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EFFECT OF PREPARTUM VITAMIN E AND SELENIUM ADMINISTRATION ON POSTPARTUM GENE EXPRESSION AND METABOLIC PROFILE OF IMMUNE AND OXIDATIVE MARKERS IN BARKI EWES

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The present study aimed at evaluating the influence of administration of vitamin E and selenium (VE/Se) on the gene expression pattern of some immune-metabolic variables as well as the oxidative stress markers in Barki ewes during the transition period. Sixty clinically healthy Barki ewes were studied. Two weeks prior to lambing, the investigated ewes were allocated into three equal-sized groups and assigned to receive one of the following supplements: (G1) received sterile saline solution (0.9 % NaCl) and considered as a control group; (G2) received a single intramuscular injection of VE/Se at a dose of 6.66 IU/kg BW VE, and 0.133 mg/kg BW Se; while G3 received the same dose of VE/Se but repeated at the time of lambing. Blood samples were collected from each ewe at the following time points: two weeks before lambing (-14), at time of lambing (0), and two weeks following the date of lambing (+14), for molecular and biochemical analyses. Our findings demonstrated that VE/Se supplementation had improved the gene expression of interleukin (*IL*)*5, IL6,* toll-like receptor 4 *(TLR4),* toll interacting rotein *(Tollip*), superoxide dismutase 1 *(SOD1),* catalase *(CAT),* acetyl-CoA carboxylase 1 *(ACACA),* fatty Acid Synthase (*FASN)* and stearoyl-CoA desaturase-1*(SCD).* A moderate degree of negative energy balance was seen at the time of lambing and two weeks later as evidenced by the values of metabolic variables including glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), and cholesterol levels. A repeated dose VE/Se supplementation provoked a significant effect on glucose, total cholesterol, NEFA, malondialdehyde (MDA), total anti-oxidant

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capacity (TAC), glutathione peroxidase (GPx) and lamb birth bodyweight compared with other groups. A prepartum supplementation of VE/Se could be considered an effective strategy for enhancing the metabolic and antioxidant status of late pregnant Barki ewes and productive performance of their lambs.

Keywords: Vitamin E/Se, immunity, transition period, Barki ewes, Gene expression.

INTRODUCTION

The transition period has been defined in ruminants as three-weeks around the time of parturition (CAROPRESE *et al*., 2006; THEODOROU *et al*., 2007; ANUGU *et al*., 2013; SUCUPIRA *et al*., 2019). During that time, tremendous metabolic and endocrine alterations are often occurring due to increase nutritional demands to combat the growth of the fetus and the initial production of milk (ANUGU *et al*., 2013; SUCUPIRA *et al*., 2019). These facts, when coupled with insufficient dietary intake and/or non-metabolic adaptation to the newly physiological situation, will likely predispose the animal to immune suppression and production of reactive oxygen species (ROS) as well as the occurrence of potential diseases such as metabolic, nutritional and infectious diseases (CAROPRESE *et al*., 2006). The increased production of ROS in the demand for endogenous and exogenous antioxidant variables can lead to oxidative stress and decrease of the neutrophil function, antibody responses and increase the production of cytokine by immune cells (SUCUPIRA *et al*., 2019).

Although several studies have dealt with the alterations of the immune status of ewes during the transition period (CAROPRESE *et al*., 2006; THEODOROU *et al*., 2007; ANUGU *et al*., 2013), the causes of immunosuppression are not completely understood. Hence, extensive studies are needed to explore the situation in periparturient dairy sheep. It has been suggested that immunosuppression could be linked to the endocrine changes associated with parturition (THEODOROU *et al*., 2007) or probably related to the increased concentrations of non-esterified fatty acids (NEFA), that result from intense negative energy balance (LE BLANC, 2010). Marked fluctuations have also occurred in cell-mediated and humoral immunity (CAROPRESE *et al*., 2006; THEODOROU *et al*., 2007).

Currently, there have been new approaches about using antioxidants, especially those relating to the immune status, to minimize the deleterious consequences which are observed during the peripartum period. Among the most known exogenous antioxidants, vitamin E (VE) and selenium (Se) can stand out in this role. The multiple functions of both nutrients extend beyond their antioxidant protection, as their supplementation at concentrations above requirements is associated with variable improvements in sheep performance and immune function (DANIELS *et al*., 2000). Vitamin E could prevent peroxidation in the susceptible subcellular membrane and prevents oxidative damage to the sensitive membrane lipids by decreasing hydrogen peroxide formation (KOYUNCU and YERLIKAYA, 2007), thereby reducing the oxidative stress and maintaining the integrity of cell membrane (KACHUEE *et al*. 2013). Selenium, on the other side, is an integral part of glutathione peroxidase which is involved in detoxification of hydrogen peroxide and lipid hydro peroxidase (MESCHY, 2000), and is a portion of selenoproteins protecting the animal's body against heavy metals and is involved in immune and neuropsychological function in animals (KACHUEE *et al*., 2013). Selenium status

of doe can directly influence the health and thriftiness of her kids as selenium is easily transported through the placenta and milk (KACHUEE *et al*., 2013).

Based on the current knowledge, there has been limited information about the effects of VE/Se on the on gene expression and innate immunity and metabolic variables in Barki ewes during the transition period. Therefore, the present study aimed to evaluate the potential effects of parenteral administration of VE/Se on the gene expression pattern of some immune-metabolic variables and oxidative stress markers in Barki ewes during the transition period. We hypothesized that variations in the tested genes and regulatory enzymes of the intermediary metabolism could provide useful tools to improve genetic selection toward livestock adaptation to harsh environments.

