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Ameliorative effects of dietary vitamin C and E supplementation on growth performance, hemato-biochemical, antioxidant and non-specific immune parameters in heat-stressed rabbits

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ABSTRACT

This study was conducted to investigate the ameliorative effect of vitamin C and E dietary supplementation on growth performance, some hemato-biochemical parameters, antioxidant status and immune response in rabbits under the hot climatic condition in Egypt. Sixty weaned New Zealand White (NZW) rabbits were randomly assigned into four equal groups as follows; the control group was fed on the basal diet without any supplement, the other experimental groups were fed on basal diets supplemented either with 250 mg/kg of vitamin C or E and their combination for eight consecutive weeks. At the end of the experimental period feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were calculated and blood samples were collected. The results revealed that vitamins C and E supplementation significantly improved FI, FBW, and BWG, otherwise, FCR was significantly reduced. Dietary supplementation either with vitamin C or E significantly decreased the RBCs count, Hb concentration, PCV%, total leukocytic count, neutrophils and neutrophils/lymphocytes ratio compared to the control. A significant reduction in the serum total protein, albumin, cortisol, GSH and SOD was recorded in all supplemented groups. Meanwhile, dietary inclusion of vitamin C or E significantly enhanced serum glucose, T3 and NO levels as well as lysozyme and bactericidal activities compared to the control. These results indicate that dietary supplementation with antioxidant nutrients as vitamin C and E separately or in combination with rabbits was efficacious in alleviating heat stress negative effects on different examined parameters in rabbits.

Keywords: Heat stress; Rabbits; Vitamin C and E; Endocrine changes; Antioxidant enzymes; Immune response.

INTRODUCTION

In the last few decades, rabbit production has been gaining great attention mainly as a source of meat, since they have high reproduction rate, small body size, rapid growth rate and high meat yields (Basavaraj et al., 2011). Under hot climatic conditions like in Egypt (from May to September) rabbits are exposed to severe heat stress in comparison to the comfort zone temperature in rabbits of around 21°C. Rabbits have non-functional sweat glands so they are highly sensitive to elevated environmental temperatures and unable to eliminate the excess of body heat. Heat stress has negative effects on productive and reproductive performance as feed intake, feed efficiency (Attia et al., 2011), and numerous hematological, biochemical, hormonal parameters are tended to be altered in response to heat stress (Ondruska et al., 2011). Also, heat stress induces oxidative damage to the cell membrane resulted in the impairment of cellular functions ((Sujatha et al., 2010). Therefore, recent studies have focused on the use of different methods that may be physical or nutritional to modify environmental conditions and combat heat load in heat-stressed animals (Daader et al., 2018).

Diet supplementation with antioxidant nutrients as vitamin C and E had a beneficial effect in averting adverse effects of heat stress. Ascorbic acid as a water-soluble antioxidant might have the ability to reduce lipid oxidation and protect cell membranes from oxidative damage (Sujatha et al., 2010). Moreover, Vitamin E is one of the cell membrane components that prevent oxidative damage. Otherwise, its antioxidant properties, it has a significant role in maintaining the immune system and reproductive function of animals (Hashem

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et al., 2013). Both vitamins act as scavengers for ROS, maintaining cell membrane integrity and prevent oxidative damage, they subsequently alleviate heat stress and maintain homeostasis (Sahin et al., 2009). Furthermore, they enhance immunity and antioxidant status under summer conditions (Jang et al., 2014). Recently, the adverse effects of heat stress conditions and their amelioration using nutritional antioxidants, mainly for growth parameters and metabolic profiles have been extensively discussed. However, there is not enough information regarding the effects of nutritional antioxidants (Vit. C and E) on antioxidant and immunity status to homeostasis in heat-stressed rabbits. Therefore, this study was planned to investigate the ameliorative effect of vitamin C and E separately or their combination on growth performance, some selected hematological and biochemical parameters as well as antioxidant status and immune response in New Zealand White rabbits reared under Egyptian summer condition.

MATERIALS AND METHODS

Animals and Experimental Design

The present study was conducted in the Rabbit Research Unit, Faculty of Agriculture, Mansoura University, Egypt. Sixty growing male New Zealand White (NZW) rabbits at 6 weeks of age with an average initial body weight of 697.4±1.28 g were randomly assigned into 4 equal groups each with three replications, 5 rabbits/ replicate. The rabbits were kept in an open-air building with exhausting electric fans on both sides. Rabbits were housed in battery cages, provided by a feeder and a stainless-steel nipple for drinking with free access to freshwater and pelleted diets. The study was carried out between June 3 and August 2, 2017. Ambient temperature and relative humidity were measured twice daily in the rabbit unit at 6 a.m. and 3 p.m. The maximum and minimum ambient temperature and relative humidity during the whole experimental period ranged between 28 to 33°C and 75 to 79%, respectively. Averages of the ambient temperature, relative humidity percentage and temperature-humidity index (THI) inside the rabbit unit were 30.5±2.5°C, 77±4% and 29.4, respectively. The value of THI indicates that rabbits were subjected to severe heat stress as it is higher than 28.9 according to Marai et al. (2001).

The control group fed on a basal diet (NRC, 1977) without any supplement (Cont). The 2nd and 3rd experimental groups were fed on diets supplemented with 250 mg/kg diet of vitamin C (Vit.C) and 250 mg/kg diet of vitamin E (Vit.E), respectively and the last one supplemented with 250 mg/kg diet of vitamin C plus 250 mg/kg diet of vitamin E (Vit.C &E). The supplemented levels of vitamin C and E were based on the studies conducted by Alba et al. (2015) and Sahin et al. (2001) respectively. All experimental procedures followed the guidelines of the Ethical Committee of Faculty of Veterinary Medicine, Mansoura University.

