The effect of vitamin E administration to dairy cows in the prepartum period on some metabolic, oxidative, and reproductive parameters

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SUMMARY

The purpose of this study was to investigate how vitamin E affected oxidative stress, metabolic biomarkers, and reproductive parameters in cows during late gestation. The study used forty healthy, multiparous cows in late gestation. The animals were divided into two groups, control (n.20) and experimental (n:20) group, ten (±5) days before birth. On the same day, 10 ml of blood sample was drawn from each animal's tail vein, and the cows in the experimental group received 3000 mg of vitamin E (DL-alpha-tocopherol acetate) intramuscularly, whereas the cows in the control group received 20 ml of 0.9% NaCl. On calving day and on the 15th day after the calving, blood samples were taken again. The serums of the blood samples taken were separated and stored at -80 C until the analysis. Malondialdehyde (MDA), Glutathione Peroxidase (GSH-Px), Catalase (CAT), Glutathione (GSH), Glucose (GLU), Triglyceride (TG), Cortisol (COR), Glutamic Oxaloacetic Transaminase (GOT), Gamma Glutamyl Transferase (GGT) and Glutamic Pyruvic Transaminase (GPT) in the samples were measured. Calving - first estrus, calving - first insemination, calving-conception, and artificial insemination per pregnancy were recorded to determine the reproductive performance of the cows involved in the study. Vitamin E administration to cows during the peripartum period was found to boost GSH and GSH-Px levels at birth while decreasing GPT levels ($p<0.05$). Furthermore, the period of calving-first estrus, calving-first insemination, and calving-conception was shortened compared to the control group, however this was not statistically significant ($p>0.05$). Vitamin E was proven to be useful in lowering birth stress and protecting the liver when given to cows in late gestational stages. It is thought that it may also be effective in improving reproductive parameters, but the study should be replicated with more animals.

KEY WORDS

Dairy cow; vitamin E; reproduction; oxidative stress.

INTRODUCTION

During the periparturient period, the immune system of high-yielding dairy cows is compromised. The most important factors causing immunosuppression during this time are metabolic stress, a negative energy balance, the onset of lactation, and vitamin, mineral, and protein deficiency due to the increased nutrient requirements for the fetus[40]. By disrupting the balance of pro-oxidants and antioxidants, these factors cause oxidative stress and immune dysfunction[2,55]. When there is an imbalance between oxidant production and the body's natural antioxidant system in dairy cows during the periparturient period, serious health problems occur[63]. In dairy cows, oxidative stress rises during the periparturient period and may play a role in the pathogenesis of periparturient diseases[57,60,62]. It has also been reported that during this time, oxidative stress in dairy cows causes metabolic stress, and that metabolic and oxidative changes can be found together[56,64].

An imbalance of reactive oxygen species (ROS) and antioxidant potential (AOP) causes oxidative stress[57]. When exposed to oxidative stress, there is a rapid increase in ROS production and a decrease in AOP[19]. The main damage caused by increased ROS is lipid peroxidation. Malondialdehyde (MDA) is produced as a result of lipid peroxidation, and monitoring MDA levels can provide insight into the organism's oxidative stress and lipid peroxidation[46].

To protect themselves from the harmful effects of oxidants, cells in the body use enzymatic antioxidant defence systems such as Glutathione Peroxidase (GSH-PX), Catalase (CAT), and non-enzymatic antioxidant defence systems such as Glutathione (GSH), Vitamin E (α-tocopherol) [49,58].

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Vitamin E (α-tocopherol) is a fat-soluble antioxidant important in the body's defense against oxidative stress[30]. Vitamin E is an antioxidant that helps to prevent or postpone the onset of some diseases by inhibiting free radical-mediated tissue damage and it also helps in immune system function[6]. Studies have shown that vitamin E significantly improves immune functions. It is reported that vitamin E indirectly regulates the immune system by reducing the production of suppressive factors, such as Prostaglandin E2 (PGE2)[37]

Cow reproductive performance has declined dramatically in recent years around the world. Although the antioxidant effect of vitamin E is well known, its effect on reproductive performance in cows is not clear. As a result, the current study aimed to assess the effect of vitamin E administration during late gestation in cows on reproductive performance together with metabolic and oxidative stress biomarkers.

MATERIALS AND METHODS

Ethical Clearance

Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee granted permission for the project (no T2022-831 dated 25.01.2022).

Animals, Feeding, Housing

The study used forty (n: 40) Holstein cows that were clinically healthy, in late gestational stages, and kept on a private farm in Tekirda province with close expected calving dates. After their late gestational stages were established by rectal palpation, the animals were randomly assigned into two groups: experimental (n: 20) and control (n: 20). All the animals were cared after, fed, and bred in the same way. Both groups were provided TMR-specific Total Mixed Ration (TMR) ad libitum twice a day during the changeover period. They always had clean drinking water available to them.

