[2] Ilori B. M.,[2] Akinjute O. F.,[2] Tiamiyu A. K[1]., Makanjuola B. A.[1]**

[1]Institute of Agricultural Research and Training, Moor Plantation, Ibadan.
[2]Department of Animal Physiology, FUNAAB, Abeokuta.
*Corresponding Author: ifeayo24@yahoo.com, +2348167330502

ABSTRACT
This study evaluated the effect of supplemental vitamin C (VC) and vitamin E (VE) used solely and in combination on one hundred and forty-four improved Fulani ecotype chickens. The birds were grouped into four dietary treatments (control, VE (125mg/kg alpha tocopherol acetate), VC (200mg/kg ascorbic acid) and VC+VE) and managed intensively. At eight weeks, three birds per sex per replicate were randomly picked for blood sampling to determine malondialdehyde concentration (MDA) and serum antioxidant enzymes: catalase (CAT), superoxide dismutate (SOD) and glutathione peroxidase (GPx) using validated analytical laboratory protocols. Analysed data revealed that lowest MDA ($P<0.05$) was recorded in VC+VE (2.75nmol/ml) compared to control (5.93nmol/ml), VC (4.52nmol/ml) and VE (4.01nmol/ml). Serum antioxidant enzymes; superoxide dismutase (SOD) and glutathione peroxidase (GPx) were also elevated in VC+VE (110.06U/ml, 151U/ml) respectively compared to control (69.09U/ml, 121.89U/ml). Supplemental VC and VE increased ($p<0.05$) serum SOD (97.32U/ml, 102.31U/ml) and GPx (141.85U/ml, 143.64U/ml) activity respectively but not as much as VC+CE. Similarly, increased CAT activity ($p<0.05$) were recorded in VC (50.87U/ml), VE (54.94U/ml) and VC+E (52.80U/ml) compared to control (38.92U/ml). Sex and interaction between supplemental dietary antioxidants were not influenced ($P>0.05$). It can be concluded from this study that a combination of VC and VE in the diet of IFEC resulted in better serum antioxidant enzymes levels and malondialdehyde concentration (MDA) which alleviates the negative effects of thermal stress in IFEC.

Key words: Antioxidants, Vitamins, Heat, Stress, Health

Description of Problem

Antioxidants play their role by taking part in series of interconnected redox antioxidant cycle called the "antioxidant network" (1). Two of the antioxidants that play pivotal role in this network are vitamin C (VC) and E (VE). Cifti *et al*. (2) tagged ascorbic acid and alpha tocopherol "protective partners" because vitamin E was not catalytically reproduced by ascorbic acid and at the aquaphobic stage glutathione catalytically reproduced vitamin E. Many of the researches undertaken to evaluate the influence of VC and VE observed complementary activity between these two antioxidants (3, 4). This can be explained by the report of Hill *et al*. (5) about the aggravated declination in clinical characteristics when there was lack of both ascorbic acid and alpha tocopherol. Asadpour *et al*. (6) in their study recommended the usage of both ascorbic acid and alpha tocopherol as effective antioxidants in combating the adverse impacts of thermal stress. The findings of Jena *et al*. (7) that broiler breeders administered alpha tocopherol acetate had considerably reduced concentrations of malondialdehyde (MDA), greater activity levels of catalase and superoxide dismutase. Hieu *et al*. (8) also revealed in his review that adding ascorbic acid to the diet of poultry helps to decrease the MDA amount while helping to increase Total-Antioxidant capacity, glutathione peroxidase, and superoxide dismutase. The positive attributes of VC and VE in poultry production and health led to its choice as dietary supplement in this nutritional intervention study conducted to combat thermal stress challenges in the newly developed Institute of Agricultural Research and Training improved Fulani ecotype chicken (IFEC).

Materials and Methods

Experimental location: This study was carried out at the Bora poultry farm of the Institute of Agricultural Research and Training (IAR&T),

Moor Plantation, Ibadan. Ibadan is in South-western, Nigeria and it lies on the geographical coordinates of 7° 23´ 16´´N and 3° 53´ 47´´E (9). Ibadan has a mean annual rainfall of 1382mm, annual mean temperature range between 21.3-31.2 ^{0}C. The relative average humidity of Ibadan is 70 – 88% (10).

Experimental design: The study was laid out as a randomized complete block design with sex as block. The effect of test ingredients (control, vitamin E (125mg/kg alpha tocopherol acetate), vitamin C (200mg/kg ascorbic acid), combination (C+E) (125mg/kg+200mg/kg) and sex were examined.

Experimental birds: One hundred and forty-four (144) day-old improved Fulani ecotype chicks were sourced from the Nigerian Indigenous Chicken Improvement Research Unit of IAR&T, Moor Plantation, Ibadan.