MATERIAL AND METHODS

Animals

A total of sixty seemingly healthy pregnant Barki ewes with an average of 4 - 6 years (mean ± SD: 4.9 ± 0.7) and a range of bodyweight 28 - 45 kg (mean ± SD: 38.5 ± 4.9) were used in this study and were confirmed pregnant via ultrasonography. The experiment was carried out in Mariut Research Station, Desert Research Center, El-Amryea, Alexandria, Egypt during the period between March and April 2018. All investigated ewes were received Albendazole as a broad-spectrum anthelmintic [Arab Veterinary Industrial Company (AVICO), Amman, Jordan] at a dose of 10 mg/Kg BW given orally. The investigated ewes were subjected to thorough clinical examination according to the standard protocols given previously (Pugh and Baird, 2012) and the findings were recorded simultaneously. All animals were clinically healthy, with no history of metabolic or concurrent ailments and were kept under identical housing and veterinary supervision throughout the study period.

All procedures were performed according to the NIH Guidelines for the Care and Use of Laboratory Animals and the guidelines of Mansoura University and those of the Desert Research Center (Egypt). The ewes were housed in semi-open shaded pens and fed on 750 g concentrate feed mixture (CFM) plus 750 g alfalfa hay/head/day, while water was always available ad libitum. The CFM was consisted of wheat bran (300 kg), soya bean (250 kg), corn (400 kg), sodium chloride (10 kg), calcium carbonate (20 kg), Premix (1 kg), Netro-Nill (0.5 kg) and Fylax (0.5 kg).

Study design

Two weeks prior to the expected time of lambing, the investigated ewes were randomly allocated into three equal-sized groups (20 ewes each), and were assigned to receive one of the following treatments: a placebo which received a sterile saline solution (0.9 % NaCl) and served as control group ***(G1)***; Myoselen E (a commercial medication contains 150 mg Vitamin E acetate as DL-Alpha tocopherol acetate, and 3 mg sodium selenite, excipients q.s.ad. 1 mL) given as a single intramuscular injection at a dose of 6.66 IU/kg BW Vitamin E and 0.133 mg/kg BW Se ***(G2)***; while ***G3;*** received the same dose of Myoselen E, but with a repeated dose regimen at the time of lambing. Both G1 and G2 received an equivalent volume of saline solution at the time of lambing.

Blood sampling and measurements

Ten milliliters of blood was collected from each ewe via jugular vein puncture at the following time points: -14 (two weeks before lambing), 0 (at time of lambing), and +14 (two weeks following the date of delivery). The samples were collected into vacutainer tube containing anticoagulant (EDTA or sodium fluoride) and without anticoagulant to yield whole blood or serum, respectively. The EDTA blood was used for real-time PCR assay; while those in plain tubes were kept overnight at room temperature and centrifuged at 3000 rpm for 15 minutes. Only clear sera were collected then aliquoted and kept frozen at -20°C for subsequent biochemical analyses of energetic and oxidative stress markers. The following commercial kits were used according to standard protocols of the suppliers to quantify beta-hydroxybutyrate (BHBA): (Cayman chemical company, CAT No: 700190, USA); glucose: (Biodiagnostic Egypt, CAT No: GL 13 20); triacylglycerol, and cholesterol: (SPINREACT Company, SPAIN); high density lipoprotein (HDL): (Spectrum company, Egypt); low density lipoprotein (LDL): (Biodiagnostic Egypt, CAT No: CH 12 31); NEFA: (FUJIFILM Wako Chemicals U.S.A.); immunoglobulin G (IgG): (AnaSpec company, CAT No: AS-55524); malondialdehyde (MDA): (Biodiagnostic Egypt, CAT No: MD2529); nitric oxide (NO): (Biodiagnostic Egypt, CAT No: NO2533); glutathione peroxidase (GPx): (Biodiagnostic Egypt, CAT No: GP 2524); total antioxidant capacity (TAC): (Biodiagnostic Egypt, CAT No: TA25 13), while serum lysozyme activity was determined using turbidimetric assay.

RNA extraction and reverse transcription

Extraction of RNA was done using Trizol™ reagent (Invitrogen, UK) according to the manufacturer's guidelines (Direct-zolTM RNA MiniPrep, CAT No: R2050). The assessment of good quantity and purity of RNA were carried using Nanodrop (UV-Vis spectrophotometer Q5000/USA). The integrity of RNA was also assessed by gel electrophoresis. An equivalent to 1 mg of RNA was transferred to cDNA with High Capacity (SensiFastTM cDNA synthesis kit, Bioline, CAT No: Bio- 65053). Reaction volume of 20 μL consisted of total RNA up to 1μg, 4 μL 5x Trans Amp buffer, 1 μL reverse transcriptase and DNase free-water up to 20 μL was used. The final reaction volume was placed in a thermal cycler with the following cycling program; primer annealing at 25°C for 10 min, reverse transcription at 42°C for 15 min followed by inactivation at 85°C for 5 min. The samples were held at 4°C.

