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Original Article

Effects of the Melatonin and Vitamin E (Alpha-Tocopherol Acetate) as Antioxidants on Biochemical Blood Parameters, Lipid Profile, and Muscle Vitamin E Concentration in Awassi Lambs Fed a High-Energy Diet and Normal Diet

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Abstract

Health specialists currently suggest low-cholesterol diets, suggesting that cholesterol in the form of high-density lipoprotein (HDL) reduces the risk of chronic atherosclerosis. The large volume of literature describes the biological roles of vitamin E and its application to preventing disease and improving the health and productive performances of farm animals. The present study aimed to evaluate the effects of vitamin E (Alpha-tocopherol acetate) supplementation and melatonin implants on biochemical blood, lipid profile and muscle vitamin E content of Awassi male lambs fed by a high and normal diet in Iraq. The lambs were divided into teen groups as control normal energy diet T1 (NED) T2 (HED) concentrated lamb fattening feed. Two levels of melatonin (18 and 36 mg implant) were applied to T3, T4, T5, and T6 treatment and 2 levels of Vitamin E (Alpha-tocopherol acetate) diet 200 mg/kg, 400 mg/kg to T7. T8. T9 and T10, respectively. Results from the present study indicate that Vitamin E 200, 400 mg/lamb/day and melatonin implantation 18 mg, 36 mg/lamb/day significantly (P<0.05) increased total protein in serum while decreasing globulin level, glucose concentration in serum, melatonin implantation 36 mg/lamb and vitamin E 400 mg/lamb/day recorded significantly (P<0.05). The same effect on decreasing cholesterol concentration in serum 42.6mg\dl, 40.5 mg\dl, respectively, compared to non-treated groups. Vitamin E 200 mg/kg/lamb recorded the lowest AST level in serum, 43.3. Lambs implanted with Melatonin 36 mg/lamb and fed a high-energy diet (T8) resulted in a significant decrease of serum ALT activity (P<0.05) in comparison to other treated groups 12.7 U/L was achieved. Lambs fed a normal energy diet with vitamin E 200 mg/kg/lamb (T4) exceeded other treated groups, decreasing ALT serum levels by 9.35 U/L. Interestingly, muscle vitamin E concentrations for lambs received 200, 400 mg/lamb/day on the 2nd, 7th and 14th days of the storage period, and fed high energy diet (T10) or normal energy diet (T5) were significantly higher compared to control group (T1, T6).

Keywords: Awassi lamb, Melatonin implants, Alpha-tocopherol acetate, Blood biochemical trait, LD muscle vitamin E concentration, Lipid profile

1. Introduction

Health specialists currently suggest low-cholesterol diets, suggesting that cholesterol in the form of high-density lipoprotein (HDL) reduces the risk of chronic atherosclerosis. The large volume of literature describes the biological roles of vitamin E and its application to preventing disease and improving the health and productive performances of farm animals. In recent years, vitamin E has frequently been used in animal nutrition to improve meat quality. In investigations with laboratory animals and human participants, the relationship between dietary vitamin E and cholesterol metabolism has been examined; inconsistent results have been discovered. In rats, supplementing with varying dietary doses of vitamin E reduced serum cholesterol levels and improved cholesterol levels in the arteries. Dietary supplementation with a-tocopheryl acetate raises the concentrations of a-tocopherol in the tissues of sheep (1, 2), but other components of the diet may also affect tissue levels. Antioxidants are any substances that delay or inhibit oxidative damage to the cellular molecule. Currently, health professionals are recommending diets low in cholesterol and indicate that cholesterol in a more significant proportion as high-density lipoprotein (HDL) reduces the risk of chronic atherosclerosis. The relationship between dietary vitamin E and cholesterol metabolism has been investigated in studies with laboratory animals and human subjects; conflicting results have been observed. In studies with rats, supplementation with various dietary levels of vitamin E decreased cholesterol levels in the serum and liver (3, 4). The efficiency of turning dietary vitamin E into muscle vitamin E is influenced by several factors, including dietary concentration, muscle type, and the type of vitamin E used (5). For these reasons, the muscle vitamin E content is highly variable, leading to inconsistencies among prior studies. In recent years, many studies have been conducted on the effects of dietary vitamin supplementation on various animal performances (6, 7). Oxidative stress due to the increased production of free radicals and reactive oxygen species, and/or a decrease in antioxidant defences, leads to damage of biological macromolecules and disruption of normal metabolism and physiology when reactive forms of oxygen are produced faster than they can safely be neutralized by antioxidant mechanisms (8). Melatonin has beneficial effects, including stimulating antioxidant enzymes and inhibiting lipid peroxidation, so it contributes to protection from oxidative damage. Melatonin is secreted primarily by the pineal gland in response to darkness (9).

