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Research Article

Effects of Graded Doses of Vitamin E on Blood and Serum Biochemistry of Sheep

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Abstract: Vitamin E (Vit. E) is primarily responsible for the increased antioxidant capacity observed in animal studies. The present research aimed to investigate vitamin E's effect on haematological and serum biochemical parameters in Kail sheep. Eighteen (18) Kail breed ewes older than two years but not yet pregnant were chosen for this experiment. The animals were randomly divided into three groups (Control, T1, and T2). During the entire experiment, the control group had access to pure water. Vitamin E was administered orally to both groups of ewes daily for 30 days, with Group T1 receiving 150mg/kg body weight and Group T2 receiving 200mg/kg body weight. Blood samples were collected on days 0, 15, and 30. The results revealed a significant increase in blood biochemistry parameters such as RBC, HGB, RDW%, WBC, LYM concentration, and LYM (%) in sheep fed Vitamin E. The serum concentration of albumin, globulin, total protein, and AST was significantly increased ($P< 0.05$). We conclude that the haematological and serum biochemical parameters in Kail sheep were enhanced after an oral dose of vitamin E.

Keywords: Kail Sheep, Vitamin E, Haematological parameters

1. INTRODUCTION

Vitamin E is a biologically active substance that primarily functions as a lipid antioxidant, preventing the proliferation of free radicals when fat is oxidized [1, 2]. It is required to develop and maintain animal health because it is an essential component of the antioxidant defence system [3]. Although only trace amounts are needed in animal diets, it plays a crucial function in farming animals [4].

Vitamin E is abundant in immune cells compared to other cells in the body, so its deficiency can impair the immune system's ability to function normally in humans and animals. By reducing free radicals, it protects humans and animals from the majority of diseases and disorders, including respiratory infections, allergic diseases (asthma), and the majority of chronic diseases [5, 6]. Vitamin E protects cells from reactive oxygen species (ROS) by reducing free radicals [7, 8]. Excessive ROS production defeats the antioxidant defence mechanism, resulting in oxidative destruction of living molecules and disruption of metabolism [9, 10]. Vitamin E is also absorbed by the small intestine (20 to 40 %) and is conveyed into the blood, generally by lipoproteins [11, 12]. Vitamin

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E reduces the risk of cardiovascular diseases in humans. A recent study found that patients with Myocardial Infarction (MI) have low levels of Vitamin E in their plasma and that supplementation with Vitamin E has a positive effect [12]. Vitamin E plays an important role in connective tissue growth, which is essential for the worth of meat. According to a study, vitamin E supplementation in broilers' diets improved the oxidative stability of breast meat and had no negative effects [7]. Lambs supplemented with Vitamin E, 15 days before slaughter improves meat and colour stability [13]. In Holstein dairy cows injection of Vitamin E and selenium increase serum albumin level, blood glutathione peroxidase activity and lactation performance [14]. When supplemented with 3000mg of Vitamin E 10 days before gestation, it reduces birth stress and protects the liver in cows [15]. Amar *et al.* performed a study on lambs by adding Vitamin E and garlic oil to their diet and found that body weight and blood parameters significantly improved [16]. A study on lambs supplemented with Vitamin E found that it dramatically affects subcutaneous fat and upregulates the genes involved in the metabolic pathway related to Vitamin E metabolism, which may be involved in meat quality [17].

Vitamin E also reverses the effect of heat stress in sheep. Sheep fed a diet high in selenium, and vitamin E have lower respiration rates, and rectal temperatures than sheep fed a diet low in selenium and vitamin E [18]. Vitamin E could boost antibody production and cellular immune responses. A study reported that Vitamin E was found in lower concentration in a group of sheep with mastitis caused by *Staphylococcus aureus* compared to the control group [19]. Vit. E deficiency completely disturbs immune functions [20]. Because of the numerous applications and roles of vitamin E, a study was designed to determine the effect of graded doses of vitamin E on blood and serum biochemical parameters in Kail sheep.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Site

The study was conducted at the Experimental Livestock Farm Khaigala, Department of Veterinary Clinical Sciences, University of Poonch Rawalakot (UPR), Azad Kashmir. This experiment included 18 non-pregnant ewes of the Kail breed ranging in age from 2-3 years. The animals were divided into three groups of six each: control, T1, and T2. During the experiment, the Control group was given clean water. Animals in Group T1 received an oral dose of 150 mg/kg body weight of Vitamin E daily for 30 days, whereas animals in Group T2 received an oral dose of 200 mg/kg body weight of Vitamin E daily for 30 days. All of the animals were fed concentrates, wheat bran, wheat straws, and maize in addition to open grazing.

