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The effect of vitamin E treatment on selected immune and oxidative parameters in Kivircik ewes suffering from transport stress

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Abstract

The study aimed to investigate the effects of vitamin E injection for the prevention of transport stress on ewes. Kivircik ewes (2–3 years old, n = 24) were randomly separated into three groups; G1 (Control) and G2 treated with 14 ml. saline as the placebo, G3 treated with 2100 IU/ind. DL-alpha-tocopherol acetate prior to transport. G2 and G3 were transported at 80 km/h for 4 h on a truck. Serum samples were obtained before (T0) and after (T1) transport. Serum cortisol, catalase, IgG, ceruloplasmin, C-reactive protein, complement component 4, interleukin-1 beta, tumour necrosis factor-alpha, glutathione peroxidase (GPx), superoxide dismutase, malondialdehyde analyses performed by ELISA, and serum alpha-tocopherol concentrations were evaluated by HPLC-UV. Wilcoxon and Kruskal–Wallis tests were used for statistical assessments (p < 0.05). Alpha-tocopherol concentrations were founded 1.22 ± 0.82, 0.27 ± 0.14 and 0.14 ± 0.07 µmol/L, respectively, in G1, G2 and G3 at T1. Alpha-tocopherol concentration decreased significantly in G2 between T0 and T1. GPx concentrations were increased twofold in G2 and G3 between T0 and T1 (p < 0.01). As a result, G2 alpha-tocopherol concentrations decreased but, the stress and oxidative parameters tested in this study were not affected by treating 2100 IU/ind. DL-alpha-tocopherol acetate before transport.

KEYWORDS
DL-alpha-tocopherol acetate, ewe, glutathione peroxidase, Kivircik, oxidative stress, transport stress

1 | INTRODUCTION

Animal welfare targets a life free of unfavourable feelings such as pain, suffering and stress, and the lack of any response to stress in animals is considered an indicator of welfare. Metabolic changes occur in animals depending on the severity of the stress (Avcı et al., 2008). Humoral immune responses in sheep are indicative of relative stress caused by both physical and emotional changes (Caroprese et al., 2006). It was reported that stress affects living life and productivity, causing negative consequences such as lipid peroxidation, protein denaturation and DNA mutations in cells and eventually reduced meat quality (Avcı et al., 2008). On the other hand, the oxidant–antioxidant balance may be disrupted in favour of free radicals as a result of the increase in enzyme activities such as lactic acid, lactate dehydrogenase and creatine phosphokinase as well as the increased levels of epinephrine and other catecholamines due to stress (Çetin et al., 2011). Sedatives, dopamine, opioids, central nervous system depressants, hormonal drugs, vitamins, minerals and amino acids are widely used to reduce the physiological and biochemical effects of stress in pets (Avcı et al., 2008).

Stress factors such as nutritional imbalances, warm and cold exposure, crowding, noise and transportation can weaken the body's defence mechanisms, therefore susceptibility to infectious diseases increases. Chemical stress regulators, for example corticosteroids, have a significant immunosuppressive effect associated with elevated prostaglandin levels and may also affect the bioavailability of nutrients. Diarrhoea, a stress-induced infection, may lead to a reduction in feed intake and thus further increase susceptibility to disease (Tengerdy, 1989). Immunity is reduced due to insufficient intake of essential trace elements such as copper, iron and zinc as well as vitamins E, A and C, all of which are essential for antioxidant defence (Sherman, 1992). Among immunostimulants, the antioxidant vitamins C and E can be taken orally in high doses at low cost and are often used together for immune-stimulating responses (Ortuño et al., 2000).

Vitamin E together with vitamins A and C also strengthens the immune response against tumours and plays a role in the prevention of cancer (Watson & Leonard, 1986; Ferguson & Philpott, 2007). The potential of vitamin E to enhance the performance of cattle increases with elevated stress. The protective effects of vitamin E on animal health may also be associated with its role in the reduction of immunosuppressive glucocorticoids. Vitamin E also has an immune-boosting effect due to altered arachidonic acid metabolism and the subsequent synthesis of prostaglandins, thromboxanes and leukotrienes. Increased levels of such compounds through endogenous synthesis or exogenous uptake under stress conditions may adversely affect immune cell function (McDowell et al., 1996). The immune-boosting effect of vitamin E was proven versus a synthetic antioxidant. Vitamin E, which is attached to the cell membrane, plays an important role in complex cell–cell interactions that trigger and regulate immune responses (Tengerdy, 1989).