The objective of the prese nt study was to compare normal and high-energy diets implanted with two levels of melatonin (18-36 mg/) and two levels of vitamin E (200 mg/kg and 400 mg/kg) on biochemical blood, lipid profile and muscle (LD) vitamin E concentration during storage period 2^{nd},7^{th} and 14^{th} day.

2. Materials and Methods

This experiment was conducted in 2021 in the private animal production farm at Bahrka, Erbil government, Iraq. Around 50 Awassi lambs at 4 to 5 months of age (determined to weigh 29±1kg) were selected for the currentstudy. Lambs were breaded under normal pasture conditions before thestudy. One week before the trial, lambs were moved to the research facility and distributed randomly to treatment groups. The feeding period was designed as 2 diet energy levels Normal Energy (T1), and High Energy Level (T6) concentrated lamb fattening feed. Two levels of melatonin (18 and 36 mg implant) were applied to T2, T3 and T7, T8 and 2 levels of Vitamin E diet 200 mg/kg, 400 mg/kg T4, T5 and T9, T10. Lambs were housed in shaded pens, and water was provided as ad libitum. Concentrated feed is formulated to meet the requirement for a gain of 200-250 g/day for lambs, according to the NRC (Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids; 2007) (Table 1). Alfalfa hay and concentrated diet (based on corn and soybean meal ratio was around 20:80. Concentrated Diet provided 2 times/day. The experiment lasted 60 days. 50 Lamb was randomly assigned to 10 experiment groups, 5 animals per each, as below:

T1: Normal Energy DIET (NDC).T2: ND+M 18mg

T3: ND+M 36mg.T4: ND+VIT E 200mg

T5: ND+VIT E 400mgT6: High Energy DIET (HEDC)

T7: HEDC+M 18mgT8: HEDC+M 36mg

T9: HEDC+VIT E 200mg. T10: HEDC+VIT E 400mg

Meat samples from the Longissimus Dorsi (LD) (150 g) were collected 24 h postmortem and kept at 4 ^{0}C, placed in trays and wrapped with oxygen-permeable PVC film, 2.5 cm thick samples were collected from the Longissimus Dorsi (LD) between the 12^{th} and 13^{th} ribs of each left half carcass and kept in the fridge at 4^0C for (7 days). Muscle vitamin E content of Longissimus Dorsi (LD) muscle was measured on days 2 and 7 of storage in the fridge at 4^0C and on day 14 after storage in the Freezer -at 20°C.

Table 1. Experiment diet ingredient and chemical composition

Ingredients	Control diet %	High-energy diet %
Corn	23.5	31
Wheat bran	7.29	13.78
Barley	50	33
Soybean meal 48%	14	15.67
Salt	0.96	0.30
Limestone	4	2
Vegetable oil	-	4
Vitamin Mineral Premix	0.25	0.25
Total	100	100
Chemical composition		
Dry matter	94.23	95.13
Ash	6.4	5.7
Crude protein	16	15.3
ME (KCal/kg)	2650	3156
Crude fat	1.796	2.296

2.1. Blood Samples Collection and Blood Parameters

50 plasma samples from experiment treatments (Lamb) were collected, and concentrations of Glucose, Total protein, Albumin, Globulin, lipid profile traits (Triglycerides, Cholesterol, HDL, LDL, VLDL) in plasma of animals of experimental treatments. Approximately 10 mL of blood samples were collected from the jugular vein of each animal before the morning feed, and 5 ml of blood was transferred to a vacutainer for serum preparation. Centrifugation was done at 3000 rpm for 15 min for serum separation to separate the plasma from the cells. Then, the serum was transferred to an Eppendorf tube for storage at −20°C until analysis. All samples were processed at the laboratory of the Departments of Animal Science, Agricultural College, Salahaddin University-Iraq, Medline private laboratory and Hilla private laboratory. Serum samples were tested using an automatic biochemical analyzerby using auto analyzer (Cobas 6000, Roche diagnostics. Germany) to estimate lipid profile (triglyceride, cholesterol, HLD, LDL, VLD), blood biochemical traits (albumin, globulin, glucose, total protein TP) and Activities of ALT, AST enzymesaccording to the method described by Rahman, Islam (10).

2.2. Estimate of Muscle Vitamin E Concentrations in LD During the Storage Period

Vitamin E analysis was performed according to the method described by Chauveau-Duriot, Doreau (11) for determining vitamin E by high-performance liquid chromatography. The muscle vitamin E content of the Longissimus Dorsi (LD) muscle was measured on days 2 and 7 of the storage period in the fridge at 4^0C and day 14 after storage in a Freezer -at 20°C.

2.3. Statically Analysis

The obtained data were subjected to a factorial two-way analysis design, the effect of two different energy levels (low and high), two levels of Melatonin implant (18, 36 mg) and two levels of Vitamin E (200, 400 mg/kg) effect of treatments and interaction between them using general linear model (GLM) SAS.2012 program as in the following model.