Quantitative Real-Time PCR

The gene expression pattern of genes encoding immunity, antioxidant and lipogenic genes were assessed using quantitative RT-PCR using SYBR Green PCR Master Mix (2x SensiFastTM SYBR, Bioline, CAT No: Bio-98002). Primer sequences of the target genes besides the annealing temperature and the size of each amplified PCR product are shown in Table 1. GAPDH housekeeping gene was used as an internal control. The reaction volume 20 µL consisting of 10 µL 2x SensiFast SYBR, 3 µL cDNA, 5.4 µl H2o (d.d water), and 0.8 µl of each primer was used. The PCR cycling conditions were as follows: 95°C for 2 min followed by 40 cycles of 94°C for 10 sec, annealing temperatures for 30 sec, and 72°C for 20 sec. A melting curve was implemented after completion of the amplification phase, to authorize the specificity of the PCR product. The expression analysis was done using the 2-ΔΔCt method (PFAFFL, 2001).

Table 1. Oligonucleotide primers sequence, accession number, annealing temperature and PCR product size of the studied genes

Gene	Oligonucleotide sequence	Accession number	Annealing temperature (C^0)	Size (bp)
IL-5	f5'- TCTGCGTTTGACCTTGGTAGCTCT-3' r5'- TCAGCAGAGTTTGATGCGTGGAGA-3'	NM_001009783.1	64	less than 155
IL-6	f5'- TGCAGTCCTCAAACGAGTGGGTAA-3' r5'- AGCCGCAGCTACTTCATCCGAATA -3'	NM_001009392.1	62	less than 155
TLR4	f5'- GGTTCCCAGAACTGCAAGTG -3' r5'- GGATAGGGTTTCCCGTCAGT -3'	AY957615	58	117
Tollip	f5'- CTGGTGCTGTCCTACACGTC-3' r5'- ACAGTGGGCATTCCTGTGAT-3'	NM_001039961	56	122
SOD1	f5,- CGAGGCAAAGGGAGATACAG-3, r5,- TCTCCAAACTGATGGACGTG -3'	M81129	60	90
CAT	f5'- GAAACGCCTGTGTGAGAAC-3' f5'- ACATAGGTGTGAACTGCGT-3'	XM_012096208.3	58	171
ACACA	f5'- ATGTGGCCTGGGTAGATCCT-3' r5'-ACGTAACACAAGGCTGATGGTG-3'	NM_001009256.1	60	261
FASN	f5'- GGAAGGCGGGACTATATGGC-3' r5'- CATGCTGTAGCCTACGAGGG-3'	XM_004013447.1	62	278
SCD	f5'- GGCGTTCCAGAATGACGTTT-3' r5'- TGAAGCACAACAGCAGGACA-3'	NM_001009254.1	58	251
GAPDH	f5'- TGACCCCTTCATTGACCTTC-3' r5'- GATCTCGCTCCTGGAAGAG-3'	NM-001034034	62	143

Statistical analysis

SPSS software (SPSS analytical program for windows version 21) was used to conduct the statistical analyses. The effect of treatment on each variable in each group was evaluated using analysis of variance with repeated measurements of the general linear model using the Mauchly's sphericity test to detect the significant variations. When there was a significant result, one-way ANOVA with post hoc Duncan multiple comparison tests was used to detect the specific variations. Chi square test used to detect the effect of treatment on the lamb performance variables. For all analyses, values of $P < 0.05$ were considered significant. Results are presented as mean ± SD**.**

RESULTS

Supplementation of ewes with a repeated dose of VE/Se has significantly up-regulated the gene expression of of interleukin (*IL*) *5, 6,* toll-like receptor 4 *(TLR4),* toll interacting protein *(Tollip*), superoxide dismutase 1 *(SOD1),* catalase *(CAT),* acetyl-CoA carboxylase 1 *(ACACA),* fatty Acid Synthase (*FASN)* and stearoyl-CoA desaturase-1*(SCD)* at +14 compared with other groups (Figure 1).

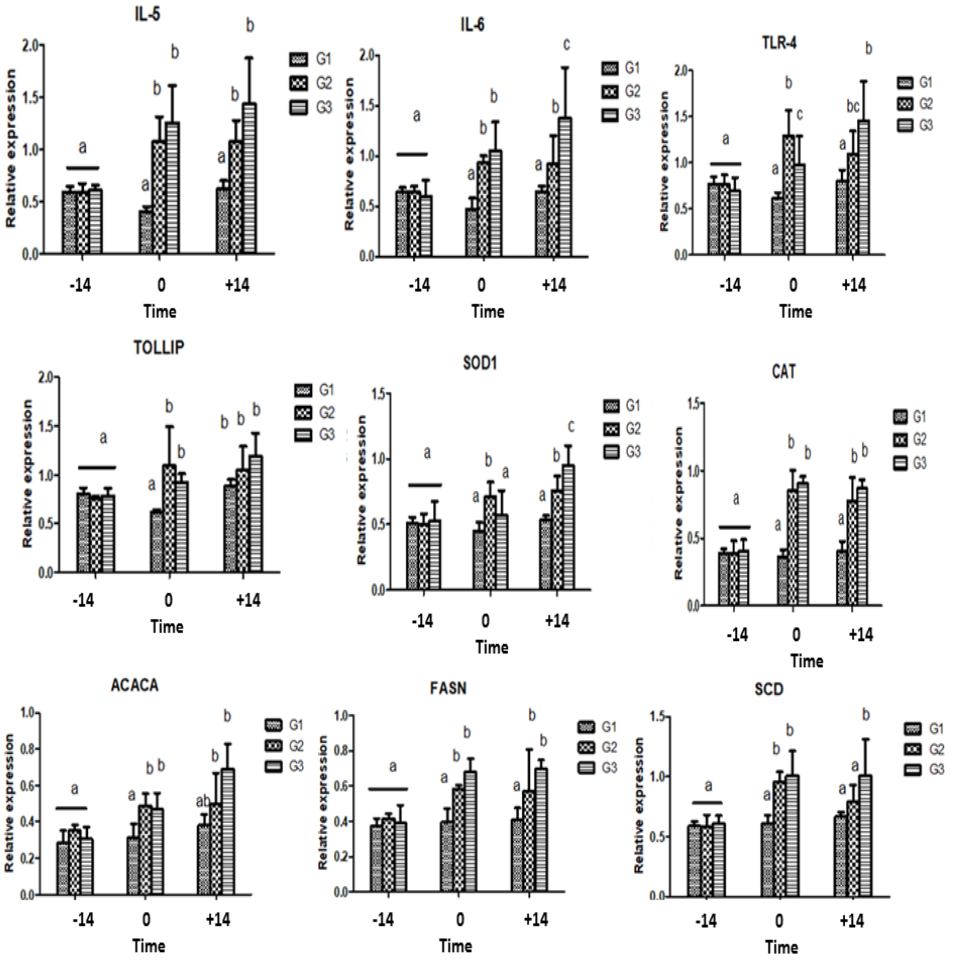
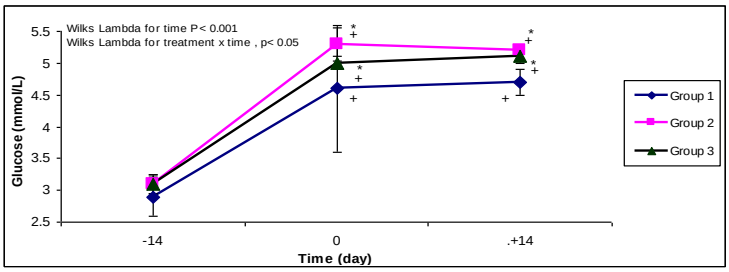