Stress-mediated activation of neutrophils may lead to autooxidative damage to the neutrophil membranes of fish fed a diet low in vitamin E, and a reduced superoxide anion production of neutrophils (Montero et al., 2001). Damage to the oxidative stress system may cause several problems in livestock. One of the main reasons for the breakdown of oxidative stress balance is transport stress. Transport stress increases serum oxidative stress concentrations and involves in a preparative way the mortality of beef cattle with Bovine respiratory diseases (Chirase et al., 2004). Goats are influenced by acute stress during transportation (Ahmad Mir et al., 2019). Besides this, noise, manoeuvres, climate and humidity can also affect animals during transport, even in short periods (Andronie et al., 2008).

Vitamin E is administered to stressful animals, lactating cows and cattle that are transported or overfed in order to the early intervention interlocking this situation with the chain of events leading to the disease. Supplementation of vitamin E is most effective for farm animals under stress. Vitamin E may also decrease the levels of stress-induced corticosteroids (Tengerdy, 1989). Supplementation of vitamin E to maintain the health of animals under stress tended to reduce the increments of blood tissue damage indicators during road transport. Thus, supplementation of vitamin E may improve the welfare of animals during road transportation (Morán et al., 2012). Vitamin E treatment effects on transport stress in sheep and goats have been mainly focused on haematological, cortisol levels, weight loss and welfare parameters (Avcı et al., 2008; Çetin et al., 2011; Ekiz et al., 2013; Horton et al., 1996; Kannan et al., 2000; Morán et al., 2017). In heat stress, supplementation of vitamin E and C is also a widespread implementation (Belhadj Slimen et al., 2019).

Moreover, brief information about the parameters measured in this paper would be useful. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase remove superoxide and peroxides from cells. They know as fundamental enzymatic antioxidants (Yardimci et al., 2013). SOD is an enzyme that converts superoxides to H_2O_2 and after that GPx enzyme converts H_2O_2 to water (Khan et al., 2016). Ceruloplasmin has also antioxidant functions by interacting with zinc and copper (Nockels, 1996). Cortisol is a very well-known stress indicator in animals. C-reactive protein (CRP) is a pro-inflammatory acute phase protein produced by the liver and a sensitive marker of systemic inflammation (Voort et al., 2013). CRP has a role in complement activation, binding to membrane phosphorylcholine, opsonization (Iliev & Georgieva, 2018). Complement component 4 (C4) proteins have a role modulator role during inflammation. Immunoglobulin G (IgG) shows the immunity level of the individuals. Interleukin 1 beta (IL-1 beta) is a pro-inflammatory cytokine that activates neutrophils, monocytes, eosinophils and basophils and triggers the production of tumour necrosis factor-alpha (TNF-alpha). TNF-alpha influences the proliferation, activation and differentiation of many cells and enhances the synthesis of IL-1 (Voort et al., 2013). Malondialdehyde (MDA) is the last product of the lipid peroxidation (Celi, 2010).

The objective of this research was to determine the effects of administering DL-alpha-tocopherol acetate by intramuscular route prior to transport on the serum antioxidant enzymes (serum catalase, ceruloplasmin, SOD, GPx), inflammation response parameter (CRP), complement activation and chemokine marker (C4), immune status (IgG), lymphocyte activation factor (IL-1 beta), systemic inflammation cytokine (TNF-alpha), lipid peroxidation marker (MDA), psychological stress parameters (cortisol) and alpha-tocopherol concentrations in kivircik ewes.