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

Where:

Y_{ijk}:experimental unit of (k) lamb for each factor diet, different levels of vitamin E, melatonin implants

M: Overall mean

A_i: Effect of i^{th} (High energy diet, Normal energy diet)

B_j:Effect of j^{th} (control, melatonin 18mg-36mg, vitamin E 200mg-400mg)

$_{Bij}$:Effect of interactions between i^{th} Diet (High energy diet, Normal energy diet), and treatment j^{th} (control, melatonin 18mg-36mg, vitamin E 200mg-400mg)

Y_{ijk}:Experimental error assumed to be NID with $(0,\sigma^2 e)$

Duncan multiple range tests (1955) were also used to test the difference between the sub-classes of each factor.

3. Results and Discussion

3.1. Lipid Profile Traits

Effects of the Melatonin and Vitamin E (Alpha-tocopherol acetate) as Antioxidants on lipid profile (Cholesterol, Triglyceride, HDL, LDL, VLDL) of Awassilambs Fed a High-Energy Diet and Normal Diet summarized in table 2. Diet was not affected significantly (*P*<0.05) on Cholesterol, Triglyceride, LDL and VLDL. Treatments had a significant (*P*≤0.05) influence on HDL levels in serum, as did a high-energy diet. Melatonin implantation 36mg/lamb and vitamin E 400mg/lamb/day recorded significantly (*P*<0.05) same effect on decreasing cholesterol concentration in serum 42.6, 40.5 mg\dl respectively compared to other treatments, VLDL mg\dl decreased with vitamin E supplementation 200 mg/lamb/day treated group fed high or normal energy diet 2.32 mg\dl, 2.36 mg\dl moreover lower triglyceride 16.6 mg\dl recorded for vitamin E 400 mg/lam/day fed high energy diet (T10). Vitamin E is a potent lipid-soluble antioxidant that is the major oxidation chain reaction–breaking compound in membranes and an important factor in the protection of polyunsaturated fatty acids in LDL against lipid peroxidation; Vitamin E contains chemicals called tocotrienol that may lower cholesterol levels. The results of the present study were in line with previous work with other species. Hermann, Ward (12) reported that human vitamin E administration in the form of synthetic Alf-Tocopherol acetate (1000 IU/week) contributed to the increase of the HDL-cholesterol plasma concentration, with decreases in the very low-density lipoprotein (VLDL) fraction of cholesterol and total triglycerides (TG).

Table 2. Effects of the melatonin and vitamin E (Alpha-tocopherol acetate) as antioxidants on lipid profile in Awassi lamb fed a high-energy diet and normal diet

Item	Cholesterol mg\dl	Triglyceride mg\dl	HDL mg\dl	LDL mg\dl	VLDL mg\dl
Diet					
Normal Diet	44.0	18.8	24.0^{b}	17.6	3.48
High Diet	46.4	18.4	26.2^{a}	17.0	3.44
Treatments					
Diet	52.0^{a}	28.0^{a}	19.9^{d}	21.3^{a}	4.85^{a}
M 18 mg	44.8^{b}	16.1^{b}	23.6^{c}	15.4^{b}	3.68^{b}
M 36 mg	42.6^{bc}	16.5^{b}	25.7^{bc}	16.4^{b}	3.32^{bc}
VIT.E 200 mg	45.9^{b}	17.2^{b}	28.4^{a}	16.7^{b}	2.34^{d}
VIT.E 400 mg	40.5^{c}	15.3^{b}	27.7^{ab}	16.6^{b}	3.12^{c}
Interaction					
NDC	52.0^{a}	28.6^{a}	19.6^{d}	22.7	5.33^{a}
ND×M 18 mg	44.8^{b}	18.1^{b}	20.7^{d}	16.2	3.83^{bc}
ND×M 36 mg	44.0^{b}	15.4^{bc}	26.1^{bc}	17.0	3.09^{cde}
ND×VIT, E 200 mg	45.7^{b}	18.3^{b}	27.9^{abc}	16.7	2.36^{e}
ND×VIT.E 400 mg	33.4^{c}	13.9^{c}	25.5^{bc}	15.4	2.79^{de}
HDC	52.1^{a}	27.4^{a}	20.2^{d}	20.0	4.38^{b}
HD × M 18 mg	44.9^{b}	14.2^{c}	26.6^{abc}	14.6	3.54^{cd}
HD ×M 36 mg	41.2^{b}	17.7^{bc}	25.3^{c}	15.8	3.55^{cd}
HD×VIT.E 200 g	46.2^{ab}	16.1^{bc}	29.0^{ab}	16.7	2.32^{e}
HD ×VIT.E 400 mg	47.6^{ab}	16.6^{bc}	29.9^{a}	17.9	3.45^{cd}
SEM*	1.23	1.15	0.838	0.625	0.207
Mean	45.2	18.6	25.1	17.3	3.46
***P*-value**					
Diet	NS**	NS.	0.0064	NS.	NS.
Treatments	0.0011	<.0001	<.0001	0.0184	<.0001
Interaction	0.0014	<.0001	0.0002	NS.	0.0001

* SEM: Standard Error Mean.