Methods

All the animals in the study were moved birth cage 10 days (10±5) before they were due to calving. On the same day 10 ml blood sample was obtained from each animal's tail vein and 3000 mg Evit (Evigen ampoule containing 300 mg DL-alpha-tocopherol acetate in each ampoule -Aksu Farma Medical Tıbbi Ürünler laç San. Ve Tic.Ltd. ti.) was administered intramuscularly to the cows in the experimental group while the cows in the control group were administered with 20 ml of 0.9% NaCl. On the day of calving and on the 15th day after calving, blood samples of 10 ml were obtained from the tail vein. The blood samples were centrifuged for 15 minutes at 3000 rpm to extract the serum, then refrigerated at -80 degrees until analysis. MDA and GSH levels, as well as CAT and GSH-Px enzyme activities, were analysed spectrophotometrically in order to show the oxidative damage and antioxidant activity in the serum samples obtained. The Biochrom Libra S22 device was used to do spectrophotometric measurements at wavelengths of 374, 412, and 532 nm. Autoanalyzer was used to perform biochemical analyses (GLU, TRI, GOT, GPT, GGT) (Gesan Chem 200). The level of serum cortisol was determined using a bovine-specific ELISA kit (Quant, Bio Tek Instruments Inc. The period of calving-first estrus, calving-first insemination, and calving-conception were measured in days, and the number of inseminations per pregnancy was calculated using farm records in order to assess the reproductive performance of the animals involved in the study.

45 days after calving was regarded as the voluntary waiting period in the study's animals. The first estrus after the voluntary waiting period was determined with a pedometer by assessing the increase in cows' activity, increase in uterine tone found by rectal palpation, and the presence of Graff follicle in the ovaries. The animals in the study were artificially inseminated with the same viable bull sperm and the same technician carried out the procedure. Pregnancy examination was performed on the 45th day after insemination using a 5MHz linear probe real-time ultrasonography device (Hasvet WED-3100V).

Statistical Analysis

In the preparation of statistical data, the SPSS (IBM SPSS for Windows ver.26) package program was utilized. The results were presented as mean (SD). The Shapiro-Wilk test was performed to determine if the study's continuous variables were regularly distributed. Because the measurements were not normally distributed, nonparametric tests were used. For all parameters, the Mann Whitney U test was employed to compare the mean and significant level of the two groups. To compare the measurements over time, the Friedman test was applied. The time difference following the Friedman test was determined using the Bonferroni Post-Hoc test. The statistical significance level was taken as 5% in the calculations. The mean and significance level of the two groups for the oxidative stress parameters, biochemical parameters, and reproductive parameters were compared using the Mann Whitney U test. To compare oxidative stress and biochemical markers with regard to time, the Friedman test was performed. The time difference after the Friedman test was calculated using the Bonferroni Post-Hoc test. In the calculations, 5% was used as the statistical significance level.

RESULTS

During the partum period, GSH and GSH-Px were shown to be higher in the Vit E group ($p>0.05$). The level of GSH in the experimental group increased significantly ($p>0.05$) over time (from pre-partum to post-partum)(Table 1).

The GLU level in the experimental group decreased significantly over time (from pre-partum to post-partum) ($p>0.05$) but there was no statistically significant difference between the groups ($p>0.05$)(Table 2).

The GOT level increased significantly ($p>0.05$) over time (from pre-partum to post-partum), but this increase did not result in a significant difference between the groups ($p>0.05$)(Table 2).

The GPT level in the experimental group declined significantly over time (from pre-partum to post-partum) ($pp>0.05$), but there was no statistically significant difference between the groups ($p>0.05$)(Table 2).

The TRI level in the experimental group decreased significantly over time (from pre-partum to post-partum) ($p>0.05$), but there was no statistically significant difference between the groups ($p>0.05$)(Table 2).

The levels of GGT, COR, MDA, and CAT were not significantly different between groups or periods ($p>0.05$)(Table 1 and Table 2).

Although the application of vitamin E shortened the period of calving-first estrus, calving-first insemination and calving-conception, this difference was not statistically significant between the groups ($p>0.05$)(Table 3).

Table 1 - Two-way Comparison of Oxidative Stress Parameters by Groups and Periods.

	PREPARTUM			PARTUM			POSTPARTUM			exp.	con.
	experiment	control	p	experiment	control	p	experiment	control	p	*p	*p
MDA (nmol/mL)	24,64±4,56	25,30±4,32	,705	21,17±4,13	20,65±2,80	,791	20,40±4,18	21,47±3,10	,597	,105	,103
CAT (kU/gr)	28,28±2,76	27,27±2,13	,199	28,26±2,90	29,33±2,80	,427	27,80±3,29	27,98±3,45	,880	,910	,314
GSH (nmol/mL)	2,55±0,51^{b}	2,88±0,44	,198	3,12±0,58^{a}	2,67±0,28	**,041**	2,77±0,39^{ab}	2,96±0,40	,173	**,033**	,190
GSH-PX (IU/gr)	20,87±7,23	21,17±5,92	**,762**	24,90±8,77	17,82±5,48	**,049**	23,32±10,80	20,89±6,55	,650	,426	,199

MDA (Malondialdehyde), CAT (Catalase), GSH (Glutathione), GSH-Px (Glutathione Peroxidase) P Mann-Whitney-U test result, *p Friedman test result, a, b Bonferroni Post-Hoc test.