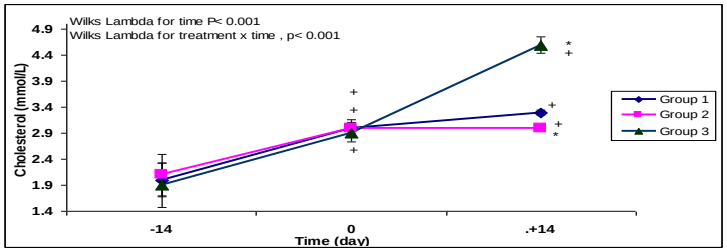
Figure 1. Relative expression patterns of immunity, antioxidant and lipogenic marker genes in the control and treated Barki sheep groups. Small alphabetical letters show significance when ($P<0.05$).

An overview of the serial measurements of serum biochemical and antioxidant profile in Barki ewes, as well as the productive performance of their lambs after supplementation with VE/Se, is illustrated in Figures 2, 3, Table 2. Clinically, the investigated Barki ewes showed no detectable clinical alterations throughout the study period and remain clinically healthy. All vital signs of investigated ewes were within the normal reference range and all ewes demonstrated normal laboring and delivered without obvious clinical illness.

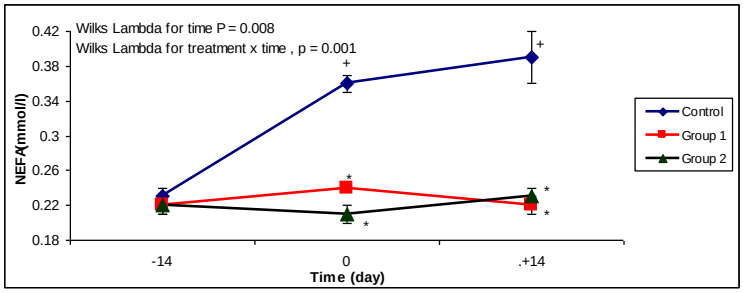
A


Wilks Lambda for time P< 0.001
Wilks Lambda for treatment x time , p< 0.05
Glucose (mmol/L)
5.5
5
4.5
4
3.5
3
2.5
-14
0
.+14
Time (day)
Group 1
Group 2
Group 3

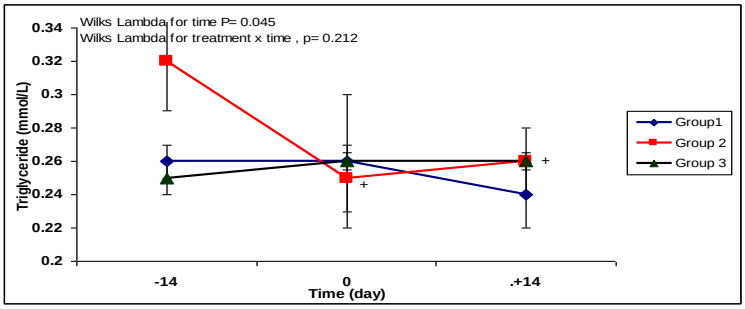
B


Wilks Lambda for time P< 0.001
Wilks Lambda for treatment x time , p< 0.001
Cholesterol (mmol/L)
4.9
4.4
3.9
3.4
2.9
2.4
1.9
1.4
-14
0
.+14
Time (day)
Group 1
Group 2
Group 3

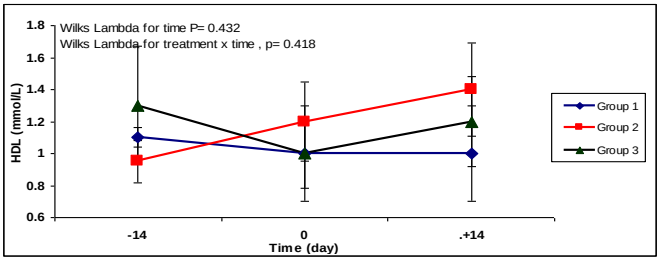
C


Wilks Lambda for time P = 0.008
Wilks Lambda for treatment x time , p = 0.001
NEFA(mmol/l)
0.42
0.38
0.34
0.3
0.26
0.22
0.18
-14
0
.+14
Time (day)
Control
Group 1
Group 2