** NS: Non-Significant at probability value (*P*≤0.05).

a, b, c: means in the same Columns with different superscripts differ significantly at probability value (*P*≤0.05). NDC: normaldietcontrol. HDC: high diet control. ND: Normal diet. HD: HighdietM18mg:melatonin.18mg.M36mg:melatonin. 36 mg.

In contrast to our findings, El-Shahat and Abdel Monem (13) found that supplementing sheep diets with 25-50 mg of vitamin E per kg feed did not affect any blood parameters. In contrast to the current findings, Hidiroglou, Wolynetz (14) found that when lambs were given significant doses of vitamin E (300 mg/lamb/d of DL-ascorbic acid) (t-tocopheryl acetate) twice a day for two months, their lipid profile did not change. The current findings are confirmed by Alipour, Vakili (15), who found that lambs given Vitamin E (DL-tocopheryl acetate at 225 IU/ week for 42 days had lower (P<0.01) plasma cholesterol concentrations than the control group.

Melatonin implantation 18 mg/lamb with high energy diet (T7) raised significantly (P<0.05) serum HDL 26.6 mg\dl in comparison to the same dose 18 mg/lamb with normal energy diet (T2) 20.7 mg\dl. Higher serum LDL 22.7 mg\dl was recorded for the non-treated group (T1) fed normal energy diet; furthermore, lower serum LDL14.6 mg\dl was recorded for Melatonin 18mg/lamb fed high energy diet. Also treated group (T7, T8) with melatonin 18mg/lamb and 36 mg/lamb recorded lower serum VLDL mg\dl compared to the non-treated group either fed high (T5) or normal energy diet (T1). Melatonin may play a function in modulating lipid metabolism in animals through its influence on the level of insulin hormone that affects lipid-carbohydrate metabolism, present findings of melatonin implantation on lipid profile in Awassi lams of this experiment agreed with a report by Darul and Kruczynska (16) whose recorded a decline in cholesterol level in dairy goats over 4 hours of melatonin dose of 0.1 mg/kg body weight treatment. Our result agrees with the findings of Kassim, Al-Rishdy (17), who indicated that the concentration of cholesterol was significantly decreased in treating ewes with melatonin at two doses of 12 and 15 mg/ head compared with control groups and significant effect of melatonin treatment on cholesterol concentrations during gestation months in Arabi ewes. Our results agree with the results of Khatun, Wani (18), who found a decrease in cholesterol concentration in 12 and 15 mg/ head melatonin-treated groups, as compared with the control group in the first and second months of gestation of ewes, might be due to variation in the body score of ewes or to the accumulation effect of melatonin treatment. Also, cholesterol concentration decreased in the first month of gestation and continued gradually to the last month. Present results supported the report of Zhang, Meng (19), who illustrated that oral administration of Melatonin 5mg/kg/BW/daily for 30 days in rats alleviated the increase of total lipids, total cholesterol and triglycerides.

Changes in dietary ME levels will undoubtedly lead to changes in nutrient metabolism, which the changes in blood biochemistry could reflect. There is limited literature on diet's influence on serum lipid profile in lamb.

3.2. Biochemical Blood Parameters

The influence of two doses of melatonin, vitamin E, and a normal, high-energy diet on the blood biochemical characteristics of Awassi lambs was presented in table 3. Diet had a significant (P<0.05) effect on total protein and serum glucose levels. The effect of high energy diet recorded a higher total protein level of 6.64 g/dl than the normal energy diet of 6.29 g/dl, and the normal diet had a higher glucose level of 70.5 mg/dl than high energy diet of 68.1 mg/dl. The effects of diet on albumin and globulin levels were non-significant (P<0.05). The treatments significantly affected total protein, globulin level, and glucose concentration in serum (P<0.05). Adding different doses of Vitamin E 200,400 mg/lamb/day and melatonin implantation 18 mg, 36mg/lamb recorded a significant (P<0.05) increase in serum total protein while decreased globulin level, glucose concentration in serum, the lower glucose level in serum 63.7 g/dl was recorded for vitamin E 400 mg/lamb/day significantly (P<0.05) in comparison to other treated groups. There was a significant (P<0.05) effect of interacted factors on total protein and glucose levels. Interaction between high energy diet with vitamin E