2.2 Blood Sampling

Blood samples were collected from experimental animals on days 0, 15, and 30. Blood samples were collected in two tubes, one with an anticoagulant, K3-ethylenediaminetetraacetic acid (K3-EDTA), for whole blood and the other without for serum isolation. The samples were kept in an icebox with ice packs before being transferred to the laboratory for further processing. Serum was stored at -20 °C after extraction [21].

2.3 Blood and Serum Parameters

Blood samples in EDTA tubes were checked for different parameters i.e., WBC count, RBC count, numbers of lymphocytes and percentage, granulocytes, haemoglobin concentration, mean cell volume, hematocrit, mean corpuscular h aemoglobin value, mean corpuscular haemoglobin concentration, MID cell, GRA percentage, and mean platelets volume [22]. A haematology analyzer (Cell-Dyn 3700; Abbott, Abbott Park, IL) processed whole blood according to the company's protocols. Suitable commercial test kits determined the serum parameters. Serum concentrations of albumin, total protein, and AST were analyzed using an ELISA reader by commercially available kits (Calbiotech, USA) [23]. The difference between albumin and total serum protein was used to calculate the concentration of globulin [24].

2.4 Statistical Analysis

Data was analyzed statistically through repeated measure ANOVA using a statistical package for social sciences (SPSS Inc, version 16, Chicago, USA). The graphing software used was Graph Pad Prism. (Graph Pad Software Inc., San Diego, CA,

USA). Data are expressed as the means ± SEM. P<0.05 was considered significant.

3. RESULTS

3.1 Blood Parameter

3.1.1. Effect on the concentration of RBCs

On day 0, the RBCs concentration (Table 1) showed that all three groups (Control, T1, and T2) had RBCs ±SE values of 3.3±1.1, 3.2 ±0.2, and 3.6±0.8, respectively. On day 15, the control and T1 groups had similar RBC values (3.7±0.8 and 3.4±0.5), whereas the T2 group (4.2±0.9) had a significant increase (P<0.05) in RBC count compared to the control group. On day 30, the RBC values in the T1 and T2 groups were 5.1±1.3 and 4.9±0.8, respectively, and showed a significant increase when compared to the control group; however, there was no significant difference between the RBC values in the T1 and T2 groups. HCT values in the T2 group followed a similar pattern (Table 1), with values increasing on day 30. Throughout the experiment, there was no significant difference in MCV and MPV concentrations (Table 1) among the three groups (Control, T1, and T2).

3.1.2. Effect on values of WBC and HGB

On day 0, the values of WBCs ±SE (Table 2) were 12.0±1.6, 13.5±2.1, and 11.9±1.7 for the control, T1, and T2 groups, respectively. On day 15, however, values of Group T1 and T2 (14.1±1.7 and 13.95±1.8, respectively) showed a significant increase (P<0.05) in WBC count as compared to the control group (12.2±1.9), and on day 30, T1 had significantly (P<0.05) higher values (12.8±1.9) as compared to the control group (11.2±1.6). Similarly, the value of the T2 group (13.5±1.2) was also increased as compared to the control group.

The HGB concentrations (Table 2) in the control and treated groups (T1 and T2) differ significantly throughout the experiment. On day 15, the T1 and T2 groups had significantly higher HGB levels (8.1±0.6 and 8.1±0.7) than the Control group (7.4±0.3). On day 30, the value of HGB in the T1 group (7.7±0.2) showed an upward trend when compared to the control group (7.6±0.5), whereas the T2 group showed significantly higher values of HGB (8.0±0.8) when compared to T1(7.7±0.2). Throughout the experiment, there were no significant differences in the MCH concentration values between and within the three experimental groups (Table 2).

3.1.3. Effect of Vit. E on other blood parameters

Results of MCHC, GRAN, LYM (Mx/Dl), and LYM (%) are arranged in Table 3. In comparison to the control group (79.5 ±4.6), the percentage value

Table 1. Effect of Vit. E supplementation on the RBC, MCV, HCT% and MPV of sheep.

