EFFECTS OF DIETARY VITAMIN E SUPPLEMENTATION ON HAEMATOLOGICAL PARAMETERS, SERUM VITAMIN E AND LIPID OXIDATION OF MAHALI GOATS IN LIBYA

Z. ZAYED [1]; N. ESTUTY [2] AND S. ABDELATTI [3]

[1] Department of Physiology, Faculty of Veterinary Medicine, Azzaytuna University.
[2] Libyan Authority For Scientific Research, Libyan Olive Tree Research Centre.
[3] Department of Basic Science, Faculty of Veterinary Medicine, Sudan
University of Science and Technology

ABSTRACT

This study aimed to investigate the impact of dietary vitamin E (DL-α-tocopherol acetate) on growth performance, amount of malondialdehyde in serum, serum vitamin E level, haematological and biochemical metabolites of Mahali goat kids. Twenty four male kids weighing 10-15 kg were randomly allocated to one of four groups to receive four different treatments of vitamin E. Group one (control, C), 2nd group received 300IU vitamin E (300 E), 3rd group received 500IU vitamin E (500 E), and 1000IU vitamin E (1000 E) in the 4th group. The animals were housed in separated pens and fed concentrate with a barley hay diet. Blood samples were weekly collected through the jugular vein for haematological and biochemical analysis. The evaluation of malondialdehyde and vitamin E content in serum was performed. The result revealed that vitamin E significantly increased red blood cells (RBC), Haemoglobin (HB), white blood cells (WBC), and differential white blood cells. Vitamin E significantly ($p<0.001$) increased level of vitamin E in serum, while, reduced total protein (TP), albumin, alanine transaminase (ALT), aspartate transaminase (AST), high density lipoproteins (HDL), cholesterol, and malondialdehyde (MDA) in the serum. In conclusion, dietary supplementation of vitamin E may effectively improved haematological, biochemical parameters, serum α-tocopherol content as well as malondialdehyde of Mahali goat kids.

Keywords: Vitamin E, oxidative stability, goats, Haematological.

INTRODUCTION

Vitamin E is an essential dietary nutrient that played a crucial factor in the health and development of animals (Castellini *et al.,* 2007).

Corresponding author: N. Estuty
E-mail address: nestuty@gmail.com
Present address: Libyan Authority For Scientific Research, Libyan Olive Tree Research Centre.

It can act as an antioxidant and prevents oxidation of polyunsaturated fatty acids (Traber and Stevens, 2011). The beneficial effects of α-tocopherol on health of the body were often attributed to reducing oxidative damage (Aitken *et al.,* 2009). The inclusion of vitamin E in the diet increased HB and total leukocyte count in the blood of broiler chickens (Shivappa *et al.,* 2013). One sign of oxidative problems was lipid peroxidation of RBC cell membranes which reduced life spans of RBC and caused anemia (Ansari *et al.,*

2015). Nutrition, heat, age, gender, race, diseases, muscle activity, season, and stress have all been shown to influence blood parameters in farm animals (Khan *et al.,* 2009). Low levels of vitamin E and increased mortality rate have been reported in the newborn kids despite pregnant goats had an acceptable levels of vitamin E (Smolders *et al.,* 2012).

Controversial results were reported on the effect of vitamin E on haematological and biochemical parameters. Yusuf *et al.* (2019), reported that dietary supplementation of 200 mg α-tocopherol significantly enhanced immune response, health status, reduced level of albumin, cholesterol, ALT, and thiobarbituric acid (TBARS) in goat kids. Whereas, another research showed that supplementation of vitamin E had non-significant effects on ALT, AST level, and macrophage, with maintain of WBC, RBC count, HB, reduced TP, and albumin in heat-stressed broiler chickens (Gharieb and Moursi., 2013). Moreover, (Adebiyi *et al.,* 2014) reported that vitamin E reduced TP, albumin, LDL, cholesterol, and malondialdehyde (MDA) after supplementation of vitamin E. Although there are many studies on the effects of vitamin E on lambs, calves, and chicken, there are very few experiments have been undertaken to study the effects of vitamin E on goats and kids (Ramadan *et al.,* 2018). However, serum concentrations of metabolites such as cholesterol, TP, albumin are commonly used to assess the nutritional status of cattle (Xuan *et al.,* 2018). The present study aimed to investigate the impact of vitamin E on hematological, biochemical, serum vitamin E, and serum malondialdehyde content of Mahali goat kids.