Growth Performance

Body weights were recorded for all rabbits at the beginning and termination of the study. Rabbits were checked daily for any mortalities and FI was recorded. The BWG and FCR were calculated.

Sample Collection

At the end of the study, 5 rabbits from each treatment were randomly picked up and blood samples were collected from ear veins. The sample was collected either with tri-potassium salt of EDTA for hematological examination or without anticoagulant for serum separation to be used in the estimation of some biochemical, antioxidant and immunological parameters.

Hematological Examination

Red blood cell counts (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV%), RBC indices, total and differential leukocytic count were determined according to Feldman et al. (2000).

Serum Biochemical Analysis

Prepared frozen serum samples were analyzed spectrophotometrically according to the enclosed pamphlets for the colorimetric estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Randox, UK). Glucose, cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol **(**HDL-C) levels were determined by kits obtained from Spinract, Spain. Total protein and albumin were detected using Stanbio (USA) kits.

Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), total antioxidant capacity (TAC), malondialdehyde (MDA) and nitric oxide (NO) were estimated in serum sample (Biodiagnostic, Egypt). Serum level of cortisol, triiodothyronine (T3) and thyroxine (T4) were determined by using an IMMULITE 1000, and IMMULITE 2000 Immunoassay System, respectively (Siemens Health diagnostic– the USA) according to the manufacturer's instructions.

Serum Immunological Parameters

Table 1: Composition and chemical analysis of the experimental diets.

Ingredients %	Amount
Soybean meal, 44%	**11.3**
Yellow corn	**3.4**
Wheat bran	**30.0**
Barley grain	**20.0**
Clover hay	**30.0**
Molasses	**1.5**
Di-calcium phosphate	**1.3**
Limestone	**1.6**
Vit.+Min. Mix.(1)	**0.3**
Sodium chloride	**0.5**
Dl- Methionine	**0.1**
Total	**100**
Calculated analysis :	
Digestible energy, kcal/kg	**2618.3**
Dry matter, %	**89.55**
Organic matter, %	**83.21**
Crude protein, %	**17.21**
Crude fiber, %	**13.52**
Ether extract, %	**3.19**
Nitrogen free extract,%	**49.29**
Ash, %	**16.79**

Each 3 Kg vitamin and mineral mixture contained 12000000 IU Vit. A, 2500000 IU Vit. D_3, 10000 mg Vit. E, 2500 mg Vit K_3, 1000 mg Vit B_1, 4000 mg Vit. B_2, 1500 mg Vit. B_6, 10 mg Vit. B_{12}, 10000 mg Pantothenic acid, 20000 mg Nicotinic acid, 1000 g Folic acid, 50 mg Biotin, 500 g Choline chloride, 60 g Manganese, 55 g Zinc, 100 mg Selenium, 1000 mg Iodine, 35 g Iron, 10 g Copper, 250 mg Cobalt, and Carrier $CaCo_3$ to 3 kg.

Table 2: Effect of Vit.C and Vit.E dietary supplementation on growth performance in rabbits under summer condition.

	Treatment			
Parameter	**Cont**	**Vit.C**	**Vit.E**	**Vit.C&E**
IBW (g)	696.2 ±1.24^a	698.2 ±1.01^a	697 ±0.95^a	698.2 ±1.93^a
FBW (g)	2013.8 ±1.69^d	2140 ± 2.51^b	2121± 2.63^c	2165 ± 1.92^a
BWG (g)	1317.6 ±.1.21^d	1441.8 ± 2.07^b	1424 ± 3.02^c	1466.8 ±2.24^a
FI (g)	4307 ±4.46^c	4342.4 ± 2.5^b	4434 ±2.28^a	4425.4 ±2.73^a
FCR (g/g)	3.27 ±0.004^a	3.01 ±0.004^c	3.11 ±0.01^b	3.02 ±0.002^c

Cont (fed on basal diet without any supplement), Vit.C (vitamin C), Vit.E (vitamin E),Vit.C&E (vitamin C and E)
Data are expressed as Mean ± SEM. The different letters in the same row indicate significant difference ($P < 0.05$) among groups.
IBW: Initial body weight; *FBW*: Final body weight; BWG: Body weight gain; *FI*: Feed intake; FCR: Feed conversion rate

Lysozyme Activity

According to the procedures of Parry et al. (1965), serum lysozyme was measured by the turbidimetric assay. The lysozyme substrate (0.75 mg of a gram-positive bacterium *Micrococcus Lysodeikticus* Lyophilized Cells) (Sigma-Aldrich) was dissolved in 1 ml of PBS, pH 5.8. A 25 µl of serum was blended with 175 µl of substrate solution in a round bottom microtiter plate at 25oC. The reduction in absorbance was read after 0 and 20 min at 450 nm using a microtiter plate ELISA reader. The lysozyme in serum was detected from a lysozyme standard curve prepared by lyophilized hen egg-white lysozyme and expressed as µg /ml (Sigma-Aldrich).

Table 3: Effect of Vit.C and Vit.E dietary supplementation on hematological parameters in rabbits under summer condition.