Parameters	Days	Groups		
		Control n=6	**T1(5g) n=6**	**T2(7g) n=6**
RBC	0 day	3.3 ± 1.1^{Aa}	3.2 ± 0.2^{Ba}	3.6 ± 0.8^{ABa}
	15 day	3.7 ± 0.8^{Aa}	3.4 ± 0.5^{Aa}	4.2 ± 0.9^{Aab}
	30 day	4.6 ± 1.1^{Aa}	5.1 ± 1.3^{Ab}	4.9 ± 0.8^{Ab}
MCV (fL)	0 day	36.6 ± 1.2^{Aa}	36.4 ± 0.4^{Aa}	36.8 ± 2.3^{Aa}
	15 day	37.4 ± 0.8^{Aa}	36.6 ± 0.7^{Aa}	37.2 ± 2.4^{Aa}
	30 day	35.9 ± 0.6^{Aa}	36.7 ± 0.7^{Aa}	37.4 ± 1.1^{Aa}
HCT%	0 day	15.7 ± 3.8^{Aa}	11.8 ± 2.0^{Aa}	14.6 ± 5.3^{Aa}
	15 day	15.6 ± 3.3^{Aa}	12.5 ± 1.96^{Aa}	14.7 ± 5.1^{Aa}
	30 day	15.1 ± 6.4^{Aa}	15.8 ± 3.8^{Aa}	18.2 ± 4.3^{Aa}
MPV	0 day	19.8 ± 4.6^{Aa}	21.1 ± 0.9^{Aa}	18.3 ± 3.7^{Aa}
	15 day	21.9 ± 0.9^{Aa}	21.4 ± 0.95^{Aa}	18.6 ± 3.8^{Aa}
	30 day	18.98 ± 5.4^{Aa}	22.2 ± 0.9^{Aa}	18.5 ± 5.95^{Aa}

^{AB} superscript denotes the significant difference between the groups (P<0.05). ^{ab} superscript denotes the significant difference (P<0.05) within the groups. T1= Treatment 1, T2= Treatment 2

Table 2. Effect of Vit. E supplementation on the WBC, HGB and MCH parameters of sheep

Parameters	Days	Groups		
		Control n=6	**T1(5g) n=6**	**T2(7g) n=6**
WBC	0 day	12.0± 1.6^{Aa}	13.5± 2.1^{Aa}	11.9 ±1.7^{Aa}
	15 day	12.2± 1.9^{ABa}	14.1± 1.7^{Aa}	13.95±1.8^{Ba}
	30 day	11.2± 1.6^{Aa}	12.8 ±1.9^{Ba}	13.5± 1.2^{ABa}
HGB (g/dL)	0 day	7.9± 0.4^{Aa}	7.1± 0.7^{Ba}	7.2 ±0.8^{Ba}
	15 day	7.4± 0.3^{Aa}	8.1 ±0.6^{Aa}	8.1 ±0.7^{Aa}
	30 day	7.6± 0.5^{Aa}	7.7 ±0.2^{Aa}	8.0±0.8^{Aa}
MCH(pg)	0 day	19.5± 4.5^{Aa}	21.3±0.9^{Aa}	19.8± 5.1^{Aa}
	15 day	20.1 ± 3.6^{Aa}	22.0±1.8^{Aa}	19.9 ± 4.8^{Aa}
	30 day	19.6 ± 8.1^{Aa}	17.5± 3.9^{Aa}	22.3± 4.9^{Aa}

^{AB} superscript denotes the significant difference between the groups (P<0.05). ^{ab} superscript denote the significant difference (P<0.05) within the groups.

of LYM at day 15 was significantly higher (P<0.05) in Group T1 and Group T2 (82.1±4.8 and 85.8±6.8, respectively). At day 30, the concentration of LYM (Mx/D1) in Group T1 (11.4±1.1) was higher than that of the control group. Throughout the experiment, the values of all other parameters in Table 3 did not change significantly (P>0.05) between and within groups.

In Table 4 we can access the RDW%, GRA%, MID%, and MID concentration data. No statistically significant differences existed between any of the groups on day 0. (Control, T1, and T2). Compared to the T1 and control groups, the MID% figure for the T2 group increased dramatically by day 15. On day 30, the RDW% in the T1 group increased (P<0.05) compared to the control group. Furthermore, there

Table 3. Effect of Vit. E supplem entation on the MCHC, LYM conc., GRAN and LYM% of sheep.