MATERIALS AND METHOD

Animals
The experiment was carried out on 24 male kids (Mahali breed) 3-4 months of age,

average life weight 10-15 kg, purchased from the local market. The study was conducted on a private farm in Tarhuna city about 80 km south of Tripoli. The animals were rested, ear tagged, and then divided randomly into four groups according to the treatment of vitamin E. Each animal was allocated in an individual well ventilated shaded separate pen, and allowed visual contact with each other with free access to water. All diets with hay to concentrate ration formulated to meet all nutrient requirements for goat kids (NRC, 2007). The concentrate ration consists of 48% crushed maize grain, 26% soybean meal, 24% wheat bran, 0.5% mineral mixture, 1.4% limestone, and 0.1% vitamins and minerals, the ration contain 46 IU/kg vitamin E.

Adaptation period
Kids had an adaptation period for two weeks before the study. They were fed a basal diet of a 75:25 mixture of hay and concentrate.

Treatments
The kids have received particular levels of α-tocopherol with concentrate and hay for each group as follows: control group (C), 2nd group (300 E) received 300 IU vitamin E/ day, 3rd group (500 E) received 500 IU vitamin E/ day, and 4th group (1000 E) had 1000 IU vitamin E/ day, the experiment lasted for 2 months.

Haematological analysis
Blood samples were weekly collected, about 10 ml of blood was drawn from the jugular vein of each kid, 4ml of each sample was released into vacuum tubes for evaluation of complete blood count (CBC) using Mindray hematology analyzer BC 2800aa (©2013 Shenzhen Mindray Bio-Mindray Electronics Co., Ltd,).

Biochemical analysis
Serum was recovered by centrifugation (10 m at 3000 rpm) for evaluation of total proteins, albumin, LDL, HDL, cholesterol, ALT, and AST using Mindray hematology analyzer BC 2800aa. The part of the serum samples stored at -20 C for evaluation of

MDA and α-tocopherol content in the serum. Both were measured by high-performance liquid chromatography, which was performed in the Animal Health Research Center in the Arab Republic of Egypt, following the method of Hoehler *et al.* (1998) for vitamin E content in the serum. Whereas, method of Buege and Aust, (1978) for evaluation of MDA.

Statistical analysis

The data obtained from the study were subjected to one-way analysis of variance ANOVA, Minitabe (release No.; South College, Pa.). Grouping information using Tukey method was used for post-hoc analysis at 95.0% confidence level. Two factors with repeated measures on one factor (weeks). Correlation and regression analysis was used to establish the relationships between dietary concentrations of α-tocopherol acetate and blood parameters. Comparison made using ANOVA to establish effects of α-tocopherol on blood parameters.

RESULTS

Blood analysis

The effects of the three levels of vitamin E on blood parameters are presented in Table (1). Vitamin E reduced the number of RBC in the three experimental groups in the first three weeks of the trial. Afterward, there was a significant (p<0.05) elevation in the number of RBC through the rest of the weeks of the trial in the three groups compared with the control. Regarding the impact of α-tocopherol on HB, it caused a decline of HB level in the three α-tocopherol supplemented groups at the first three weeks of the trial. Subsequently, the level of HB had significantly (p<0.05) increased again in the vitamin E supplemented groups to the end of the trial. In addition, vitamin E significantly (p<0.001) reduced the percentage of PCV in the treated groups. However, there was a significant rise

(p<0.05) in the number of WBC in 300 E and 500 E groups compared with the control. Whereas, α-tocopherol at level 1000 E had no significant (p>0.05) effect on WBC. Vitamin E had a highly significant (P<0.001) elevation in the number of monocytes and eosinophils, while a non-significant increase in the number of lymphocytes. Vitamin E supplementation had a significant (p<0.05) increase in number of basophils. Whereas, vitamin E caused a significant (p<0.05) decrease in number of neutrophils in 500 E and 1000 E groups. While, a non significant decrease in number of neutrophils in 300 E group compared with the control.

Blood biochemicstry

Data in Table (2) revealed that α-tocopherol supplementation caused significant (P<0.001) decrease in the concentration of TP in the treated groups compared with the control. Vitamin E significantly (p<0.001) reduced level of albumin in the vitamin E supplemented groups compared with the control. There was a significant decrease (p<0.05) in ALT level in 500 E treated group, whereas, a non-significant impact in 300 E and 1000 E groups. Moreover, vitamin E caused significant lowring (p<0.05) in AST content in 300 E and 500 E treatments compared with the control, while there was a non-significant reduce of AST in 1000 E treatment.

Dietary α-tocopherol significantly minimized (P<0.001) level of cholesterol in the three treated groups compared with the control. Regarding LDL, there was a non-significant impact of LDL on the vitamin E supplemented groups compared with the control. Turning to HDL, α-tocopherol caused a significant (P<0.001) reduction in HDL content in the all treated groups compared with the control. Furthermore, vitamin E significantly (p< 0.001) boosted the amount of vitamin E in the serum of the three groups of α-tocopherol compared with the control.