200 mg/lamb/day (T9) significantly (*P*<0.05) increased total protein in comparison to other interacted groups, on the other hand, higher glucose concentration in serum significantly (*P*<0.05) reduced for normal energy diet with vitamin E 400 mg/lamb/day treated groups (T5) than other treated groups, furthermore albumin level not affected by diet, treatments and interaction between a diet with treatments. According to the results of the present study, supplementing extra antioxidants (vitamin E 200-400 mg/lamb/day and melatonin 36 mg/lamb) to Awassi lambs dramatically improved total serum protein levels and reduced glucose levels in serum. The stimulating effect of melatonin on blood biochemical traits may protect against oxidative damage during the fattening period in Awassi lamb. It is suggested that melatonin may play a role in controlling lipid metabolism in the animals through its influence on the level of insulin hormone that affects lipid-carbohydrate metabolism. Mahmoud, Abdel-Raheem (20), (21) recorded similar findings and found that in Ossimi rams, treated groups with 450 mg of Vitamin E for 1 month, there was a substantial rise in serum total protein concentration. Present results in line with Kasapidou, Enser (22) showed that a quadratic response was observed in blood glucose, proteins and globulins (*P*<0.05), whereas the albumin/ globulin ratio, was linearly reduced (*P*<0.01) when four levels of a natural vitamin E dietary inclusion of Herbal E-50 (Nuproxa) at 0, 1, 2 and 3 g/kg dry matter (DM) for 60 days on blood metabolites, evaluated in (Hampshire x Suffolk) lambs. Our findings were in contrast with the results of Shinde, Dass (23), who observed that supplementation of vitamin E (300 IU) in the diet did not affect serum glucose levels in buffalo calves, total serum protein (TP) and globulin, but not albumin, were increased (*P*<0.05) for lambs of treated groups vs control and decomposition. On the other hand, the present findings contrast with reports by Ziaei (24) reported that supplementation of diets with different levels of vitamin E (20 and 50 mg/kg of feed) in Raieni goats had a non-significant effect (*P*>0.05) on albumin or total protein, but vitamin E supplementation at the level of 50 mg/kg tended to (*P*=0.132) increased blood glucose, present findings concur with the reports of El-Shahat, and Abdel Monen (13) who illustrated that supplementation of ewe diets with vitamin E had no significant effect on any blood parameters. Also. Al-Judi (25) established that there was a non-significant difference in total protein ratio between the treated group with 1g/day for 6 weeks vitamin E and non-treated vitamin E groups in Awassi sheep, while the ratio of albumin decreased significantly after 2 and 4 weeks for vitamin e treated group, with a significant increase in the globulin ratio at the same period. Our results contrast with Kassim, Al-Rishdy (17), who indicated that no significant differences between different doses of 9 mg, 12 mg and 15 mg/head of melatonin were obtained between groups in total protein and albumin concentrations after 1 hour of melatonin treatment. Our results align with Zhang, Meng (19), who found that Oral administration of Melatonin 5 mg/kg BW/daily for 30 days in rats attenuates the decrease in total proteins and glucose levels and reduces oxidative stress.

Dietary energy is essential to nutrient intake, digestion, metabolic efficiency and production performance. Blood biochemical indices can reflect the changes in livestock's metabolic, growth and development status. Present results are in line with results recorded by Wang, Zhang (26), who established that Hu Lambs were fed diets with 5 levels of metabolizable energy 9.17, 9.59, 10.00, 10.41 and 10.82 MJ/kg for 60 days, with an increase of dietary ME level linearly increased the concentrations of serum total protein (*P*<0.001), glucose level decreased in serum (*P*=0.004). While our results, in contrast with Chauhan, Ponnampalam (27), who found that there was a significant (*P*=0.002) effect of diet on plasma glucose levels of Merino lambs, lambs on High energy 11.8 MJ ME/kg diet showed the greater concentration of plasma glucose compared to Low energy 7.8 MJ ME/kg diet lambs.

Table 3. Effects of the melatonin and vitamin E (Alpha-tocopherol acetate) as antioxidants onbiochemical blood parameters in Awassi lamb fed a high-energy diet and normal diet