Distribution of day-old chicks to treatments: The day-old chicks were tagged, allowed to acclimatize and grow for 4 weeks after which they were weighed using a digital weighing balance and sexed into cocks and hens. Their weights were used to group them into treatments and replicates with equal weight distribution, a total of 144 chicks, 12 per replicate (4 hens and 8 cocks), resulting in 36 birds per treatment group served as the initial opening stock for the study.

Management of experimental birds: Chicks were reared in a poultry pen disinfected prior to their collection. Birds were fed a starter ration that supplied 22% crude protein (CP) and 2970 Kcal/kg metabolizable energy (ME) up to 8 weeks of age, the feed met the recommendation of the National Research Council (11). Clean water was provided *ad libitum*. Standard vaccination and medication schedule for chickens was followed throughout the experiment as described by Oladunjoye *et al*. (12).

Sampling and preparation of blood: Three birds per sex per replicate were randomly picked for blood sampling on the 8th week of the study. Using sterilized needle and syringe, blood (3ml) was drawn from the jugular vein and dispensed into vials without anti-coagulant and thereafter submerged in an icebox and transported to the laboratory. Blood samples were centrifugated at a speed of 4000 rotations/minute for 10 minutes for the separation of the serum and afterwards stored at -20 °C until biochemical analysis in the Bioscience Laboratory of the Institute of Agricultural Research and training, Ibadan.

Determination of antioxidant enzymes activity

Catalase: Catalase activity (CAT) in serum was measured using the method of Aebi (19). In brief, 100 µl of H_2O_2 (10 mM) was added to 100 µl of serum, mixed thoroughly, incubated (20 °C) and assayed immediately for the change of absorbance within 1 min at 240 nm by spectrophotometry. One unit of CAT was defined as the amount required to decompose 1µmol of H_2O_2 within 1 min.

Glutathione peroxidase (GPx): GPx activity in samples were evaluated using an adapted technique of Paglia and Valentine (20), 100 µl of serum was mixed with 200 µl of glutathione reductase (5 units/ml), 50µl of glutathione (40 mM), and 620 µl of K-P buffer (0.25M). To this mixture, 10 µl of 20 mM NADPH (in 1 % Na_2CO_3) and 20 µl of 15 mM cumene hydroperoxide were added. Changes in absorbance were observed at 340 nm for 3 min with a spectrophotometer and one unit of GPx was defined as µmol NADPH oxidized/min.

Superoxide dismutase (SOD): Activity of superoxide dimutase was evaluated by colorimetry (21). A mixture of 10 µl serum, 3 ml Tris-HCl buffer, and 6.1 µl the pyrogallol (50 mM in 10 mM HCl) was observed immediately using a spectrophotometer within 1 min at 325 nm to monitor change in absorbance. One unit of SOD activity was defined as the amount of the enzyme inhibiting the autoxidation by 50 %.

Evaluation of lipid peroxidation status

Lipid peroxidation status in serum was measured as malondialdehyde (MDA) equivalents using the technique of Ohkawa *et al*. (22). In brief, serum (100 µl) were treated with sodium sodecyl sulfate (0.2 ml, 8.1 %), acetic acid solution (1.5 ml, 20 %), and aqueous solution of thiobarbituric acid (TBA) (1.5 ml, 0.8 %). The mixture was heated (95°C, 60 minutes) and cooled to room temperature. An equal volume of 1-butanal was added and the mixed vigorously. Fluorescence intensity of the organic layer (upper layer) was measured at excitation and emission wavelengths of 515 and 553 nm, respectively. A standard curve was

prepared using tetraethoxypropane, which would yield an equimolar amount of MDA.

Data analysis

Data were subjected to two-factor Analysis of Variance (ANOVA) using the General Linear Model procedure of Minitab® statistical software (13). Statements of significance was based on $p<0.05$. Tukey's Honesty significant difference test was employed for comparison of significantly different treatment means. The model for statistical analysis is as shown below.

$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$

Where:

Y_{ijk} = Traits of interest

μ = Overall mean

A_i = Influence of i^{th} dietary supplementation of vitamin, (i = control, ascorbic acid, alpha tocopherol and combination)

B_j = Effect of j^{th} Sex (j = male, female)

AB_{ij} = Effect due to interaction between vitamin supplements and sex

e_{ijk} = Random error

Result and Discussion

The effect of dietary inclusion of VC, VE and their combination on serum antioxidant enzymes and malondialdehyde concentration (MDA) is presented in Tables 1-3. MDA is an important indicator of oxidative stress as it is the end product of lipid peroxidation, lowest MDA ($P<0.05$) recorded in VC+VE (2.75nmol/ml) compared to control (5.93nmol/ml), VC (4.52nmol/ml) and VE (4.01nmol/ml) suggests a more efficacious role in combating/alleviating the impacts of heat stress at the cellular level if combined compared to individual usage. These buttresses the outcome of authors like Attia *et al*. (14), Surai *et al*. (15) and Ijadunola *et al*. (16) that reported in earlier studies the synergistic effects of combining VC and VE in alleviating thermal stress challenges in chicken. Serum antioxidant enzymes; superoxide dismutase (SOD) and glutathione peroxidase (GPx) which are known to help balance the production of free radicals and reduce the rate of lipid peroxidation through their scavenging activity (17) were elevated in VC+VE (110.06U/ml, 151U/ml) respectively.