2 | MATERIALS AND METHODS

2.1 | Animals, analyses and sampling information

The study was performed at the Research, Training and Application Farm of Balıkesir University in Balıkesir, Turkey (39° 32′ 8.5236″ N, 28° 0′ 38.8224″ E) on 20 May 2017. The kivircik ewes (2–3 years old, Body Weight 51 ± 2 kg) were provided from the same farm. Ewes were randomly divided into three equal groups (n = 8 per group). All groups provided the same ration (2 kg corn silage, 1 kg dried alfalfa and 0.5 kg concentrate feed) and free access to water before 30 days of the experiment. All individuals underwent health assessment, and no clinical health problem was observed.

Daily oral supplement of 40 mg/kg alpha-tocopherol is generally recommended in lambs (Kaya, 2007; McDowell et al., 1996). Based on this reference, the DL-alpha-tocopherol acetate dose to be used in the study was determined. Vitamin E (2100 IU/ind. DL-alpha-tocopherol acetate) was administered by the intramuscular route (Evigen® Ampule, 2 ml containing 300 mg DL-alpha-tocopherol acetate; Aksu Farma) to G3 10 min before transport. The same volume (14 ml) of sterile saline solution (0.9% NaCl, Biofleks® Osel) was injected IM into G1 and G2 at the same time. Caravaggi et al., (1968) founded that IM injection of 100 mg/ind alpha-tocopherol acetate produced a rapid increase in 4 h in serum tocopherol concentration in sheep. G2 and G3 were transported in the truck with a non-slip plastic floor covered with straw at a space allowance of more than 0.32 m^2 per animal at 9:00 h (max. 80 km/h for 4 h). The temperature, humidity and driving speed, and route travelled were similar for each ewe.

Serum was collected from the *vena jugularis* with the aid of a 21 G vacuum-tube cannula into 8 ml gel blood tubes from all groups before administering sterile saline solution or vitamin E (T0) and five minutes after (T1) transport. Serum samples were taken to the laboratory within 30 min, maintaining the temperature at 4°C with a polystyrene box. Serum tubes were centrifuged at room temperature for 10 min at 3000 *g* (Hettich® Rotofix 32A). The serum parts were taken into the tubes and stored at −86°C until the analyses were performed.

The serum catalase (Cayman®), SOD (Cayman®), GPx (Sunlogbiotech®), Ceruloplasmin (Sunredbio®), Immun status: IgG (Sunredbio®), CRP (Cusabio®), C4 (Mybiosource®), IL-1 beta (Sunredbio®), TNF-alpha (Sunredbio®), MDA (Cayman®), analyses were performed by Enzyme-linked immunosorbent assay (ELISA) commercial kits. Cortisol concentrations were determined by an auto biochemistry analyser (Fujifilm® DRI-Chem NX500i). ELISA kits and biochemistry analyser maintenance and validation procedures were performed according to the manufacturer's recommendations. Materials for validation and internal quality control solutions were provided by the kit manufacturers.

Alpha-tocopherol concentrations were determined using a commercial kit (Immuchrom®) on high-pressure liquid chromatography-ultraviolet (HPLC-UV) according to the manufacturer's instructions by a commercial laboratory (Delta Analiz).

All samples were tested in duplicate, and 0.05% differences were accepted between dublicate analyses. The intra-assay coefficient of variation (CV) was determined by duplicate analyses of certain samples and was below 4.1%, inter-assay CV (7.2%) was determined by analysing six samples on three different occasions.

2.2 | Statistical analysis

The variables were investigated using visual and analytical methods to determine whether or not they are normally distributed. Descriptive analyses were reported by using medians and interquartile range for the non-normally distributed variables. Since parameters were not normally distributed, the Wilcoxon test was conducted to compare two measurement points and the Kruskal–Wallis test was conducted group differences in the same measurement time. The Mann–Whitney *U* test was performed to test the significance of pairwise differences using Bonferroni correction to

TABLE 1 Mean serum antioxidant enzyme catalase (U/ml), ceruloplasmin (U/ml), superoxide dismutase (SOD) (U/ml), glutathione peroxidase (GPx) (U/ml)) concentrations of the ewes before and after transport