Parameter	Days	Groups		
		Control	**T1(5g)**	**T2(7g)**
		n=6	**n=6**	**n=6**
MCHC(g/dL)	0 day	53.1± 12.2^{Aa}	57.6 ± 5.5^{Aa}	53.9± 13.6^{Aa}
	15 day	53.7± 11.0^{Aa}	60.1± 6.1^{Aa}	54.1± 13.8^{Aa}
	30 day	51.3 ±24.2^{Aa}	50.4±11.3^{Aa}	58.9±12.2^{Aa}
LYM(Mx/Dl)	0 day	9.6±1.3^{Aa}	10.3±1.3^{Aa}	9.7± 0.9^{Aa}
	15 day	9.7±1.6^{Aa}	10.8±1.4^{Aa}	9.9 ±0.9^{Aa}
	30 day	9.7± 1.4^{Aa}	11.4 ± 1.1^{Ba}	10.4±1.4^{ABa}
GRAN	0 day	0.5±0.2^{Aa}	0.6± 0.5^{Aa}	0.3±0.1^{Aa}
	15 day	0.5±0.3^{Aa}	0.4±0.3^{Aa}	0.4±0.1^{Aa}
	30 day	0.4±0.3^{Aa}	0.4±0.1^{Aa}	0.4±0.1^{Aa}
LYM%	0 day	79.9± 4.1^{Aa}	79.5± 4.9^{Aa}	72.2± 31.6^{Aa}
	15 day	79.5± 4.6^{Aa}	82.1±4.8^{Aa}	85.8±6.8^{Ab}
	30 day	86.3±1.4^{Aa}	86.9±6.2^{Aa}	89.4±2.7^{Ab}

^{AB} superscript denotes the significant difference between the groups (P<0.05). ab superscript denote the significant difference (P<0.05) within the groups.

Table 4. Effect of Vit. E supplementation on the RDW%, GRA%, MID, and MID% of sheep.

Parameter	Days	Groups		
		Control n=6	T1(5g) n=6	T2(7g) n=6
RDW%	0 day	7.2±1.2^{Aa}	5.6±1.4^{Aa}	7.7±4.7^{Aa}
	15 day	7.4±1.4^{Aa}	5.9±1.4^{Aa}	9.4±5.0^{Aa}
	30 day	9.1±4.6^{Aa}	10.7±4.6^{Ab}	10.1±4.9^{Aa}
GRA%	0 day	4.4±1.5^{Aa}	2.9±0.8^{Aa}	3.2±1.6^{Aa}
	15 day	4.7± 2.2^{Aa}	3.6±1.3^{Aa}	3.3±1.6^{Aa}
	30 day	3.8±2.1^{Aa}	3.1±0.3^{Aa}	3.9±0.9^{Aa}
MID Conc.	0 day	1.9±0.5^{Aa}	4.3±5.8^{Aa}	1.5±0.7^{Aa}
	15 day	1.9±0.5^{Aa}	4.5±5.7^{Aa}	1.5±0.7^{Aa}
	30 day	1.4±0.4^{Aa}	1.9±0.5^{Aa}	1.5±0.6^{Aa}
MID%	0 day	15.6±2.9^{Aab}	14.5±2.9^{Aa}	11.6±5.0^{Aa}
	15 day	12.8±2.5^{Aa}	12.7±3.2^{Aa}	15.3±5.4^{Aa}
	30 day	11.5±2.9^{Ab}	12.9±4.2^{Aa}	13.8±2.4^{Aa}

^{AB} superscript denotes the significant difference between the groups (P<0.05). ^{ab} superscript denotes the significant difference (P<0.05) within the groups.

was no significant difference between groups on days 0-15 or 30-day MID concentration or GRA % (P>0.05) (Table 4).

3.2 SERUM PARAMETERS

3.2.1. Effect on albumin, globulin, AST, and total protein concentration

Figure 1 demonstrates the effect of vitamin E supplementation on the levels of several enzymes in the blood. Both albumin and total protein concentrations on day 0 were similar (P>0.05) for the Control, T1, and T2 groups. On day 15, however, the T1 and T2 groups' albumin concentration and total protein were higher than the control group's by a significant margin (P<0.05). Additionally, T2 exhibited a higher albumin concentration than T1 and control on day 30, with a significance level of P<0.05. On day 30, there was an increasing tendency in the T1 group's total protein concentration (P>0.05), whereas the T2 group's total protein concentration improved significantly compared to the control group. (Fig 1A, B).