Table 1: Effects of different dietary levels of vitamin E on blood parameters of male goat kids (n=6).

Parameters	Control	300 E	500 E	1000 E	SED	Statistical Significant
Hematological Parameters						
RBC ($\times 10^{12}/\mu l$)	2.1[a]	1.4[b]	1.6[ab]	1.4[b]	0.19	p<0.05
HB(g/dl)	12.0[a]	10[bc]	10.8[ab]	10.4[bc]	0.5	P<0.05
PCV (%)	5.0[a]	3.1[b]	3.3[b]	3.3[b]	0.5	P<0.001
WBC($\times 10^{9}$ /L)	44[c]	56[b]	62[a]	42[c]	6.8	p<0.05
Neutrophil (cells μl^{-1})	0.42[a]	0.34[ab]	0.29[bc]	0.31[bc]	0.06	P<0.05
Eosinophil (cells μl^{-1})	0.08[c]	0.07[c]	0.13[a]	0.10[b]	0.03	P<0.001
Basophil (cells μl^{-1})	0.014[c]	0.016[bc]	0.023[a]	0.02[ab]	0.004	P<0.05
Monocyte (cells μl^{-1})	0.21[d]	0.37[c]	0.56[b]	0.72[a]	0.22	p< 0.001
Lymphocyte (cells μl^{-1})	0.62[a]	0.73[a]	0.75[a]	0.64[a]	0.06	N.S.

RBC: red blood cell, HB: haemoglubin, WBC: white blood cells, PCV:pack cell volume. Values in the same raw labeled with different superscript letters (a-d) differ significantly.

Table 2: Effects of different dietary levels of vitamin E on serum biochemical parameters of male goat kids (n=6).

Parameters	Control	300 E	500 E	1000 E	SED	Statistical Significant
Biochemical analysis						
Total protein (g/dl)	7.0[a]	6.3[cd]	6.5[bc]	6.7[b]	0.29	p<0.001
Albumin (g/dl)	5.4[a]	4.5[c]	4.9[bc]	4.9[b]	0.27	p<0.001
ALT (u/l)	23[ab]	22[bc]	20[c]	25[a]	2.9	p<0.05
AST (u/l)	42[a]	31[c]	37[b]	38[ab]	5.2	P<0.05
Cholesterol (mg/dl)	103[a]	87[b]	83[b]	86[b]	12.5	p<0.001
LDL (mg/dl)	75.9[a]	76[a]	74.7[a]	81.6[a]	5.9	P>0.05
HDL (mg/dl)	31[a]	29[b]	28.5[bc]	28[c]	1.6	P<0.001
Vit E in serum(μg/ml)	4.1[d]	5.2 [ab]	5.3[a]	5.1[bc]	0.16	p<0.001
MDA in serum (n.molml)	7.2[a]	6.4[b]	4.7[c]	3.7[d]	0.07	P<0.001

ALT: alanine transaminase, AST: aspartate transaminase, HDL: high density lipoproteins, LDL: low density lipoproteins,
NS: non-significant, MDA: malondialdehyde. Values in the same raw labeled with different superscript letters
(a-d) differ significantly.

On the other hand, vitamin E significantly (p<0.001) reduced the amount of MDA in the serum in all vitamin E supplemented groups compared with the control. Group 1000 E was the most effective treatment followed by 500 E and 300 E group, the differences between vitamin E groups were significant.

DISCUSSION

There is an essential need for antioxidant supplementation in the diet of goats to protect antioxidant balance in the blood (Minetti et al., 2007). The result confirmed the important of antioxidant supplementation in increasing the concentration of RBC. The effects of the three levels of vitamin E on RBC significantly (p<0.05) increased RBC count in all treatments of vitamin E compared with the control. This was agreed with Yusuf et al. (2019) in goat kids. In contrast, it was disagreed with Netto et al. (2017) who suggested that vitamin E had no significant impact on RBC count in Saanen goats. Regarding haemoglobin, the inclusion of the different amounts of vitamin E had significantly (p<0.05) raised HB concentration in the vitamin E supplemented groups compared with the control. This result was in accordance with Yusuf et al. (2019) in goat kids. Vitamin E reduced the level of PCV in the three groups of α-tocopherol compared with the control. This outcome was agree with the study of (Yusuf et al., 2019). The level of PCV in this study was very low, this could be due to the fluctuation in level of PCV in goat kids in less than one year age (Egbe-Nwiyi et al., 2000).