		T0			T1			*p*-value		
								Between	Intra	
Parameter	Group	Mean ± SD	Median	SEM	Mean ± SD	Median	SEM	T0 - T1	T0	T1
Catalase (U/ml)	G1	10.40 ± 2.75	10.90	0.98	10.97 ± 3.81	11.04	1.35	0.88	0.59	0.70
	G2	9.96 ± 2.75	10.87	1.45	10.08 ± 4.24	10.80	1.50	0.77		
	G3	8.77 ± 3.48	8.77	1.23	12.90 ± 5.10	11.40	1.81	0.09		
Ceruloplasmin (U/ml)	G1	79.92 ± 62.75	64.05	22.19	74.40 ± 77.99	52.50	27.57	0.88	0.71	0.43
	G2	97.43 ± 83.36	62.45	29.47	98.70 ± 79.32	61.10	28.05	0.77		
	G3	60.72 ± 41.48	55.81	14.67	56.94 ± 42.70	42.70	17.19	0.77		
SOD (U/ml)	G1	32.12 ± 3.09	33.00	1.09	32.85 ± 2.84	34.00	1.01	0.67	0.36	0.11
	G2	32.85 ± 2.58	32.93	0.91	35.37 ± 1.84	35.50	0.65	0.04		
	G3	33.57 ± 3.41	34.00	1.21	35.00 ± 2.77	35.00	0.98	0.34		
GPx (U/ml)	G1	49.93 ± 20.74	57.90	7.33	44.86 ± 27.83	47.58	9.84	0.48	0.05	0.31
	G2	29.73 ± 18.56[b]	28.00	6.56	62.03 ± 16.36[a]	64.90	5.79	0.01*		
	G3	34.18 ± 17.59[b]	39.99	6.22	62.36 ± 6.31[a]	64.25	2.23	0.01*		

Note: Values in the same row, appearing in pairs differ at a, b.

G1 (Control): 14 ml saline injection and not transported, G2: 14 ml saline injection and transported, G3: 14 ml vitamin E injection and transported.

Abbreviations: SD, standard deviation; SEM, standard error of the mean.

*$p < 0.05$.

adjust for multiple comparisons. The limit of statistical significance was assumed as $p < 0.05$. Statistical analyses were conducted with IBM® SPSS® (V. 21).

3 | RESULTS

The mean May temperature was reported as 17.7°C by the Turkish National Meteorological Agency. Only in G2 group SOD concentration increased from 32.85 ± 2.58 U/ml to 35.37 ± 1.84 U/ml between T0 and T1 ($p = 0.04$). GPx concentration founded higher (49.93 ± 20.74 U/ml) than transported groups (G2 = 29.73 ± 18.56 U/ml, G3 = 34.18 ± 17.59 U/ml) at T0. While the control group had no significant change, GPx concentrations altered twofold in transported groups (G2 and G3) ($p = 0.01$) (Table 1). Cortisol concentrations increased in all groups at T1 and founded above reference limits (42–82 nmol/L) (Jackson & Cockcroft, 2002; Table 2). In G3, cortisol concentrations founded 62.19 ± 23.46 and 94.11 ± 32.01 nmol/L in T0 and T1 respectively ($p = 0.05$).

CRP levels decreased in G1 and G2 between T0 and T1 ($p = 0.01$; Table 3). CRP levels founded higher in G3 compared to G2 at T1 ($p = 0.005$). C4 mean concentrations were founded highest in G2 at T0, and this trend was observed also at T1 ($p > 0.05$). Groups mean MDA concentrations were homogenous, and no significant change was observed after transport (Table 4). The control group had the highest serum alpha-tocopherol concentrations between all groups at T1 ($p = 0.004$). G3 and G2 differences founded statistically different comparing to control group at T1 ($p = 0.005$, $p = 0.001$ respectively). However, transported groups of alpha-tocopherol concentrations were observed in a decreasing trend between T0 and T1 (Table 5).

4 | DISCUSSION

Importing live farm animals from overseas countries and road transport inside countries are very common, but it is very well known that it may constitute stress for animals. This study aimed to find the effects of vitamin E injection prior to short term transport in ewes.

In agreement with the present study, Çetin et al. (2011) did not observe any change in serum catalase levels of transported yearling lamps.