On day 15, globulin values were found to be significantly higher in the T1 and T2 groups

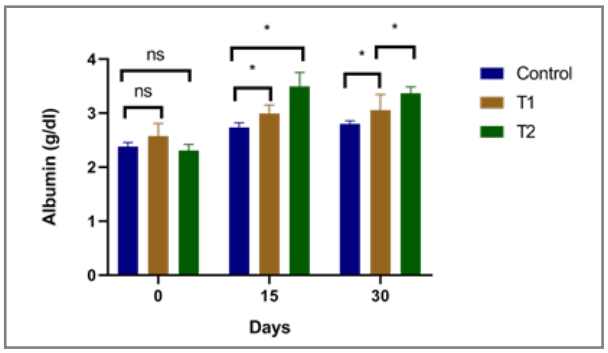

Fig 1A: Effect of graded doses of Vit. E on serum Albumin

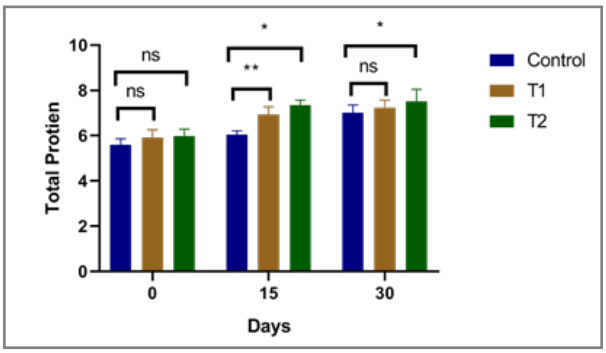

Fig. 1B. Effect of graded doses of Vit. E on total protein

compared to the control group. On day 30, globulin values were trending upward in the T1 group and increased significantly in the T2 group compared to the control group (P>0.05). (Fig 1C).

The AST enzyme value on days 15 and 30 increased in the T1 and T2 groups (P< 0.05) compared to the control group. There was no difference in AST enzyme levels between the control, T1, and T2 groups on day 0. (Fig 1D).

4. DISCUSSION

Vitamin E is crucial to the antioxidant defence system and vital to animal and human development and health [25–27]. As an antioxidant, it helps protect the cell membranes of immune system cells and has been linked to disease prevention. The effects of vitamin E supplementation on sheep blood and serum biochemistry were investigated in this study.

Vitamin E supplementation significantly affected the HCT/PCV and HGB values in this experiment. It has been demonstrated that supplementation with vitamin E significantly improved (P<0.05) PCV in sheep [28]. Mohri *et al.* found that calves fed with Se and Vitamin E in the age of third and fourth weeks had higher HCT and HGB values/levels. This study reported that HGB and PCV values increase significantly in treated groups as compared to control groups (p<0.05) [29]. Our findings are similar to these.

Previous data indicated no differences in PCV between treated and control lambs when supplemented with vitamin E, which contradicts the current findings [30]. Intake of Vitamin E did not affect rats' HCT value or HGB concentration [31]. Mohri *et al.* found that Se and Vitamin E supplementation had a negligible effect on HCT and HGB [29]. Because of the various doses utilized, these results do not correspond with the data we obtained.

The current studies found that vitamin E supplementation significantly improved (P<0.05) WBCs and RBCs in the treated group. A previous study reported that white blood cells had higher values in the test group in calves due to the antioxidant effect of vitamin E, thus increasing RBC and WBC lifespan [32]. Similarly, the administration of vitamin E to rats increased the number of white blood cells in the treated group compared to the untreated group [31]. Broiler breeders' white blood cell and lymphocyte counts improve when vitamin E is added to their diet [33]. In a study, Vit. E supplementation in poultry reduced the toxic effect of chromium by boosting haematological parameters (RBC, WBC, Hb, PCV, and MCHC) at a significant level [34]. Vitamin E and selenium supplementation dramatically increased red blood cell, white blood cell, and neutrophil counts in Markhoz offspring, as reported by Shokrollahi *et al.* [35]. Finding agreed with research by H_Asadi *et al.*, which found that vitamin AD3E injections in Arabi rams increased red blood cell (RBC) concentration [36].