Supplemntation of antioxidants have no deleterious influence on immune response of goats (Yusuf et al., 2019). The two groups 300 E and 500 E significantly boosted the level of WBC, which in concur with Shokrollai et al. (2013) in goats kids. In contrast, α-tocopherol at 1000 IU had no significant effect on number of WBC which agree with Netto et al. (2017) in Saanen goats. The number of WBC in this study was high, this might be due to the kids possess protective system provides a potent defense against diseases (Opera et al., 2010). In the current study vitamin E had no significant influence on number of lymphocytes in the vitamin E supplemented groups. This was in agreement with study of (Yusuf et al., 2019). Supplementation of antioxidants helped in regulation of immune response to parasitic infection and allergy (Sokol and Medzhitov, 2010). Vitamin E had a significant increase on basophils in both treated groups 500 E and 1000 E. while, vitamin E had a non significant impact on number of basophils in 300 E group , this was agree with the result of (Yusuf et al., 2019).

From the table (2), vitamin E caused a significant (P<0.001) depletion in concentration of TP in the three groups of α-tocopherol treatment. This was in disagreement with Ziaei, (2015) who concluded that α-tocopherol had a non-significant effect on level of TP of Raieni goats. However, vitamin E significantly (P<0.001) reduced albumin level in the three treatments of α-tocopherol compared with the control. This was in agreement with Ramadan et al. (2018) in goats, whereas, was in disagreement with Rahmani et al. (2015), who reported that vitamin E significantly elevated serum albumin in cows. Elevation of ALT and AST might be a consequences of hepatic necrosis (Bollard et al., 2009), or liver cell damage (Soltan et al., 2008). In the present study, vitamin E had significantly (P<0.05) reduced ALT level in 500 E group. This was go along with Yusuf et al. (2019) in goat kids. Moreover, there was a significant (P<0.05) reduction in serum AST level in both treatments 300 E and 500 E groups. The result was disagree with Yusuf et al., (2019) who concluded that vitamin E caused non significant impact on AST level in serum. By comparison, vitamin E had no significant influence on ALT and AST in 1000 E group. The values of both AST and ALT in the three treatment groups

are still in the normal range according to the research of Sharp and Corp, (2011).

An increase in the amount of serum cholesterol was an indicator for the elevation of free radical damage in the body (Bansal and Jaswal, 2009). Vitamin E in this study caused a significant (p<0.001) reduction in the cholesterol levels in the three groups of α-tocopherol. This was in agreement with Yusuf et al. (2019) in goat kids, whereas, in disagreement with Ziaei, (2015), who reported that vitamin E had a non-significant effect on the cholesterol levels in Raieni goat. Regarding LDL, supplementation of goat kids with 300 IU, 500 IU and 1000 IU had no significant impact on LDL levels in the treated groups, which was disagree with Netto et al. (2017) who concluded that vitamin E reduced LDL in Saanen goats. While, it was disagreed with Ziaei, (2015), who suggested that vitamin E had increased amounts of LDL in Raieni goats. However, the influence of vitamin E on HDL was significantly (p<0.001) decreased HDL in the three groups of α-tocopherol compared with the control. It was in disagreement with Ziaei, (2015) who proposed that vitamin E had increased HDL concentration in Raieni goats. Supplementation of α-tocopherol had a highly significant (P<0.001) increased the level of vitamin E in the serum of the various treated groups of α-tocopherol, which concuring with Dobbelaar et al. (2010) and Anugu et al. (2013) in dairy heifers and ewes respectively.

Malondialdehyde is produced by lipid oxidation caused by oxidative damage (Mougeot et al., 2009). Vitamin E improved the oxidative status of the kids in the three groups of α-tocopherol in the present study by decreasing the amount of MDA in serum. These outcomes were agree with the result of Yusuf et al. (2019) on goat kids. Whereas, it was in disagreement with the result of Bouwstra et al. (2008), who proposed that α-tocopherol did not reduce MDA concentration in the blood of periparturient heifers. The reduction of MDA in erythrocytes could be due to inhibition of lipid peroxidation in erythrocyte membranes (Jena et al., 2013). Obviously, from the results, group 500 E was the more effective treatment in improved the blood parameters than the other two treatments of vitamin E, except in reducing level of MDA, group 1000 E reduced the amount of MDA more than group 500 E.

IN CONCLUSION

The results of this study suggested that dietary supplementation of vitamin E in the three treated groups showed improvement of RBC, HB, boosted the state of the immunity and serum vitamin E content. Whereas, it reduced albumin, TP, ALT, AST, cholesterol, and MDA. The results of haematological and biochemical parameters of the present study showed that the three levels of vitamin E had no adverse impact on haematological, biochemical metabolites, improved level of vitamin E content and MDA in serum. High enzymatic activity and low MDA concentration is a good indication on better antioxidants status in goat kids.

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