	Treatment			
Parameter	**Cont**	**Vit.C**	**Vit.E**	**Vit.C &E**
RBCs (10^6/µL)	7.58 ± 0 .27 [a]	6.57 ± 0.13 [b]	6.34 ± 0.21 [b]	6.79 ± 0.15 [b]
Hb (g/dl)	13.58 ± 0.32[a]	11.73 ± 0.39 [b]	11.34 ± 0.47[b]	11.54 ± 0.27 [b]
PCV (%)	40.4 ± 0.93 [a]	37.6 ± 0.51 [b]	36.80 ± 0.66[b]	37.40±0.81 [b]
MCV (fl)	55.27 ±02.97[a]	57.28 ±1.21 [a]	56.75 ±2.37[a]	55.13 ± 0.97[a]
MCH (pg)	18.09 ±0.68[a]	18.67 ±0.88[a]	18.12 ±1.03 [a]	17.01 ±0.33 [a]
MCHC (%)	32.96±1.5[a]	32.62 ±1.53[a]	31.89± 0.97[a]	30.9 ±0.87[a]
TLC (10^3/µL)	14.57 ±0.47 [a]	11.53±0.39 [b]	10.34±0.76 [bc]	9.44 ±0.55[cd]
Neutrophil (10^3/µL)	6.44 ±0.3 [a]	4.20 ±0.57 [b]	3.40 ±0.22 [c]	3.20 ±0.12 [c]
Lymphocyte (10^3/µL)	7.90 ±0.30 [a]	7.06 ±0.33 [a]	6.71 ±0.65 [a]	6.73 ±0.26 [a]
Monocyte (10^3/µL)	0.35±0.06 [a]	0.28 ±0.05 [a]	0.23 ±0.07 [a]	0.25 ±0.04[a]
N/L ratio	0.82±0.05 [a]	0.60±0.05 [b]	0.52±0.06 [b]	0.48±0.04 [b]

Cont (fed on basal diet without any supplement), Vit.C (vitamin C), Vit.E (vitamin E),Vit.C&E (vitamin C and E)
Data are expressed as Mean ± SEM (n=5). The different letters in the same row indicate significant difference ($P < 0.05$) among groups.
RBCs: Red blood cell counts; *Hb*: hemoglobin concentration; *PCV %*: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; TLC: Total leukocytic count; *N/L ratio*: Neutrophil / Lymphocyte ratio

Table 4: Effect of Vit.C and Vit.E dietary supplementation on some selective biochemical parameters in rabbits under summer condition.

	Treatment			
Parameter	**Cont**	**Vit.c**	**Vit.E**	**Vit.C&E**
ALT (U/L)	19.29±1.45[a]	20.79 ±1.21[a]	20.34 ±1.63[a]	20.85±1.63[a]
AST (U/L)	25.60 ±0.44[a]	24.7±1.08[a]	24.53±0.45[a]	23.75±0.70[a]
Glucose (mg/dl)	79±0.4.69[b]	127± 06.9[a]	119±5.27[a]	121±1.83[a]
Cholesterol (mg/dl)	88.35±1.68[ab]	90.37±2.11[a]	83.42±3[bc]	80.81±1.67[c]
Triglycerides (mg/d)l	60.64±0.38[a]	69.77±1.07[a]	70.65±1.56[a]	68.54±0.34[a]
HDL-C (mg/dl)	29.24±1.43[a]	31.65±1.25[a]	29.31±1.34[a]	29.62±0.86[a]
Total Protein (g/dl)	6.25±0.04 [a]	5.96±0.16[b]	5.90±0.09[b]	5.7±0.03[b]
Albumin (g/dl)	3.29±0.08[a]	3.03±0.11[b]	2.85±0.08 [b]	2.95±0.04[b]
Globulin (g/dl)	2.96±0.05[ab]	2.93±0.13[ab]	3.04±0.05[a]	2.79±0.09[b]
A/G ratio	1.11±0.05[a]	1.04±0.07[ab]	0.93±0.03[b]	1.06±0.03[ab]

Cont (fed on basal diet without any supplement), Vit.C (vitamin C), Vit.E (vitamin E),Vit.C&E (vitamin C and E)
Data are expressed as Mean ± SEM (n=5). The different letters in the same row indicate significant difference ($P < 0.05$) among groups.
ALT, Alanine aminotransferase; *AST*, aspartate aminotransferase; *HDL-C*, high density lipoprotein-cholesterol; *A/G ratio*, Albumin to Globulin ratio

Bactericidal Activity

Bactericidal activity was performed as proposed by Welker et al. (2007), 200

microliters of serum or Hank's Balanced Salt Solution as control was added to duplicate wells of a 96 round bottom well microtiter plate and incubated for 2.5 h at 37 °C with 50 µL of 24 h live culture of *E.col (*1X108 CFU/ml*)*. Twenty-five µL diphenyltetrazolium bromide solution ((MTT; 2 mg/ml, Sigma) was added to each well, then incubated at room temperature for 30 min to permit formazan formation. After that, the supernatant was removed, and 200 µL of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. Then, with a microtitre plate ELISA reader, the absorbance was read at 560 nm and reported as absorbance units.