While road transport showed a significant influence on GPx concentrations (G2: T0: 29.73 ± 18.56 U/ml, T1: 62.03 ± 16.36 U/ml; G3: T0: 34.18 ± 17.59 U/ml, T1: 62.36 ± 6.31 U/ml, the control group (G1: T0: 49.93 ± 20.74 U/ml, T1: 44.86 ± 27.83 U/ml) did not affect. While GPx levels founded in normal ranges (30.2–48.0 E.U./g Hb) at T0, only the control group serum GPx level was between normal ranges (Osame et al., 1990). In agreement with this study, 30 IU dl-alpha-tocopherol/kg BW repeated IM injection to pregnant ewes 12 times following the vaccination was not change serum extracellular GPx levels but altered serum alpha-tocopherol and IgG levels on ewes (Anugu et al., 2013). Parallel to our findings, 360 IU supplemental vitamin E to ewes 5–6 weeks before lamping did not occur any significant change in GPx levels (Dønnem et al., 2015).

Cortisol concentrations observed an increasing trend in all groups between T0 and T1 in agreement previous findings (Ahmad Mir et al., 2019; Avcı et al., 2008; Horton et al., 1996; Morán et al., 2017; Swanson & Morrow-Tesch, 2001). Elevation of cortisol levels explained by the activation of the sympathetic nervous system (Ahmad Mir et al., 2019) and handling stress (Knowles, 1999). Our findings support these two ideas. On the other hand, vitamin E administration to calves and cows decreased plasma cortisol levels (Gupta et al., 2005; Khan et al., 2016; Mudroň et al., 1996; Nockels, 1996; Reddy et al., 1985). In contrast with these reports in the present study, the reduction did not observe in cortisol levels in the alpha-tocopherol supplemented group. As indicated by Morán et al. (2017) and Swanson and Morrow-Tesch (2001) cortisol levels increased by transport stress. Administering time of vitamin E may be influenced and may not show enough bioactivity on cortisol level.

Although serum CRP levels were higher than the reference ranges that Iliev and Georgieva (2018) has reported in G1 and G3 groups at T0, they were founded between reference ranges (5.23 ± 0.42–9.11 ± 0.64 mg/L) that Vojtic and Krajnc (2000) has been reported at T1. In all groups, a decreasing trend was observed between T0 and T1 in G1 and G2 this trend was found statistically significant. Vitamin E is known as a reducing factor of CRP (Prasad, 2006; Saboori et al., 2015; Singh et al., 2005). In this study, all groups CRP levels decreased. In a study with twenty dogs, 3 h of transport

TABLE 2 Serum cortisol (nmol/L) concentration of the ewes before and after transport

		T0			T1			*p*-value		
								Between	Intra	
Parameter	Group	Mean ± SD	Median	SEM	Mean ± SD	Median	SEM	T0 - T1	T0	T1
Cortisol (nmol/L)	G1	75.08 ± 27.21	71.73	9.62	109.56 ± 59.27	104.83	20.96	0.32	0.74	0.46
	G2	81.52 ± 57.13	58.35	20.20	91.73 ± 72.69	61.11	25.70	0.88		
	G3	62.19 ± 23.46	56.28	8.29	94.11 ± 32.01	99.04	11.32	0.05		

Note: G1 (Control): 14 ml saline injection and not transported, G2: 14 ml saline injection and transported, G3: 14 ml vitamin E injection and transported.

Abbreviations: SD, standard deviation; SEM, standard error of the mean.

TABLE 3 Inflammation response parameter (C-reactive protein (CRP) (mg/L), complement activation and chemokine marker (complement component 4 (C4) (mg/L)), immunglobulin G (IgG) (g/L), lymphocyte activation factor (interleukin 1 beta (IL-1 beta) (pg/ml)), systemic inflammation cytokine (tumour necrosis factor-alpha (TNF-alpha) (ng/L)) concentrations of the ewes before and after transport