Table 1: Effect of supplemental dietary antioxidants on serum malondialdehyde and antioxidant enzymes concentration in improved Fulani ecotype chickens

Parameter	Control	Vit. C	Vit. E	Vit. C+E	SEM	p-value
Malondialdehyde (nmol/ml)	5.93^{a}	4.52^{b}	4.01^{b}	2.75^{c}	0.239	0.001
Superoxide dismutase (U/ml)	69.09^{c}	97.32^{b}	102.31^{ab}	110.06^{a}	2.260	0.001
Catalase (U/ml)	38.92^{b}	50.87^{a}	54.94^{a}	52.80^{a}	1.190	0.001
GPx (U/ml)	121.89^{c}	141.85^{b}	143.64^{b}	151.70^{a}	2.040	0.001

^{abc} Means within same row bearing different superscripts differ significantly ($p<0.05$) Vit. C- Vitamin C, Vit. E-Vitamin E, Vit. C + E – Vitamin C + E, GPx - Glutathione peroxidase, SEM - Standard Error of Mean

Table 2: Effect of sex on serum antioxidant enzymes and malondialdehyde concentration in improved Fulani ecotype chickens

Parameter	Hen	Cock	SEM	p-value
Malondialdehyde (nmol/ml)	4.25	4.36	0.169	0.669
Superoxide dismutase (U/ml)	94.68	94.71	1.600	0.989
Catalase (U/ml)	49.04	49.73	0.840	0.566
GPx (U/ml)	140.35	139.19	1.450	0.574

Vit. C- Vitamin C, Vit. E-Vitamin E, Vit. C + E – Vitamin C + E, GPx - Glutathione peroxidase, SEM - Standard Error of Mean

Table 3: Interactive effect of sex and supplemental dietary antioxidants on serum malondialdehyde and antioxidant enzymes concentration in improved Fulani ecotype chickens

Parameters	Control		Vit. C		Vit. E		Vit. C + E			
	Hen	Cock	Hen	Cock	Hen	Cock	Hen	Cock	SEM	p-value
MDA (nmol/ml)	6.07	5.79	4.48	4.57	4.17	3.85	2.70	2.80	0.338	0.875
SOD (U/ml)	68.19	70.00	95.02	99.61	103.52	101.09	112.10	108.01	3.200	0.518
Catalase (U/ml)	39.42	38.41	49.58	52.16	54.42	51.18	55.48	54.41	1.680	0.386

GPx (U/ml)	119.08	124.71	143.12	140.58	145.94	141.34	148.63	154.77	2.890	0.151

Vit. C – Vitamin C; Vit. E - Vitamin E; Vit. C+E - Vitamin C + E; ERP - Early reproductive phase; GPx – Glutatinine peroxidase, MDA – Malondialdehyde, SOD – Superoxide dismutase SEM - Standard Error of Mean

Also, compared to control (69.09U/ml, 121.89U/ml), supplemental VC and VE increased ($p<0.05$) serum SOD (97.32U/ml, 102.31U/ml) and GPx (141.85U/ml, 143.64U/ml) activity respectively. Similarly, increased CAT activity ($p<0.05$) were recorded in VC (50.87U/ml), VE (54.94U/ml) and VC+E (52.80U/ml) compared to control (38.92U/ml). This result clearly indicates that mixing vitamin C and E in the diet of improved Fulani ecotype chickens served the purpose of ameliorating the effect heat stress by acting synergistically, lowering MDA and increasing antioxidant enzymes (SOD, CAT and GPx) in the combination group thereby working against reactive oxygen species and reducing lipid peroxidation. This corroborates the studies of Jena *et al*. (7) and Hashem *et al*. (18) that reported elevated serum SOD, GPx and Catalase with lowered MDA in chicken fed diet supplemented with ascorbic acid and alpha tocopherol mixtures. This results also further validates the report of Attia *et al*. (14) who had recorded positive impacts of ascorbic acid and alpha tocopherol acetate used in combination to combat the negative effects of lipid peroxidation which resulted from increased reactive oxygen species when chicken were exposed to adverse situations such as thermal stress. Table 2 and Table 3 were not significantly influenced ($p>0.05$).

Conclusion and Application

This study concluded that a combination of VC and VE in the diet of IFEC resulted in better serum antioxidant enzymes levels and malondialdehyde concentration (MDA).

This study recommends the usage of a combination of VC (200mg/kg) and VE (125mg/kg) for combating and alleviating the negative effects of thermal stress in IFEC.

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