Item	Protein g/dl	Albumin g/dl	Globulin g/dl	Glucose mg/dl	AST (U/L)	ALT (U/L)
Diet						
Normal Diet	6.29^{b}	3.11	2.86	70.5^{a}	49.8	12.9
High Diet	6.64^{a}	3.35	2.68	68.1^{b}	51.5	13.6
Treatments						
Diet	5.28^{b}	3.02	3.21^{a}	74.5^{a}	60.1^{a}	16.6^{a}
M 18 mg	6.56^{a}	3.39	2.42^{b}	71.0^{b}	50.3^{b}	13.5^{b}
M 36 mg	6.58^{a}	3.27	2.81^{ab}	69.6^{bc}	48.2^{bc}	13.4^{b}
VIT.E 200 mg	7.05^{a}	3.19	2.73^{b}	67.5^{c}	43.3^{c}	10.4^{c}
VIT.E 400 mg	6.84^{a}	3.31	2.69^{b}	63.7^{d}	51.8^{b}	12.4^{bc}
Interaction						
NDC	5.13^{c}	2.82	3.19	76.3^{a}	60.5^{a}	16.1^{ab}
ND×M 18 mg	6.32^{b}	3.34	2.48	70.6^{bcd}	49.6^{bcd}	14.0^{abc}
ND×M 36 mg	6.61^{b}	3.59	3.02	73.5^{ab}	44.3^{cd}	14.1^{abc}
ND×VIT,E 200 mg	6.67^{b}	3.10	2.92	68.4^{cde}	42.9^{d}	9.35^{d}
ND×VIT.E 400 mg	6.72^{ab}	2.73	2.70	63.5^{f}	54.1^{ab}	11.3^{cd}
HDC	5.44^{c}	3.21	3.23	72.8^{abc}	59.8^{a}	17.2^{a}
HD×M 18 mg	6.81^{ab}	3.44	2.36	71.3^{bcd}	50.9^{bcd}	13.0^{abcd}
HD×M 36 mg	6.55^{b}	2.95	2.60	65.6^{ef}	52.2^{abc}	12.7^{bcd}
HD×VIT.E 200 g	7.43^{a}	3.28	2.55	66.7^{def}	43.7^{cd}	11.4^{cd}
HD×VIT.E 400 mg	6.96^{ab}	3.89	2.68	64.0^{ef}	50.7^{bcd}	13.5^{abcd}
SEM*	0.158	0.102	0.0788	0.996	1.51	0.579
Mean	6.46	3.23	2.77	69.3	50.7	13.2
***P*-value**						
Diet	0.0311	N.S.**	N.S.	0.0238	N.S.	NS.
Treatments	<.0001	NS.	0.0280	0.0002	0.0009	0.0074
Interaction	0.0007	NS.	NS.	0.0009	0.0054	0.0352

* SEM: Standard Error Mean.** NS: Non-Significant at probability value ($P \leq 0.05$).a, b, c: means in the same Columns with different superscripts differ significantly at probability value ($P \leq 0.05$).NDC: normal diet control. HDC: high diet control. ND: Normal Diet. HD: High Diet. M18 mg: melatonin 18 mg. M36 mg: Melatonin. 36 mg.

3.3. Activities of ALT, AST Enzymes in Serum

Enzymes are biocatalysts in specialized protein structures with catalytic properties. In clinical enzymology, enzymes such as transaminases (AST and ALT) are crucial for diagnosis (28, 29). The evaluation of blood metabolites is important as an indicator of animal health. For example, increased liver enzymes (ALT, AST) in the blood indicate liver damage. Effects of the Melatonin and Vitamin E (Alpha-tocopherol acetate) as Antioxidants on Activities of ALT, AST Enzymes in the Serum of Awassi lambs fed a High-Energy diet and normal diet vitamin E for 60 days summarized in table 3. There were no significant variations ($P>0.05$) in serum ALT and AST enzyme activity between the normal and high-energy diets. Treatments had a significant impact ($P<0.05$) on serum ALT and AST enzymes; vitamin E 400 mg/kg/lamb recorded a significantly ($P<0.05$) lower ALT level (12.4 U/L) in serum compared to the other treated groups, whereas vitamin E 200 mg/kg/lamb recorded the lowest AST level in serum 43.3 U/L. The Interacted factors significantly ($P<0.05$) affected ALT, AST activity in serum, lambs fed high energy diet with vitamin E 200 mg/kg/lamb (T9) recorded significantly ($P<0.05$) lower ALT level 11.4 U/L in serum, in addition, lambs fed normal energy diet with vitamin E 200 mg/kg/lamb (T4) exceeded other treated groups in decreasing ALT levels in serum by 9.35 U/L. Lambs implanted with Melatonin 36mg/lamb and fed a high-energy diet (T8) resulted in a significant decrease in serum ALT activity ($P<0.05$) In comparison to other treated groups 12.7 U/L was achieved. In addition, lambs fed a high or normal energy diet and supplied vitamin E200 mg/lamb/day (T4, T9) had dramatically lower AST concentrations in serum (43.7, 42.9 U/L), respectively than other treated groups (P<0.05). Melatonin 36mg/lamb in combination with a normal energy diet (T4) resulted in a significant (P<0.05) decrease in AST levels in serum (44.3 U/L). As a result, it can be determined that daily doses of vitamin E 200-400 mg/lamb and melatonin 36 mg/lamb had a beneficial effect on liver function; this suggests that the reduced concentration of ALT in the Awassi lamb supplemented with vitamin E and implanted with melatonin in the current study was likely caused by improved antioxidant status. Our findings were supported by Das, Mani (30), who indicated that vitamin E supplementation to the diet of goats at higher doses of 100 IU and 150 IU/kg dry matter (DM) showed a protective effect (P<0.05) against AST for 12 months feeding period. Our finding is also in line with Oleshchuk, Ivankiv (31), who found that melatonin 10 mg/kg body weight significantly decreased activities of AST and ALT in the serum of Sprague-Dawley male rats. In agreement with our findings, Zhang, Meng (19) recorded that Oral administration of Melatonin 5 mg/kg BW/daily for 30 days in rats alleviated the increases in the plasma of the ALT and AST activity.