Our data showed that the lymphocyte counts of the experimental animals improved dramatically (P< 0.05) after receiving vitamin E supplements. Our findings are consistent with those of Hassan and Mustafa, who found that giving ram lambs vitamin E improved their lymphocyte counts [28]. Vitamin E intake may be related to the protection of organelles and cell membranes through the vitamin's antioxidant effects on lymphocyte cell numbers [37]. However, Shokrollahi *et al.* discovered the opposite in Markhoz's offspring, finding that varying doses of vitamin E and selenium treatment had a negligible impact on lymphocyte counts [35].

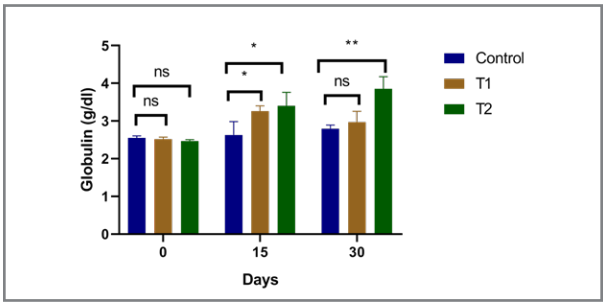

Fig. 1C. Effect of graded doses of Vit. E on serum Globulin

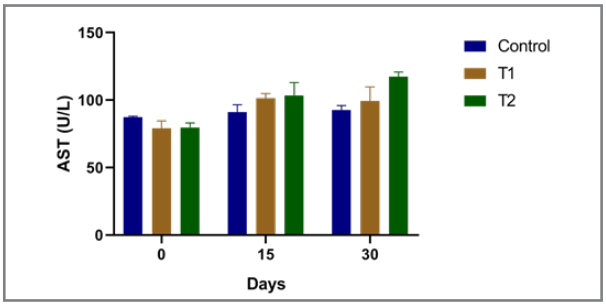

Fig. 1D. Effect of graded doses of Vit. E on serum AST

A study that was done on rats showed that there was no statistically significant difference (P>0.05) in mean cardiac output (MCV), mean cardiac hemodynamic output (MCHC), or mean cardiac output (MCH) between groups that were given or not given vitamin E [31].

The albumin, globulin, AST, and total protein levels increased by a statistically significant amount (P<0.05) in our experiment when Vitamin E was provided as a supplement. Previous research indicated that vitamin E and selenium significantly affected albumin and total protein concentrations in sheep serum [38]. Serum total protein concentration in Markhoz's progeny was reported to increase rapidly after supplementation with vitamins E [35]. El-Shahat and Abdel Monem found that taking vitamin E and selenium supplements increased globulin and tissue-specific protein levels by a statistically significant amount (P<0.05) [39]. Vitamin E plays a vital role in maintaining protein and albumin synthesis and a substantial part in cellular protein synthesis [40]. Vitamin E's capacity to boost immunoglobulins may be responsible for the rise in albumin and globulin concentration. These results accord with those found by Abdelatif *et al*. in their research on Nubian goats [32]. We hypothesized that this caused the overall increase in protein concentration that we saw in our experiment.

5. CONCLUSION

The results of the current study suggest that vitamin E has beneficial effects on several essential blood and serum parameters in sheep. RBC, WBC, HGB, LYM, RDW%, albumin, globulin, total protein, and AST were all improved significantly by vitamin E supplementation. It was concluded that vitamin E enhanced important blood parameters; hence, it is necessary for a variety of body functions as well as the sustenance of good animal health.

6. FINANCIAL SUPPORT

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7. CONFLICT OF INTEREST

The author declared no conflict of interest.