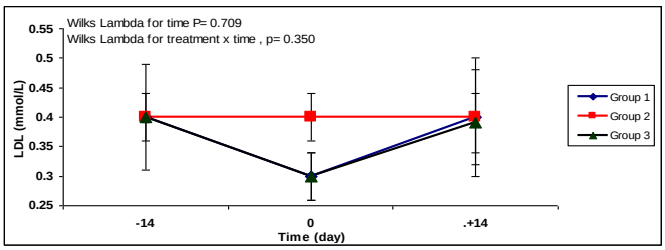
D


Wilks Lambda for time P= 0.045
Wilks Lambda for treatment x time , p= 0.212
Triglyceride (mmol/L)
0.34
0.32
0.3
0.28
0.26
0.24
0.22
0.2
-14
0
.+14
Time (day)
Group1
Group 2
Group 3

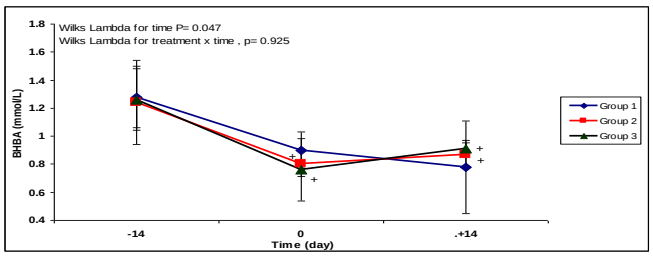
E



F



G



H

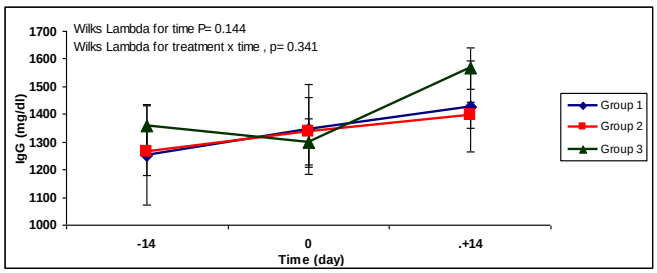
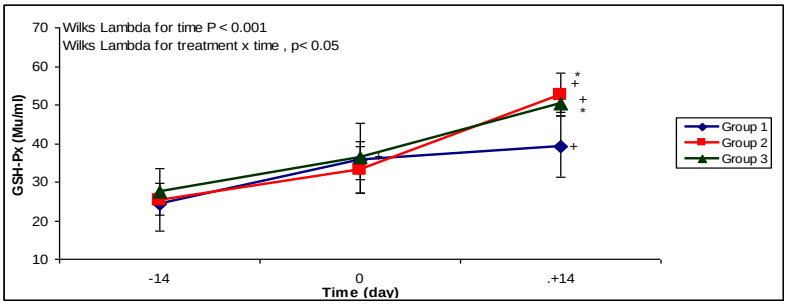
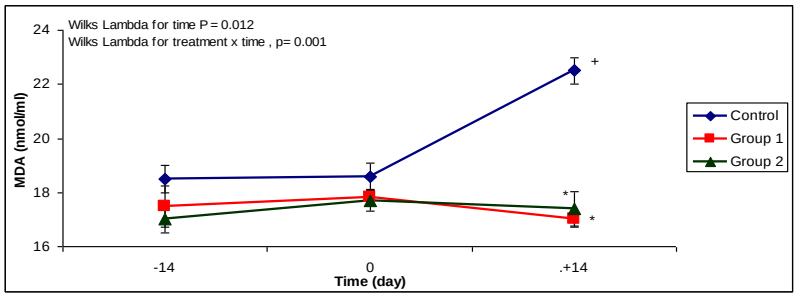


Figure 2 (A- H).Time course of blood glucose (mmol/L) (A), cholesterol (mmol/L) (B), NEFA (mmol/L) (C) and triacylglycerol (mmol/L) (D), HDL (mmol/L) (E), LDL (mmol/L) (F), NEFA (mmol/L) (G) and IgG (mg/dL) (H) concentrations in clinically healthy Barki ewes (Group 1) and in those received either single dose of Vit E/Se (Group 2) or a repeated dose (Group 3). (|—|). Mean ± SD. The superscript (*) indicates a significant difference within the groups, while the superscript (+) indicates a significant difference over time

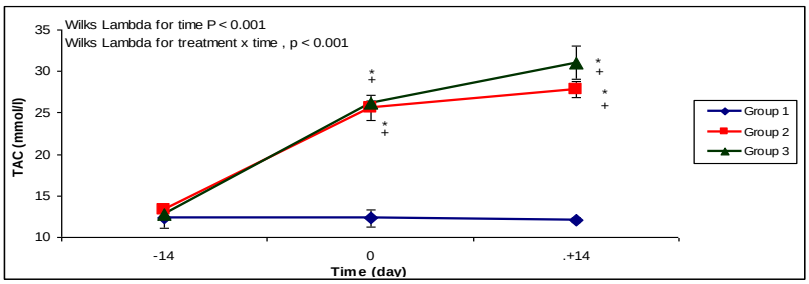
A


Wilks Lambda for time P < 0.001
Wilks Lambda for treatment x time , p< 0.05
GSH-Px (Mu/ml)
70
60
50
40
30
20
10
-14
0
.+14
Time (day)
Group 1
Group 2
Group 3