Contrary to present results, Alipour, Vakili (15) illustrated no effect of vitamin E (DL-α-tocopheryl acetate 225 IU) once a week in Baluchi male lambs for 42 days, on blood metabolites such as plasma levels of ALT and AST. There are very few studies on the effects of diet and melatonin treatments on the activity of ALT and AST enzymes, and none have been reported in the scientific literature for Awassi lambs.

3.4. Vitamin E (Alfa-Tocopherol) Concentration in the Muscle Longissimus Dorsi (LD)

Several factors influence the efficiency of processing dietary vitamin E in the muscle vitamin E concentration; those are muscle type, and the type of vitamin E utilized, including the dietary concentration of Vitamin E (5, 32). Another element that influences α-tocopherol deposition in muscle is the duration of food intake. Studies have demonstrated increased tissue α-tocopherol concentrations following dietary vitamin E supplementation in Awassi lambs. Effects of the Melatonin and Vitamin E (Alpha-tocopherol acetate) as Antioxidants on vitamin concentration in LD randomly taken at days 2, 7 and 14 in the storage period of Awassi lambs fed a High-Energy diet and normal diet vitamin e for 60 days summarized in table 4. Muscle vitamin E concentrations were significantly increased by dietary vitamin E supplementation.

Table 4. Effects of the melatonin and vitamin E (Alpha-tocopherol acetate) as antioxidants on muscle vitamin E content (mg/g) in m. Longissimus Dorsi (LD) in Awassi lamb fed a high-energy diet and normal diet

Item	1st day	7th day	14th day
Diet			
Normal Diet	3.91	3.27	2.96
High Diet	4.16	3.13	3.07
Treatments			
Diet	2.94^{c}	2.46^{c}	2.25^{c}
M 18 mg	3.27^{c}	2.47^{c}	2.30^{c}
M 36 mg	2.87^{c}	2.72^{c}	2.62^{bc}
VIT.E 200 mg	4.82^{b}	3.23^{b}	3.09^{b}
VIT.E 400 mg	6.30^{a}	5.59^{a}	4.68^{a}
Interaction			
NDC	3.43^{cd}	2.64^{bc}	2.37^{bc}
ND×M 18 mg	3.06^{cd}	2.66^{bc}	2.44^{bc}
ND×M 36 mg	2.33^{d}	2.17^{c}	2.05^{c}
ND×VIT,E 200 mg	4.55^{bc}	3.29^{b}	3.10^{b}
ND×VIT.E 400 mg	6.20^{a}	5.59^{a}	4.82^{a}
HDC	2.45^{d}	2.28^{c}	2.13^{c}
HD×M 18 mg	3.49^{cd}	2.28^{c}	2.16^{c}
HD×M 36 mg	3.40^{cd}	3.27^{b}	3.19^{b}
HD×VIT.E 200 g	5.09^{ab}	3.29^{b}	3.08^{b}
HD×VIT.E 400 mg	6.40^{a}	5.60^{a}	4.55^{a}
SEM*	0.333	0.282	0.223
Mean	4.043	3.298	2.992
***P*-value**			
Diet	NS**	NS**	S** NS**
Treatments	<.0001	<.0001	<.0001
Interaction	0.0006	<.0001	<.0001

* SEM: Standard Error Mean.
** NS: Non-Significant at probability value (P≤0.05).
a, b, c: means in the same Columns with different superscripts differ significantly at probability value (P≤0.05).
NDC: normal diet control. HDC:high diet control.ND:Normal diet
HD:Highdiet.M18mg:melatonin18mg.M36mg:melatonin 36 mg.