Statistical Analysis

Our results were expressed as mean ± standard error. Data were analyzed by ANOVA using SPSS software statistical program (SPSS For Windows (ver.20.00, USA). The groups have statistically differed if $P < 0.05$. Graphs were drawn using GraphPad Prism version 5 (GraphPad Software Inc. La Jolla, CA, USA)

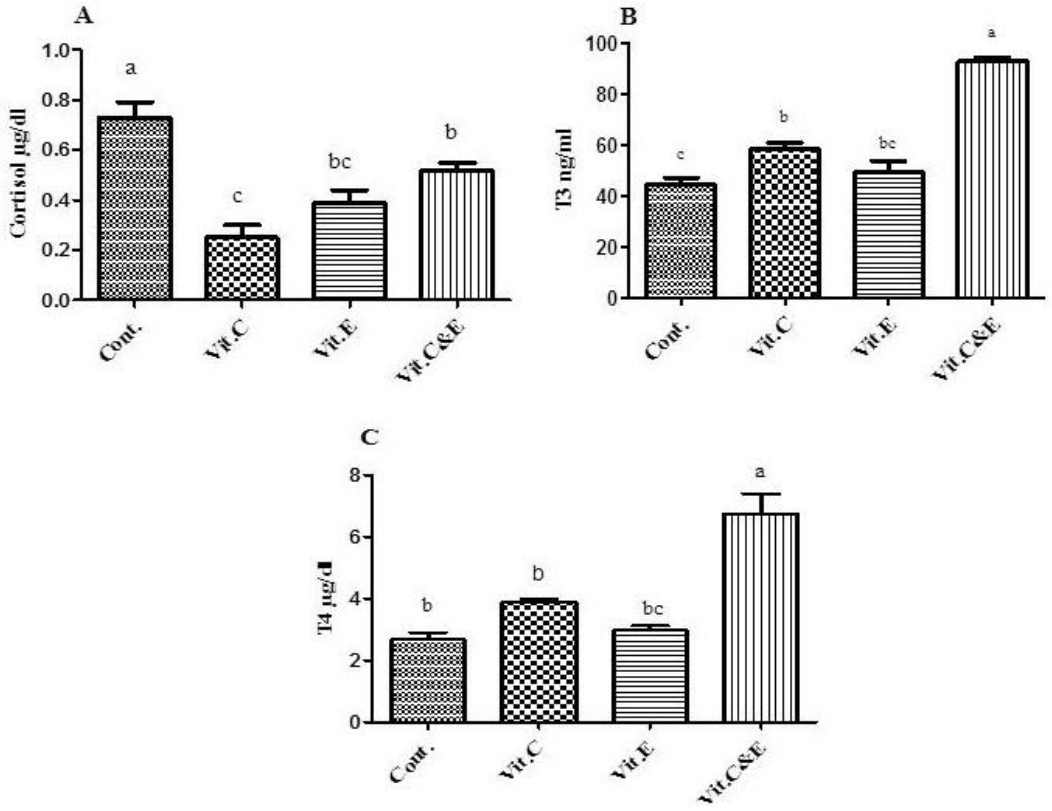

Fig 1: The effect of Vit.C and Vit.E dietary supplementation on serum levels of (Mean ± SEM) **A.** Cortisol, **B.** Triiodothyronine (T3) and **C.** Thyroxine (T4) in rabbits under summer condition. Means with different superscripts are significantly differed ($P < 0.05$).

RESULTS

Growth Performance Parameters

As shown in table 2, FBW and BWG were significantly increased in the Vit.C group followed by Vit.E and the highest improvement was observed in the combined supplemented group. Also, FI was significantly increased in all supplemented groups and the highest value was recorded in Vit.E and Vit.C &E group. In contrast, FCR was significantly reduced in all groups especially upon vitamin C supplementation

(Vit.C and Vit.C &E) compared to the control group.

Hematological Parameters

The vitamin supplementation either alone or in combination reduced significantly RBCs count, Hb concentration and PCV % compared to the control group. Also, the total leukocytic count was significantly reduced in all supplemented groups compared to the control one and the lowest count was recorded in the Vit.C & E group. The neutrophils count and neutrophils/lymphocyte (N/L) ratio were significantly attenuated in all investigated groups compared to the control. Furthermore, neutrophils were fell much lower in Vit.E and Vit.C & E than Vit.C treated group (Table 3).