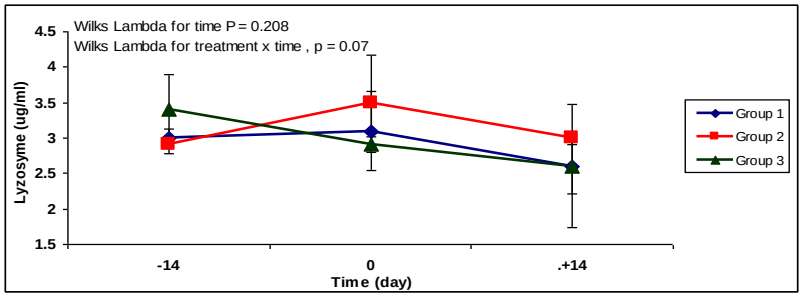
B


Wilks Lambda for time P = 0.012
Wilks Lambda for treatment x time , p= 0.001
MDA (nmol/ml)
24
22
20
18
16
-14
0
.+14
Time (day)
Control
Group 1
Group 2

C


Wilks Lambda for time P < 0.001
Wilks Lambda for treatment x time , p < 0.001
TAC (mmol/l)
35
30
25
20
15
10
-14
0
.+14
Time (day)
Group 1
Group 2
Group 3

D


Wilks Lambda for time P = 0.208
Wilks Lambda for treatment x time , p = 0.07
Lyzosyme (ug/ml)
4.5
4
3.5
3
2.5
2
1.5
-14
0
.+14
Time (day)
Group 1
Group 2
Group 3

E

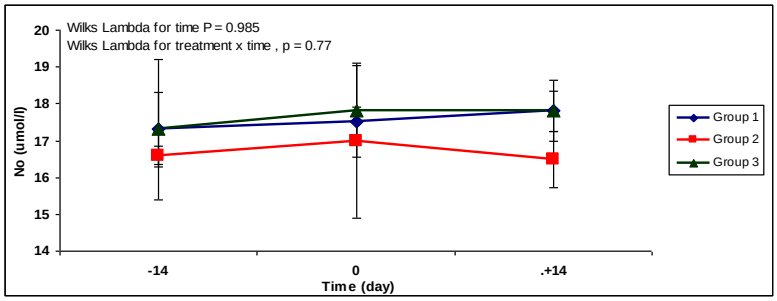


Figure 3 (A – E). Time course of Serum GPX (Mu/ml) (A), MDA (nMol/ml) (B), TAC (mmol/l) (C), lysozyme (ug/ml) (D), NO (umol/l) (E)concentrations in clinically healthy Barki ewes (Group 1) and in those received either single dose of Vit E/Se (Group 2) or a repeated dose (Group 3). (|—|). Mean ± SD. The superscript (*) indicates a significant difference within the groups, while the superscript (+) indicates a significant difference over time

Table 2. Effect of vitamin E and selenium supplementation during the periparturient period on the productive performance of lambs

	Groups			Lamb	Sex of lambs			No of ewes bearing lambs		
	No of ewes	No of lambed ewes	Total no of lambs	birth weight (kg)	Male	female	Ratio (male/female)	Mortality rate of lamb (%)	Single (%)	Twin (%)
G1	20	20 (100%)	28	2.1± 0.08[a]	15	13	1.15	16 (57.1%)	12 (60%)	8 (40%)
G2	20	20 (100%)	20	2.9± 0.09[b]	12	8	1.5	4 (20%)	20 (100%)	0 (0%)
G3	20	20 (100%)	20	3.5± 0.09[b]	16	4	4	0.0 (0%)	20 (100%)	0 (0%)

*Means with different superscripts (a, b) in the same column differ significantly (P< 0.05)

Biochemically, blood glucose levels were significantly affected by time (P < 0.001) and by treatment x time (P <0.05) in supplemented groups (G2 and G3) at lambing time (0) and at (+14) compared with the control group (Fig. 2-A). The highest values of blood glucose were observed in G2 at both time measurements compared to other groups (Fig. 2-A). There was a significant effect of time (P < 0.001) and treatment x time (P < 0.001) of VE/Se supplementation on serum cholesterol levels which were observed noticeably in G3 at (+14) compared with other groups (Fig. 2-B). Serum NEFA concentrations were significantly affected by time (P = 0.008) and by treatment x time (P = 0.001) in both supplemented groups at lambing time and (+14) (Fig. 2-C) with no significant variation between G2 and G3 at the time points compared with control groups (P> 0.05). Serum triacylglycerol and BHBA concentrations were significantly

affected by time (P = 0.045 and 0.047), respectively (Fig 2- D, G), while, serum concentrations of HDL, LDL, and IgG were not significantly affected by time nor treatment x time after supplementation of VE/Se (Fig 2- E, F, H).