Interestingly, the increase was dose-dependent because vitamin E levels were highest in the vitamin E 400 mg/lamb/day group in the LD muscle. Vitamin E concentrations in the 200, 400 mg/lamb/day treated lambs at days 2, 7 and 14 of the storage period with high energy diet (T10) and normal energy diet (T5) were significantly higher when compared to the control group (T1, T6), Differences between high energy and normal energy diet was not significant ($P \leq 0.05$), at 2^{nd}, 7^{th} and 14^{th} days of storage period. Treatments significantly affected vitamin E concentration in LD muscle at days 2, 7 and 14 of the storage period. The Vitamin E 200mg/lamb/day treated group recorded significantly higher vitamin E concentration in LD muscle than other treated groups**.** Higher α-tocopherol concentrations in meat are associated with greater dietary vitamin E content and/or longer supplementing time. Our findings were in line with Liu, Lanari (5), who showed that dietary vitamin E supplementation increases muscle vitamin E content in male Aohan fine-wool lambs 5 months old when supplemented, 20, 100, 200, 1,000, 2,000, or 2,400 IU/sheep/d vitamin E for 12 months, vitamin E concentration was highest in the 200 IU/sheep/d group in comparison of the control group. Our results were in agreement with the finding ofLiu, Lanari (5), who established that fed steers with diets supplemented with 250, 500, or 2000 mg all-rac-α-tocopherol acetate/calf/d the mean muscle α-tocopherol concentrations were 1.39, 2.27 and 4.95 mg/g, respectively, in the LD muscle. Present results were supported by Lauzurica, de la Fuente (33), who recorded that α-tocopherol concentrations in meat increased with increasing dietary vitamin E content of Santa Inês and Dorper crossbreed lambs were intramuscularly treated with 0, 10 or 20 IU of DL-alpha-tocopherol per kg of diet. Body weight for 75 days, the deposition of vitamin E in muscle depends on the length and the level of vitamin E supplementation.

On the other hand, our findings were in line withKasapidou, Enser (22), who reported that supplementation with 30 mg, 60 mg, 120 mg, 250 mg or 500 mg α-tocopheryl acetate/kg dry matter (DM) for 63 days of Suffolk×Charollaiswether lambs increased muscle α-tocopherol content with increasing dietary vitamin E content, and muscle vitamin E concentrations were positively correlated with dietary vitamin E levels. In addition, the present results agreed with Kasapidou, Enser (22), who measured the evolution of α-tocopherol concentration in muscle using 6–8 month-old lambs at different levels of supplementation with synthetic vitamin E (30, 150, 275, and 400 IU of all-rac tocopherol) or on green pasture through the feeding period (up to 8 weeks), registering a continuous increase with time in each treatment, except in that consist of vitamin E 30 IU. The muscle vitamin E concentration in the present study was higher than in other studies, possibly because of high doses of vitamin E 400 mg/day/lamb over 60 days.

Melatonin implantation effect on muscle vitamin E content was insignificant ($P \leq 0.05$) compared to non-treated groups of 3.40 mg/g, 3.27 mg/g, and 3.19 mg/g, respectively, at previous storage periods 2^{nd}, 7^{th} and 14^{th} days. There are certain studies on the effects of melatonin on muscle vitamin E concentrations; thus, our findings will be unique.

The results of the presented experiment were listed as follows:

1. Adding different doses of Vitamin E 200,400 mg/lamb/day and melatonin implantation 18mg, 36mg/lamb/day significantly ($P<0.05$) increased total protein in serum and decreased globulin level and glucose concentration in serum.
2. The lower glucose level in serum 63.7mg\dl was recorded for vitamin E 400mg/lamb/day significantly ($P<0.05$) in comparison to other treated groups, and albumin level was unaffected by diet, treatments, interactions between Normal energy diets with vitamin E 200mg/lamb/day treated group (T4) had reduced VLDL levels in serum.
3. Melatonin implantation 36mg/lamb and vitamin E400mg/lamb/day recorded significantly ($P<0.05$) the same effect on decreasing cholesterol concentration in

serum (42.6, 40.5 mg\dl), respectively, compared to other treatments.

4. Vitamin E supplementation to the Diet of Awassi lambs at an inclusion rate over the number of nutritional recommendations showed a notable effect on muscle vitamin E content in Awassi lamb when supplemented for 60 days, the dietary vitamin E content of 400 mg/lamb/d at day 2, 7 and 14 of storage period with high (T10) and normal energy diet (T5) seemed to be the best dose in this study.

5. Lambs administered 400 mg/kg/lamb of vitamin E had significantly (*P*<0.05) the lowest serum ALT and AST activity.

6. Lambs implanted with Melatonin 36mg/lamb and fed a high-energy diet (T8) resulted in a significant (*P*<0.05) decrease in serum ALT activity in comparison to other treated groups 12.7 U/L was achieved.

Authors' Contribution

Study concept and design: K. I. M. and F. K.
Acquisition of data: K. I. M.
Analysis and interpretation of data: F. K.
Drafting of the manuscript: K. I. M. and F. K.
Critical revision of the manuscript for important intellectual content: K. I. M. and F. K.
Statistical analysis: K. I. M. and F. K.
Administrative, technical, and material support: K. I. M. and F. K.

Ethics

The study design was approved by the ethics committee of the University of Yuzuncu Yıl, Van, Turkey.

Conflict of Interest

The authors declare that they have no conflict of interest.

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