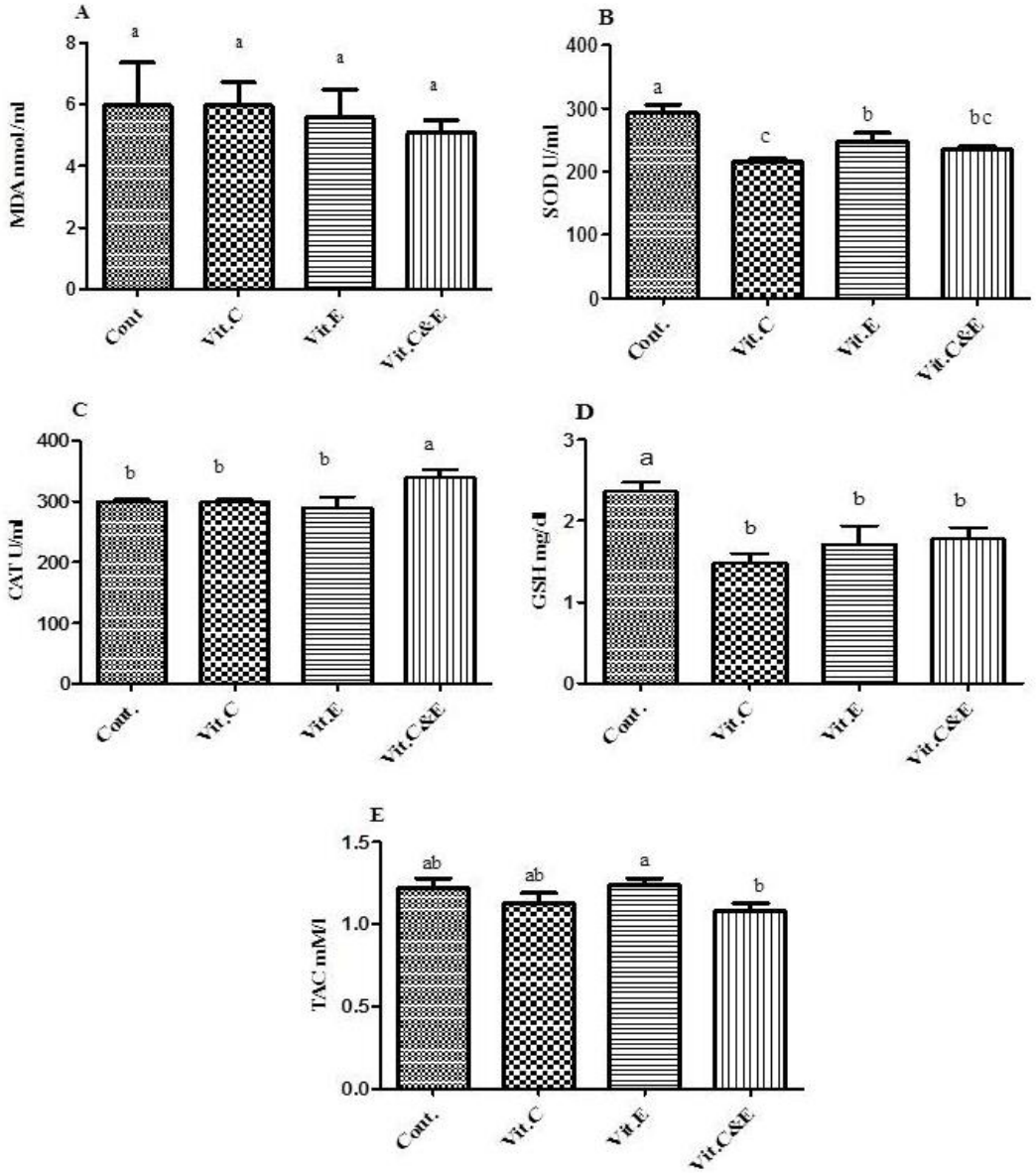

Fig 2: The effect of Vit.C and Vit.E dietary supplementation on serum (Mean ± SEM) **A.** Malondialdehyde (MDA) level, **B.** SOD activity, **C**. Catalase (CAT) activity, **D.** Reduced glutathione (GSH) level and **E**. Total antioxidant capacity (TAC) in rabbits under summer condition. Means with different superscripts are significantly differed ($P < 0.05$).

Biochemical Parameters and Endocrine Response

As presented in Table 4, no relevant changes were found in the serum activities of ALT and AST as well as serum levels of TG, HDL, and globulin in the different examined groups. Conversely, serum glucose level was significantly increased in all supplemented groups compared with the control. On the other hand, serum cholesterol level was significantly reduced only in the Vit.C &E treated group. Total protein and albumin levels were significantly reduced in all supplemented groups with respect to the control one.

Serum cortisol level was significantly reduced in all traits, where the lowest level was recorded in rabbits fed diets supplemented with vitamin C. Conversely, serum T3 level was significantly elevated upon dietary supplementation either with vitamin C or E and the more pronounced effect recorded in Vit.C &E group. Serum T4 level was elevated ($P < 0.05$) only in rabbits fed diets incorporated with both vitamin C& E mixture (Fig.1).

Oxidative Stress and Antioxidant Biomarkers

The influences of dietary vitamin C & E supplementation on oxidative stress and antioxidant biomarkers are graphically presented in Fig. 2. The serum MDA level insignificantly varied among the experimental groups. Meanwhile, rabbits fed diets supplemented with vitamin C or E showed a marked reduction in the serum SOD activity and GSH level compared with the control groups. No relevant changes were detected in CAT activity and TAC level in rabbits fed vitamin C or E supplemented diets, except for CAT, which was significantly increased in Vit. C &E