The activity of serum GPx was significantly affected by time (P < 0.001) and treatment x time (P < 0.05) in supplemented groups at (+14) compared with those of control. The maximal concentration of serum GPx was observed in G3 compared with G2 at the same time point (Fig.3-A). Serum MDA concentrations were significantly affected by time (P=0.012) and by treatment x time (P= 0.001) in G2 and G3 at (+14) compared with those of control group (Fig. 3-B). Both supplemented groups showed the lowest concentrations of MDA compared with the control ewes at (+14). Values of TAC were significantly affected by time (P < 0.001) and by treatment x time (P < 0.001) in both supplemented groups at (0) and (+14) compared with the control ewes (Fig. 3-C). The maximal values of TAC were recorded in G3 at the same time points compared with those of G2 with no significant variation between the two supplemented groups (Fig. 3-C). For serum concentrations of NO and lysozyme activity, VE/Se supplementation did not provoke either effect over time or across the treatment regimen (Fig 3-D, E). Lambs born to ewes supplemented with VE/Se had a statistically higher body weight at birth than those lambs born to control ewes (Table 2). The percentage of lambing rate was equal in all groups (100%), and the incidence of lamb mortality was higher in the control ewes than those of G2 and G3. The sex ratio (male/female) and the incidence of ewes bearing twins were higher in G3 than those of G2 and control group.

DISCUSSION

The objective of the present study was to potential impact of prepartum supplementation of VE/Se on postpartum oxidative stress indices and immune-metabolic variables in Barki ewes. To our knowledge, very limited studies are yet available focusing on the potential effect of VE/Se supplementation on the gene expression of immune-inflammatory markers and lipogenic genes in transition ewes. The present study demonstrated that supplementation of VE/Se elicits positive energetic property, improved immunity and provoked antioxidant activity.

Supplementation of Barki ewes with VE/Se has modulated the gene expression of lipogenic genes (*ACACA*, *FASN* and *SCD*) with the highest up-regulation were noticed in G3. The effect of VE on the expression profile of subcutaneous fat and thoracic muscle lipid-related genes was studied by GONZÁLEZ-CALVO *et al*. (2017). According to results, the authors found that VE supplementation can up-regulate the expression pattern of the lipid biosynthesis process, cholesterol, and sterol and steroid biosynthesis genes and it down-regulated stress response-related genes.

Ewes in the present study showed a moderate degree of negative energy balance (NEB) at time of lambing and two weeks later as evidenced by the values of metabolic variables including glucose, NEFA, BHBA, and cholesterol levels. However, ewes receiving VE/Se had high levels of serum cholesterol compared with control ewes. These results were inconsistent with those shown in previous reports (MOHRI *et al*., 2011; SHI *et al*., 2017 and SHERIEF *et al*., 2019) but unlike to those reported by others (NEMAT, 2015) who did not observe any correlations between VE/Se supplementation and serum cholesterol levels during the transition period.

Interestingly, ewes that received VE/Se had a comparatively low value of serum NEFA and BHBA than the control group. It can, therefore, be suggested that supplementation of VE/Se could improve the energy balance in lactating ewes. The extent of that increase was highly renovated with the level of adipose tissue lipolysis (HOLTENIUS *et al.*, 2003). In contrast, the higher value of serum NEFA that was observed in non-supplemented ewes at the lambing time and at (+14) could be attributed to the increase of depletion of lipid reserves existed because of the increased energy demand and increase the utilization of fat storage reserves to meet the rapid and dramatic energy responses in the ewes (MOHRI *et al*., 2011; AVCI and KIZIL 2013; SHI *et al*., 2017 and SHERIEF *et al*., 2019). Our findings demonstrated insignificant alterations of serum triacylglycerol, HDL, LDL levels in supplemented ewes compared with the control group. These findings were in line with that recorded by NEMAT (2015) and being away from that reported by others (FALKOWSKA *et al*. 2000; SHERIEF *et al*., 2019).

Blood glucose levels were significantly affected by supplementing VE/Se which was coincided with those given elsewhere (MOHRI *et al*., 2011; NEMAT, 2015; SHI *et al*., 2017 and SHERIEF *et al*., 2019). Although VE/Se has greatly affected the carbohydrates metabolism, the mechanism of action behind that is not yet evident. It has been suggested that VE/Se can increase blood glucose levels either directly or indirectly through accelerating thyroxin and triiodothyronine hormones (MOHRI *et al*., 2011). No significant effect of parenteral administration of VE/Se on blood glucose level was evident by others (SOLIMAN *et al*., 2012).

In the current study, it has shown that repeated doses of VE/Se supplementation has improved oxidant/antioxidant status via increase of GPx activity, TAC, and decreased circulating MDA significantly up-regulated the expression pattern of the antioxidant marker genes (*SOD1* and *CAT*). The findings of GPx were agreeable with the previous study in cattle, buffalo and sheep, respectively (CHAUHAN *et al*., 2014). It has shown that exposing sheep for high VE containing diet can significantly up-regulate both white blood cell glutathione peroxidase and red blood cell lysate glutathione peroxidase activity (CHAUHAN *et al*., 2015). GPx activity is a powerful indicator of selenium status and reflects the long-term selenium status in red blood cells while serum selenium concentrations are suggesting of fast and short-lived changes in its levels (PAVLATA *et al*., 2000). Our observations of TAC were in line with the results given recently (SHERIEF *et al*., 2019). In the former study, the authors observed a significant elevation of the values of TAC in experimental ewes compared with the control ones during the postpartum period which established that the maternal supplementation of VE/Se during the late stage of gestation and early lactation can ameliorate the antioxidant status of Ossimi ewes. In contrast, the findings of MDA levels were in line with that reported in Ossimi ewes (SHERIEF *et al*., 2019) and being away from that reported by (SHAKIRULLAH *et al*., 2017) who stated that supplementation of VE/Se at a dose of 50 mg and 0.3 mg/kg of diet for 4 weeks provoked no significant effect on MDA level of Damani and Balkhi sheep.