8. REFERENCES

1. E. Herrera, and C. Barbas. Vitamin E: Action, metabolism and perspectives. *Journal of Physiology and Biochemistry* 57: 43–56 (2001).
2. M.G. Traber, and J. Atkinson. Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine* 43: 4–15 (2007).
3. A.E. Azab, A.A Adwas, A.S.I. Elsayed, A.S.I. Elsayed, and F.A. Quwaydir. Oxidative stress and antioxidant mechanisms in human body. *Journal of Applied Biotechnology & Bioengineering* 6: 43–47 (2019).
4. E.M. Idamokoro, A.B. Falowo, C.E. Oyeagu, and A.J. Afolayan. Multifunctional activity of vitamin E in animal and animal products: A review. *Animal Science Journal* 91: 13352 (2020).
5. E.M. Ibrahim. 59 Effect of parenteral supplementation of vitamin E plus selenium on nutrient digestibility, productive performance and some serum biochemical indicators of lambs. *Egyptian Journal of Sheep & Goat Sciences* 12: 59–70 (2017).
6. S.N. Meydani, and J.B. Blumberg. Vitamin E and the Immune Response. *Nutrient Modulation of the Immune Response* 223–238 (2020).
7. S.G. Harsini, M. Habibiyan, M.M. Moeini, and A.R. Abdolmohammadi. Effects of dietary selenium, vitamin E, and their combination on growth, serum metabolites, and antioxidant defense system in skeletal muscle of broilers under heat stress. *Biological Trace Element Research* 148: 322–330 (2012).
8. N. Noguchi, and E. Niki. Chemistry of Active Oxygen Species and Antioxidants. *Antioxidant Status, Diet, Nutrition, and Health* 1–20 (1999).
9. M. Trevisan, R. Browne, M. Ram, P. Muti, J. Freudenheim, A. M. Carosella, and D. Armstrong. Correlates of Markers of Oxidative Status in the General Population. *American Journal of Epidemiology* 154: 348–356 (2001).
10. V. Unsal, T. Dalkiran, M. Çiçek, and E. Kölükçü. The Role of Natural Antioxidants Against Reactive Oxygen Species Produced by Cadmium Toxicity: A Review. *Advanced Pharmaceutical Buletin* 10: 184-202 (2020).
11. N.Y. Toker. Blood serum Vitamin A and E concentrations and distribution into lipoprotein fractions of pregnant sheep and newborn lambs. Revue De Médicine.Véterinaire 158: 413-417(2007).

12. M. Ziegler, M. Wallert, S. Lorkowski, and K. Peter. Cardiovascular and Metabolic Protection by Vitamin E: A Matter of Treatment Strategy. *Antioxidants* 9: 935 (2020).
13. L.N. Leal, J.A. Beltran, M. Belles, J.M. Bello, L.A.D. Hartog, W.H. Hendriks, and J.M. Tereso. Supplementation of lamb diets with vitamin E and rosemary extracts on meat quality parameters. *Journal of the Science of Food and Agriculture* 100: 2922–2931(2020).
14. M. Zahrazadeh, A. Riasi, H. Farhangfar, and S.A. Mahyari. Effects of close-up body condition score and selenium-vitamin E injection on lactation performance, blood metabolites, and oxidative status in high-producing dairy cows. *Journal of Dairy Sci*ence 101: 10495–10504 (2018).
15. K. Yenilmez, H. Atalay, M. Cellat, M. Etyemez, G. Atmaca, and F.E. Gürsel. The effect of vitamin E administration to dairy cows in the prepartum period on some metabolic, oxidative and reproductive parameters. *Large Animal Review* 28: 235–240 (2022).
16. A. Raeed, M. Thamer, W. Jasim, M. Ali, and W.K. Ahmad. Effect of garlic oil and vitamin E on productive performance and some hematological and biochemical blood parameters of Awassi male and female lambs. *Kufa Journal for Agricultural Sciences* 12: 9–16 (2020).
17. L.G. Calvo, E. Dervishi, M. Joy, P. Sarto, R.N. Hernandez, M. Serrano, J.M. Ordovas, and J.H. Calvo. Genome-wide expression profiling in muscle and subcutaneous fat of lambs in response to the intake of concentrate supplemented with vitamin E. *BMC Genomics* 18: 1–19 (2017).
18. J.A. Rooke, J.J. Robinson, and J.R. Arthur. Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *The Journal of Agricultural Science* 142: 253–262 (2004).
19. N.D. Giadinis, N. Panousis, E.J. Petridou, V.I. Siarkou, S.Q. Lafi, K. Pourliotis, E. Hatzopoulou, and G.C. Fthenakis. Selenium, vitamin E and vitamin A blood concentrations in dairy sheep flocks with increased or low clinical mastitis incidence. *Small Ruminant Research* 95: 193–196 (2011).
20. A. Azzi. Many tocopherols, one vitamin E. *Molecular Aspects of Medicine* 61: 92–103 (2018).
21. Shakirullah, M.S. Qureshi, S. Akhtar, and R.U. Khan. The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress. *Applied Biological Chemistry* 60: 585–590 (2017).
22. K.P. Maraba, V. Mlambo, A.O. Yusuf, U. Marume, and A. Hugo. Extra dietary vitamin E – selenium as a mitigation strategy against housing-induced stress in Dohne Merino lambs: Effect on growth performance, stress biomarkers, and meat quality. *Small Ruminant Research* 160: 31–37 (2018).
23. E.B. Soliman. Dose-response of vitamin e and selenium injection on growth performance, physiological and immune responses of ossimi lambs. *Egyptian Journal of Sheep and Goats Sciences* 10: 1–14 (2015).
24. S.G.A. Ramadan, S.D.H. Mahboub, M.A.Y. Helal, and M. A. Sallam. Effect of Vitamin E and Selenium on Performance and Productivity of Goats. *International Journal of Chemical and Biomedical Science* 4: 16–22 (2018). http://www.aascit.org/journal/ijcbs (accessed 4 January 2023)
25. E.J. Underwood. Trace elements in human and animal nutrition. Ed. 3. *Trace elements in human and animal nutrition (*1971)
26. L.R. McDowell. Minerals in animal and human nutrition. *Minerals in animal and human nutrition (*1992)
27. P.F. Surai. Selenium in nutrition and health. www.nup.com (accessed 5 January 2023).
28. W.I. Hasan, and K.N.S. Mustafa. Effect of vitamin e and selenium injection on some physiological characteristics and biochemical parameters in local ram lamb. *Journal of Duhok University* 23: 34–43 (2020).
29. M. Mohri, A. Ehsani, M.A. Norouzian, M.H. Bami, and H.A. Seifi. Parenteral selenium and vitamin E supplementation to lambs: Hematology, serum biochemistry, performance, and relationship with other trace elements. *Biological Trace Element Research* 139: 308–316 (2011).
30. M. Aksakal, M. Naziroğlu, and M. Çay. The Effects of Selenium and Vitamin E on some Heamatological and Biochemical Values of Blood in Lambs. *Turkish Journal of Veterinary and Animal Sciences* 20: 185–190 (1996).
31. T. Fujihara, and E.A. Orden. The effect of dietary vitamin E level on selenium status in rats. *Journal of Animal Physiology and Animal Nutrition* 98: 921–927 (2014).
32. A.M. Abdelatif, and M.Y. Ibrahim. Seasonal Variation in Erythrocytic and Leukocytic Indices and Serum Proteins of Female Nubian Goats Transportation stress in farm animals View project Physiological responses to trauma/haemorrhage : Effects of gender and gonadectomy View