		T0			T1			*p*-value		
								Between	Intra	
Parameter	Group	Mean ± SD	Median	SEM	Mean ± SD	Median	SEM	T0 - T1	T0	T1
CRP (mg/L)	G1	14.75 ± 16.04[a]	9.34	5.67	4.55 ± 2.08[b]	4.23	0.74	0.01*	0.27	0.01*
	G2	7.46 ± 6.03[a]	6.04	2.13	3.49 ± 2.23[b]	3.13[B]	0.79	0.01*		
	G3	12.79 ± 8.43	9.93	2.98	10.60 ± 6.58	10.85[A]	2.33	0.67		
C4 (mg/L)	G1	64.51 ± 32.55	61.30	11.51	47.98 ± 20.50	40.50	12.60	0.16	0.09	0.35
	G2	98.57 ± 58.84	77.50	20.81	81.78 ± 73.29	55.65	25.91	0.77		
	G3	43.97 ± 32.58	36.74	11.52	49.26 ± 20.50	38.00	7.25	0.40		
IgG (g/L)	G1	9.17 ± 8.59	7.29	3.04	10.90 ± 8.92	8.53	3.16	0.77	0.59	0.53
	G2	16.58 ± 16.16	9.65	5.72	8.59 ± 11.27	4.39	3.99	0.32		
	G3	13.04 ± 8.24	13.00	2.91	9.06 ± 7.77	6.52	2.75	0.40		
IL–1 beta (pg/ml)	G1	60.95 ± 51.71	49.90	18.28	50.92 ± 62.21	26.70	22.00	0.48	0.92	0.20
	G2	89.36 ± 76.97	75.30	12.58	76.60 ± 47.30	73.10	14.90	0.88		
	G3	61.15 ± 35.57	50.75	12.58	52.04 ± 42.14	36.40	14.90	0.40		
TNF-alpha (ng/L)	G1	158.42 ± 98.09	131.72	34.68	157.15 ± 122.27	125.20	43.23	0.26	0.57	0.89
	G2	214.38 ± 141.28	172.00	49.95	180.81±129.89	131.70	45.92	0.77		
	G3	144.94 ± 79.85	121.50	28.23	139.21 ± 83.58	110.50	29.55	0.67		

Note: Values in the same row, appearing in pairs differ at a, b, values in the same column, appearing in pairs differ at A, B.

G1 (Control): 14 ml saline injection and not transported, G2: 14 ml saline injection and transported, G3: 14 ml vitamin E injection and transported.

Abbreviations: SD, standard deviation; SEM, standard error of the mean.

*$p < 0.05$.

TABLE 4 Serum malondialdehyde (MDA) (μmol/L) concentration of the ewes before and after transport

		T0			T1			*p*-value		
								Between	Intra	
Parameter	Group	Mean ± SD	Median	SEM	Mean ± SD	Median	SEM	T0 - T1	T0	T1
MDA (μmol/L)	G1	0.50 ± 0.28	0.47	0.10	0.40 ± 0.08	0.41	0.03	0.61	0.10	0.21
	G2	0.27 ± 0.16	0.25	0.06	0.32 ± 0.08	0.32	0.03	0.31		
	G3	0.41 ± 0.16	0.41	0.06	0.39 ± 0.16	0.38	0.06	0.88		

Note: G1 (Control): 14 ml saline injection and not transported, G2: 14 ml saline injection and transported, G3: 14 ml vitamin E injection and transported.

Abbreviations: SD, standard deviation; SEM, standard error of the mean.

resulted in a high increase of CRP levels after transportation (Fazio et al., 2014). Although, G3 CRP levels were founded higher than G2 ($p < 0.05$), parallel with our findings (G1 and G2 groups CRP levels were not statistically significant), in a recent study, it was found that CRP levels were not affected by heat and transportation stress in goats (Al-Dawood, 2017). CRP-related ruminant medicine data are very rare in the literature; thus, more studies should be carried on this topic.

In contrast with this study under long-term transport stress (approximately 22 h.), vitamin E and selenium, the administration reduced serum MDA levels (Control: 35 ± 15 μmol/L, vitamin E and selenium treatment: 13 ± 5 μmol/L) in Holstein dairy cows (Aktas et al., 2011). Çetin et al. (2011) reported increased SOD, MDA, GPx activities in 10 h. and 24 h. transported yearling lamps and although 5 h. transport did not cause significant change and concluded 5 h transport stress is not enough to observe the antioxidant enzyme and MDA concentration differences. The reason that no change was observed in the present study in serum MDA levels may be related to the length of transport time.