Here, ewes that received a repeated dose of VE/Se exhibited a high IgG, but without significant difference compared with other groups, at (+14) compared to other groups. These findings were similar to the findings observed in sheep (DANIELS *et al*., 2000; MOEINI and JALILIAN 2014), but unlike to the finding reported by REDDY *et al*., (1987) who found a trend for higher titer levels of IgG for the treatment cows which were given 125 IU of VE daily compared with control ones. It has known that IgG is the isotype that represents 85% of the total Ig in the

blood and colostrum of sheep. It is therefore suggested that supplementation of VE/Se might enhance the ability of immune cells to produce Ig which later can be transferred to the mammary gland. To date, there have been few studies describing the effects of VE on the innate immunity in ruminants. Few studies have evaluated the effect of VE/Se supplementation on lysozyme activity. Lysozyme activity has been found to reflect the ability of the serum to lyse potential pathogens (FIRTH *et al*., 2005). Our findings showed that serum lysozyme activity was not significantly affected by VE/Se. This finding was in agreement with that obtained in ewes (SARITHA *et al*., 2013) and disagreed with others (JAKUBOWSKI *et al*., 2004) who reported that lysozyme activity was shown to be responsive to VE supplementation in rats.

The present study demonstrated that VE/Se supplementation could significantly increase the bodyweight of the lambs at birth compared to lambs born to non-supplemented ewes. These results went in parallel with the findings reported elsewhere in ewes (KOYUNCU and YERLIKAYA, 2007; EL-SHAHAT and ABDEL MONEM, 2011; SHERIEF *et al*., 2019). Unlike our findings, several studies showed that supplementation of VE/Se elicited no significant effect on in birth weight of lambs (MOEINI and JALILIAN, 2014). The differences in the supplemented level of VE/Se and the nutritional status of the investigated animals concerning VE/Se intake could elucidate these different findings.

The current study noted that the incidence of lamb mortality was lower in ewes receiving repeated doses of VE/Se compared to other groups. These results were consistent with that given by MUNOZ *et al*. (2009) who found that lambs from VE/Se supplemented ewes show rapid advancement to stand and nurse compared to lambs from non-supplemented ewes, thereby leading to decrease in the mortality rate of lambs. In the same line, the sex ratio (male/female) and the incidence of ewes bearing twin was also higher in G3 than other groups. These findings were in part similar to that given in Egyptian Baladi ewes (EL-SHAHAT and ABDEL MONEM, 2011).

CONCLUSIONS

The results herein demonstrated that VE/Se supplementation had improved the gene expression of tested immune markers, antioxidants and lipogenic markers of Barki ewes. Besides, supplementing pregnant Barki ewes with a repeated dose VE/Se had provoked a significant effect on metabolic variables and oxidative stress indices as well as lamb birth bodyweight. We suggest that VE/Se supplementation during the periods of late gestation and early lactation could be considered an effective strategy for enhancing the metabolic and antioxidant status of late pregnant Barki ewes and productive performance of their lambs.

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EFEKAT PRENATALNE PRIMENE VITAMINA E I SELENA NA EKSPRESIJU GENA I METABOLIČKI PROFIL IMUNIH I OKSIDATIVNIH MARKERA KOD OVCE BARKI

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Izvod

Ova studija imala je za cilj da proceni uticaj primene vitamina E i selena (VE / Se) na ekspresiju gena nekih imuno-metaboličkih promenljivih kao i markere oksidativnog stresa kod ovaca Barki tokom prelaznog perioda. Proučavanno je 60 klinički zdravih Barki ovaca. Dve nedelje pre jagnjenja, ispitivane ovce su raspoređene u tri grupe jednake veličine i primale su jedan od sledećih dodataka: (G1) su primile sterilni fiziološki rastvor (0,9% NaCl) i smatrale se kontrolnom grupom; (G2) primili su jednu intramuskularnu injekciju VE / Se u dozi od 6,66 IU / kg BV VE i 0,133 mg / kg BV Se; dok je grupa G3 primila istu dozu VE / Se, koja je ponovljena u vreme jagnjenja. Uzorci krvi prikupljeni su od svake ovce u sledećim vremenskim tačkama: dve nedelje pre jagnjenja (-14), u vreme jagnjenja (0) i dve nedelje nakon datuma jagnjenja (+14), za molekularne i biohemijske analize. Naši nalazi su pokazali da je suplementacija VE / Se poboljšala gensku ekspresiju interleukina (IL) 5, IL6, TLR4, roteina (*Tollip*), superoksid dismutaze 1 (SOD1), katalaze (CAT), acetil-CoA karboksilaza 1 (ACACA), masno-kiselinske sintaze (FASN) i stearoil-CoA desaturaza-1 (SCD). Umeren nivo negativne energetske ravnoteže primećen je u vreme jagnjenja i dve nedelje kasnije, što dokazuju vrednosti metaboličkih promenljivih, uključujući glukozu, ne-esterifikovane masne kiseline (NEFA), beta-hidroksibutirat (BHBA) i nivo holesterola. Ponovljena doza VE / Se dovela je do značajnog uticaja na glukozu, ukupni holesterol, NEFA, malondialdehid (MDA), ukupni antioksidativni kapacitet (TAC), glutation-peroksidazu (GPk) i telesnu težinu jagnjeta na rođenju u poređenju sa drugim grupama. Prenatalna suplementacija VE / Se može se smatrati efikasnom strategijom za poboljšanje metaboličkog i antioksidativnog statusa kasno trudnih Barki ovaca i proizvodnih performansi njihovih jagnjadi.

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