project (2009). https://www.researchgate.net/publication/242210780 (accessed 31 January 2023).
33. M. Zaghari, V. Sedaghat, and M. Shivazad. Effect of vitamin E on reproductive performance of heavy broiler breeder hens. *Journal of Applied Poultry Research* 22: 808–813 (2013).
34. V. Kandpal, D. Kumar, and R. Bisht. Protective Effect of Vitamin E on Haematological Parameters in Chronic Toxicity of Hexavalent Chromium in Laboratory Chicks. *Journal of Drug Delivery and Therapeutics* 9: 388–392 (2019).
35. B. Shokrollahi, M. Mansouri, and H. Amanlou. The effect of enriched milk with selenium and vitamin e on growth rate, hematology, some blood biochemical factors, and immunoglobulins of newborn goat kids. *Biological Trace Element Research* 153: 184–190 (2013).
36. F.A.A. Al-Asadi, H.N. Habib, and A.F. Hassan. Effect of the injection of vitamins AD3E and the seasons on some blood traits, biochemical components and hormones of Arabi rams. *Journal of Animal Behaviour and Biometeorology* 8: 74–81 (2020).
37. M.M. Moeini, and M.T. Jalilian. Effect of selenium and vitamin E injection during late pregnancy on immune system and productive performances of sanjabi ewes and their lambs. *Global Journal of Animal Scientific Research* 2: 210–219 (2014).
38. J.A. Rooke, J.J. Robinson, and J.R. Arthur. Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *Journal of Agricultural Sciences* 142: 253–262 (2004).
39. K. El-Shahat, and U.M.A Monem. Effects of dietary supplementation with vitamin E and/or selenium on metabolic and reproductive performance of Egyptian Baladi ewes under subtropical conditions. *World Applied Sciences Journal* 12: 1492-1499 (2011). scholar.cu.edu.eg, (accessed 31 January 2023).
40. M.W. Clarke, J.R. Burnett, and K.D. Croft. Vitamin E in Human Health and Disease. *Critical Reviews in Laboratory Clinical Sciences.* 45: 417-450 (2008).