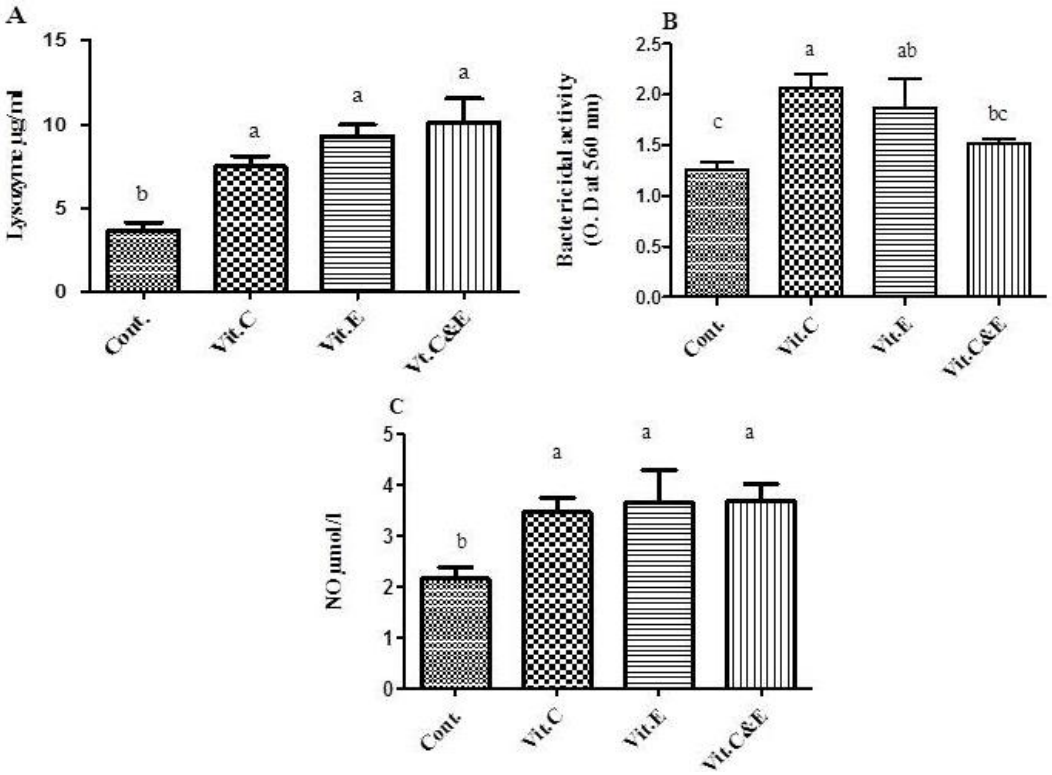

Fig 3: The effect of Vit.C and Vit.E dietary supplementation on some selected serum immunological parameters (Mean ± SEM) in rabbits under summer condition **A.** lysozyme activity **B.** Bactericidal activity and **C.** Nitric Oxide (NO) level. Means with different superscripts are significantly differed ($P < 0.05$).

group compared with the other experimental groups.

Immunological Parameters

As displayed in Fig. 3 Vitamin E and C supplementation improved the adverse effects of heat stress on the immune response as serum NO, lysozyme and bactericidal activities were significantly increased in all supplemented groups, except the bactericidal activity insignificantly changed in Vit. C &E group compared with the control one. Moreover, no significant difference was evident between the Vit.C and Vit.E supplemented groups.

DISCUSSION

Animal physiology, health, production, and reproduction are markedly affected by heat stress. The high ambient temperature in NZW rabbits negatively influences growth performance as it suppresses total and daily weight gains (Ondruska et al., 2011; El Saidy et al., 2016). This is possibly attributed to the excessive production of reactive oxygen species which oxidize and destroy cellular biological molecules (Liu et al., 2011). The results of the present study showed that both Vit.C and Vit.E improved FBW and BWG, especially in the co-treated group. Our results agree with a prior finding of Megbuwe et al. (2018) who recorded that dietary inclusion of different levels of vitamins E and C separately or in combination up to 200 mg /kg of diet in rabbit bucks improved the growth parameters by suppressing action of reactive oxygen species and the effects of oxidative and thermal stress. Furthermore, dietary supplementation with vitamin C can diminish heat load and improve the growth performance of heat stress either by increase feed intake or enhance the digestibility of nutrients or by reducing the synthesis of corticosteroid hormones consequently, inhibiting their negative effects (Lohakare et al., 2005). Moreover, vitamin C supplementation for rabbits under hot conditions significantly improves final body weight (Yassein et al., 2008). Similarly, Alba et al. (2015) recorded that dietary inclusion of vitamin C at 250 mg/kg of diet improved feed efficiency, final weight, and body weight gain in heat-stressed broiler chickens. In contrast to our findings, Jang et al. (2014) reported that dietary supplementation with vitamin C and E in birds under summer conditions have no effect on growth performance, feed intake and feed to gain ratio.

Hematological examination, especially N/L ratio recorded to be a sensitive indicator to detect the health status and effect of stressors in poultry and other livestock (Minka and Ayo, 2011). In our study dietary supplementation with vitamin C or E significantly reduced the elevated RBCs, Hb and PCV % which indicate hemoconcentration that referees to severe dehydration as reported in livestock during heat stress (Sejian et al., 2017). Under high ambient temperature, the release of adrenocorticotropic hormone occurs (Sujatha et al., 2010), resulting in increased total leukocytes, neutrophils count and N/L ratio. Since, corticosteroids stimulate the release of polymorphonuclear leukocytes from bone marrow, delayed apoptosis, and decrease the release of polymorphonuclear leukocytes into the tissue (Dyavolova et al., 2014). Our results revealed that total leukocytic and neutrophils count as well as N/L ratio was diminished in all supplemented groups compared to the control non-supplemented rabbits, which may be referred to reduced serum cortisol level with vitamin C or E supplementation (Sahin et al., 2009). The most improvement in total leukocytic and neutrophils count was recorded in Vit.E and combined treated groups.

In the present study, there was no marked difference in ALT, AST, TG, HDL and cholesterol levels in Vit.C and Vit.E supplemented groups compared with the control. Except for the cholesterol level, which was significantly decreased in the combined treated group (Vit.C &E)? Our

results are following El-Kholy et al. (2017) who observed that hot environmental conditions didn't alter AST and ALT activities in growing Japanese quails. Albumin, total protein, total cholesterol, VLDL cholesterol, and triglyceride, were not significantly influenced by heat exposure or by vitamin C treatment (Seven et al., 2009). In the same context, Sahin et al. (2001) recorded that serum ALT and AST activities were not altered by dietary vitamin E inclusion in broiler chicks reared under heat stress. In contrast to our findings, serum cholesterol and triglycerides level were decreased in vitamin E treated bucks (Hashem et al., 2013).

Our results showed that total protein and albumin levels were significantly reduced in all supplemented groups concerning the control group. Conversely, AL-Zafry and Medan, (2012) reported that vitamin E and selenium didn't affect the total protein, albumin, and globulin in heat-stressed rabbits.