Alpha-tocopherol is known as the fundamental and potent lipid-soluble antioxidant in plasma (Singh et al., 2005). In this study, SOD activity only increased significantly without vitamin E supplemented and transported group. Alpha-tocopherol injection acts a preservative role in the increase of SOD levels in transported groups between T0 and T1. In the study that supplemented vitamin E as an antioxidant in cross-bred dairy cows, SOD activity

TABLE 5 Serum alpha-tocopherol (μmol/L) concentration of the ewes before and after transport

		T0			T1			*p*-value		
								Between	Intra	
Parameter	Group	Mean ± SD	Median	SEM	Mean ± SD	Median	SEM	T0 - T1	T0	T1
Alpha-tocopherol (μmol/L)	G1	0.78 ± 1.62	0.20	0.57	1.22 ± 0.82	1.16	0.29	0.16	0.06	0.004*
	G2	0.65 ± 0.56[a]	0.43	0.20	0.27 ± 0.14[Ab]	0.26	0.05	0.01*		
	G3	0.21 ± 0.20	0.16	0.07	0.14 ± 0.07[B]	0.12	0.03	0.39		

Note: Values in the same row, appearing in pairs differ at a, b, values in the same column, appearing in pairs differ at A, B.
G1 (Control): 14 ml saline injection and not transported, G2: 14 ml saline injection and transported, G3: 14 ml vitamin E injection and transported.
Abbreviations: SD, standard deviation; SEM, standard error of the mean.
*$p < 0.05$.

founded increased in the vitamin E supplemented group (Belhadj Slimen et al., 2019). Another study reported that water buffalo SOD levels decreased related to transport stress (EL-Deeb & El-Bahr, 2014). The present study concludes this stressor was not enough to evaluate the SOD levels.

There is very limited research on serum alpha-tocopherol concentrations of sheep. Our findings are below the reference limits comparing to Storer (1974) and reported results of Toker (2007). In this study, a significant decrease was observed in the G2 which was not treated with alpha-tocopherol but transported group. Besides, the control group showed the highest alpha-tocopherol concentration after transportation stress-induced. Alpha-tocopherol supplementation was prevented transported ewes from a dramatic decrease of alpha-tocopherol concentration. Nevertheless, in the control and G3 groups, no changes were observed in the present study between T0 and T1. Biochemical values may change due to ration, gender, environmental conditions, physical condition, collection method and sample storage before analysis (Finno & Valberg, 2012). These conditions may influence this study's serum alpha-tocopherol concentrations. In the literature search, information related to serum alpha-tocopherol reference values are rare and this topic is needed to develop in further studies.

Several limitations may have influenced the results obtained herein. The lack of significant differences and higher variances in the parameters during the applications might be due to the high variability observed within each group. Antioxidants may have an immunomodulatory effect that supports or prevents the release of pro-inflammatory cytokines depending on the dose, antioxidant type or stimulation. This study did not have a chance to compare these dependents. Hemogram parameters could allow elaborating on the discussion of the results. Parameters that are evaluated in the present study are variable and short life; therefore, one more sampling point in the middle of the transport may show the trend clearer. For financial reasons, the number of subjects was relatively small in the groups. Enzymatic parameters can be easily influenced by individuals and environmental conditions further studies should incorporate more subjects to procure less variance in measured parameters.

5 | CONCLUSION

In this study, limited information can produce on the effects of treatment of alpha-tocopherol ten minutes before transport; therefore, further studies are needed on this topic. Vitamin E sources, treatment way, treatment time before transport, combination with selenium and different vitamins (such as vitamin C) can be performed in further studies on this topic.

Consequently, the single-dose 2100 IU/ind. Even though, DL-alpha-tocopherol injection was decreased the falling trend of serum alpha-tocopherol, in general, failed in reducing transport stress in this study conditions. In future studies, treating a higher concentration of single-dose vitamin E injection or oral alpha-tocopherol supplement over a longer period before transport can be recommended.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The experimental procedures were approved with the certification (02.10.2017, 2017/06) by the Local Ethical Committee for Animal Studies (Veterinary Control Central Research Institute, Turkey).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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