Serum glucose level was significantly increased in all supplemented groups compared with heat stress non-treated group, as hypoglycemia was recorded in New Zealand White bucks under heat stress during summer months that indicate depletion of energy source to maintain body temperature (Attia et al., 2011). Also, hypoglycemia may be caused either by increased glucose utilization to produce more energy for greater respiratory muscular activity after thermal exposure or may be associated with the decrease in insulin and thyroxine, which are related to metabolic changes during heat stress (Sejian et al., 2017). Our findings supported by Hashem et al. (2013) who reported that bucks treated with vitamin E (150 mg/kg diet for ten weeks) had a higher level of plasma glucose indicating the availability of energy for different physiological and biochemical functions. Similar results were recorded in heat-stressed rabbits treated with vitamin E and selenium (AL-Zafry and Medan, 2012). Also, supplementation with vitamin C (100 g/tons of feed) in broilers for weeks significantly reduced serum cholesterol and glucose concentration (Sujatha et al., 2010).

In our study, dietary supplementation with vitamin C or E in rabbits under summer condition significantly reduced serum cortisol level, especially upon vitamin C treatment as vitamin C may reduce the synthesis of corticosteroid hormones (Alba et al., 2015). Similarly, Sahin et al. (2009) and Daader et al., (2018) recorded that, supplementation of these two vitamins may diminish the response to heat stress by reducing the serum corticosterone level in heat-stressed animals. In the current study, vitamin C or E significantly elevated serum T3 level and the more pronounced effect was recorded in a co-treated group. Meanwhile, the T4 level improved only in the co-treated group. This could be referred to as the antioxidant and immunomodulatory effects of bot vitamins (Sujatha et al., 2010; Daader et al., 2018). Similar results were observed by Daader et al. (2018) in growing rabbits reared under heat stress and dietary supplemented with vitamin C and E.

In the present investigation, serum MDA level, CAT and TAC were insignificantly affected by heat stress while SOD and GSH significantly increased, which may indicate that heat stress activates antioxidant enzymes compensatory to counteract increases ROS in response to heat stress (Bhusari et al., 2008). These results are in line with those of Seven et al. (2009) who declared that heat exposure in broiler chickens significantly elevates either plasma activities of SOD, CAT, and GSH. Our results are partially in agreement with previous studies by Yun et al. (2012) who recorded that cyclic heat stress for 7 days in rats insignificantly affects hepatic activities of SOD and GST, and the level of MDA, but significantly increase GPX activity, which was reduced with vitamin C treatment. Moreover, the inclusion of Vit.C to the diet increased GPX level (Alba et al., 2015) and significantly reduced elevated

plasma SOD activity upon heat exposure (Seven et al., 2009).

This study demonstrated that vitamin E and C supplementation significantly increased NO, lysozyme and bactericidal activities compared to the control non-treated group. Since heat stress negatively affects the immune response and the reduction in NO and lysozyme concentration under the effect of high temperatures may be correlated with the increase in corticosterone concentrations, consequently reduce the lysozyme gene transcription (Panarelli et al., 1994) and inhibit NO synthase (Gulati et al., 2006) resulted in the reduction of their concentrations. Similarly, Cui et al. (2109) found that serum lysozyme activity was significantly reduced in pig exposed to chronic heat stress. Therefore, improvement in immune response upon vitamin C and E supplementation may be referred to as their antioxidant properties and potential to reduce plasma corticosterone concentration and suppressed the mRNA expression of pro-inflammatory cytokines (Jang et al*.,* 2014; Alba et al., 2015)

CONCLUSION

A comprehensive understanding of the present study imposes the opinion that dietary supplementation of potential antioxidants as vitamin C and E could ameliorate the adverse effects of heat stress, improving growth performance, hemato-biochemical changes, antioxidant status and immune response in NZW rabbits under summer conditions. Our findings also favored the usage of their combination to combat the negative effects of heat stress, as it seems to be more effective in improving endocrine changes, antioxidant and immunity status.

Conflict of interest

The authors of this article have no conflict of interest to declare.

REFERENCES

1. Alba M, Esmaeilipour O , Mirmahmoudi R (2015). Effects of *Withania coagulans* fruit powder and vitamin C on growth performance and some blood components in heat stressed broiler chickens. Livestock Science,173: 64-68.
2. Al-Zafry SR, Medan MS (2012). Effects of vitamin E and selenium complex on heat-stressed rabbits. SCVMJ, 17: 129-138.
3. Attia YA, Al-Hanoun A, Bovera F (2011). Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months. Journal of Animal Physiology and Animal Nutrition (Berl), 95: 17-26.
4. Basavaraj M, Nagabhushana V, Prakash N, Appannavar MM, Wagmare, P, Mallikarjunappa S (2011). Effect of dietary supplementation of Curcuma Longa on the biochemical profile and meat characteristics of broiler rabbits under summer stress. Veterinary world, 4: 15-18.
5. Bhusari S, Hearne LB, Spiers DE, Appannavar, MM, Wagmare, P, ,Mallikarjunappa, S (2008). Transcriptional profiling of mouse liver in response to chronic heat stress. Journal of Thermal Biology, 33: 157-167.
6. Cui Y, Wang C, Hao Y*,* Gu X, Wang H (2019). Chronic heat stress induces acute phase responses and serum metabolome changes in finishing pigs. Animals, 9(7): 395.
7. Daader AH, Al Sagheer AA, Gabr HA, El Moniem EA (2018). Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants. Spanish Journal of agricultural research, 16(3): 12.
8. Dyavolova M, Gudev D, Yanchev I, Moneva P, Marinova P, Popova T

(2015). Effect of acute heat stress on some hematological parameters, trace elements and meat quality in rabbits. Proceedings of the International Symposium on Animal Science, Belgrade-Zemun.417-422.

9. El-Kholy MS, El-Hindawy MM, Alagawany M, El-Kholy MS, El-Hindawy MM, Alagawany M, El-Hack MEA, El-Sayed SAH (2017). Dietary supplementation of chromium can alleviate negative impacts of heat stress on performance, carcass yield, and some blood hematology and chemistry indices of growing Japanese quail. Biological trace element research, 179: 148-157.
10. El Saidy NR, Allam FE, Balabel TM, El-Midany SA (2016). Evaluation of using honey, cool water and levamisole against heat stress on different traits of rabbits under Egyptian summer conditions. World's Veterinary Journal , 6: 10-18.
11. Feldman BF, Zinkl JG , Jain VC, (2000). Schalm's Veterinary Hematology 5th ed. Lippincott Williams and Wilkins. Canada; PP: 1145-1146.
12. Hashem NM, El-Hady AA, Hassan O (2013). Effect of vitamin E or propolis supplementation on semen quality, oxidative status and hemato-biochemical changes of rabbit bucks during hot season. Livestock Science,157:520-526.
13. Jang IS, Ko YH, Moon YS, Sohn SH (2014). Effects of vitamin C or E on the pro-inflammatory cytokines, heat shock protein 70 and antioxidant status in broiler chicks under summer conditions. Asian-Australasian journal of Animal Sciences, 27: 749-756.
14. Gulati K, Ray A, Masood A, Vijayan, VK (2006). Involvement of Nitric oxide (NO) in the regulation of stress susceptibility and adaptation in rats. Indian Journal of Experimental Biology, 44: 809 -815.
15. Liu HW, Dong XF, Tong JM, Zhang Q (2011). A comparative study of growth performance and antioxidant status of rabbits when fed with or without chestnut tannins under high ambient temperature. Animal Feed Science and Technology, 164: 89-95.
16. Lohakare JD, Kim JK, Ryu MH, Hahn, TW, Chae BJ (2005). Effects of vitamin C and vitamin D interaction on the performance, immunity, and bone characteristics of commercial broilers. Journal of Applied Poultry Research, 14:670–678.
17. Marai IFM, Ayyat MS, El-Monem UA (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. Tropical Animal Health and Production, 33:451-462.
18. Megbuwe OJ, Addass PA , Ahemen T (2018). Growth performance of rabbit bucks fed graded levels of vitamins C and E in the guinea savannah region of Nigeria. International Journal of Agriculture and Earth Science (IJAES) 4:2489-0081.
19. Minka NS, Ayo JO (2011). Modulating role of vitamins C and E against transport induce stress in pullets during the hot-dry conditions. ISRN Veterinary Science, Article ID 497138, 7 pages.
20. NRC (National Research Council), 1977. Nutrient Requirements of Rabbits. 2nd rev. eds. The National Academies Press. Washington, DC https://doi.org/10.17226/35.
21. Ondruska L, Rafay J, Okab AB, Ayoub MA, Al-Haidary AA, Samara EM, Parkanyi V, Chrastinova L, Jurcik R, Massanyi P, Lukas, W, Supuka P, (2011). Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits. Veterinarni Medicina, 56, 180-186.
22. Panarelli M, Holloway CD, Mulatero P, Fraser R, Kenyon CJ (1994). Inhibition of lysozyme synthesis by dexamethasone in human mononuclear leucocytes: an index of glucocorticoid sensitivity. The

Journal of Clinical Endocrinology & Metabolism, 78: 872-877.
23. Parry RM, Chandau RC, Shahani RM (1965). A rapid and sensitive assay of muramidase. Proceedings of the Society for Experimental Biology and Medicine, 119:384-386.
24. Sahin N, Sahin K , Kucuk O (2001). Effects of vitamin E and vitamin A supplementation on performance, thyroid status, and serum concentrations of some metabolites and minerals in broilers reared under heat stress (32ºC). Veterinarni Medicina Czech 46:286-292.
25. Sahin N, Tuzcu M, Orhan C, Onderci M, Eroksuz Y, Sahin K (2009). The effects of vitamin C and E supplementation on heat shock protein 70 response of ovary and brain in heat-stressed quail. British Poultry Science, 50: 259-265.
26. Sejian V, Kumar D, Gaughan JB, Naqvi SM (2017). Effect of multiple environmental stressors on the adaptive capability of Malpura rams based on physiological responses in a semi-arid tropical environment. Journal of Veterinary Behavior, 17: 6-13 .
27. Seven PT, Yılmaz S, Seven I, Cercı IH, Azman MA, Yılmaz M (2009). Effects of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. Acta Veterinaria Brno, 78:75-83.
28. Sujatha V, Korde JP, Rastogi SK, Maini S, Ravikanth K, Rekhe DS (2010). Amelioration of heat stress induced disturbances of the antioxidant defense system in broilers. Journal of Veterinary Medicine and Animal Health, 2: 18-28.
29. Welker TL, Lim C, Yildirim-Aksoy M, Klesius PH (2007). Growth, immune function, and disease and stress resistance of juvenile Nile tilapia (*Oreochromis niloticus*) fed graded levels of bovine lactoferrin. Aquaculture, 262:156-162.
30. Yassein SA, Mahmoud KGM, Maghraby N, Ezzo OH (2008). Hot climate effects and their amelioration on some productive and reproductive traits in rabbit does. World Rabbit Science, 16:173-181.
31. Yun SH, Moon YS, Sohn SH, Jang IS (2012).Effects of cyclic heat stress or vitamin C supplementation during cyclic heat stress on HSP70, inflammatory cytokines, and the antioxidant defense system in Sprague Dawley rats. Exp Anim., 61: 543-553.