Table 2 - Two-way Comparison of Biochemical Parameters by Groups and Periods.

	PREPARTUM			PARTUM			POSTPARTUM			exp.	con.
	experiment	control	p	experiment	control	p	experiment	control	p	*p	*p
GGT (U/L)	18,20±8,43	21,50±7,92	,306	20,40±9,50	46,70±94,19	,734	22,60±5,25	28,40±9,97	,130	,343	,313
GOT (U/L)	66,30±14,74^{b}	75,28±4,40^{b}	,070	95,74±24,41^{a}	90,69±11,61^{a}	,326	106,53±52,95^{a}	108,72±18,23^{a}	,253	**,044**	**,001**
GPT (U/L)	22,60±5,68^{a}	20,30±6,95	,272	19,60±5,02^{ab}	17,10±5,78	,426	18,20±7,16^{b}	20,40±6,83	,494	**,008**	,105
GLU (mg/dL)	71,00±9,01^{a}	68,30±9,66	,495	65,40±27,60^{ab}	85,40±46,96	,450	51,10±15,13^{b}	52,00±14,34	,623	**,028**	,063
TRI (mg/dL)	18,10±6,33^{a}	18,80±12,05	,910	5,14±3,72^{b}	4,30±1,89	,882	4,14±2,67^{b}	4,0±3,38	,636	**,016**	,077
COR (ng/mL)	80,99±86,44	102,15±79,87	,446	91,16±73,80	68,04±91,84	,238	82,45±80,67	84,41±77,18	,838	,934	,759

GGT(γ-Glutamyl Transferase), GOT (Glutamic-Oxaloacetic Transaminase), GPT (Glutamic Pyruvic Transaminase), GLU (Glucose), TRI (Triglyceride), COR (Cortisol) P Mann-Whitney-U test result, *p Friedman test result, a, b Bonferroni Post-Hoc test.

Table 3 - Comparison of Reproductive Parameters by Groups.

	experiment	control	p
Calving - First Estrous (days)	83,71±34,84	96,14±32,32	,139
Calving - First Insemination (days)	100,00±29,95	121,14±24,99	,096
Calving - Conception (days)	113,14±35,92	129,43±38,46	,443
Number of inseminations per pregnancy (number)	1,43±0,53	1,43±0,79	,762

P Mann-Whitney-U test result.

DISCUSSION

Malondialdehyde (MDA) is the end product of lipid peroxidation and the changes in MDA concentration are used as a biomarker of oxidative stress[15]. Sharma et al.[54] reported that MDA levels were higher in dairy cows in the early lactation period compared to the late gestational period, Konvicna et al.[36] reported that it was high within a week after calving; and Colakoglu et al.[21] reported that it began to increase 21 days before birth and peaked at the time of calving. Khatti et al.[35] found that applying Evit+Se to pregnant cows lowered MDA levels significantly, whereas Mokhber-Dezfouli et al.[38] found that vitamin E treatment reduced MDA concentration 4 hours after calving, however vitamin E application had no effect on MDA levels in the current study. This is thought to be because of the difference in dose and frequency of administration. In a similar study, Bouwstra et al.[13] found that supplementing with vitamin E reduced liver MDA levels in cows, but it had no effect on blood MDA levels.

Catalase (CAT) is an antioxidant enzyme that helps to reduce the harmful effects of reactive oxygen species (ROS)[31]. According to Sharma et al.[54], the blood CAT level in early lactating cows are substantially greater than in animals in late gestational stages. According to Chandra et al.[18], as the calving date approaches, the CAT level rises to counterbalance the rising oxidative stress. While Khatti et al.[35] found that the CAT level reached its highest at the time of calving, but it was lower in the group given E vit +Se, Aggarwal et al.[3] found that supplementing cows with vitamin E in late gestational stages significantly raised CAT levels. Although the CAT level was higher at calving in the current study, vitamin E had no effect on it.

Glutathione (GSH) is a non-enzymatic low molecular weight antioxidant found in plasma, other extracellular fluids, lipoproteins, and membranes. GSH is thought to be a better indicator than intracellular GSH-Px (Bernabucci et al.[9]. It is commonly used to assess the antioxidant capability of cells since it is the principal non-enzymatic regulator of intracellular redox homeostasis[43]. In the early stages of lactation, cows' GSH levels decrease[42]. Vitamin E administration during the prepartum period boosted GSH levels at birth, according to Brzezinska-Slebodzinska et al.[14] Prepartum vitamin E administration was found to significantly increase serum GSH levels at the time of birth ($p<0.05$) in the current study. This shows that vitamin E treatment during late gestation could help post-calving cows maintain their cellular redox status.

Glutathione Peroxidase (GSH-Px) is an important enzyme that protects cells against increased oxidative stress at calving[1]. Kon-

vicna et al.[36] and Festiala et al.[23] reported that GSH-Px levels decreased in response to increasing oxidative stress after calving. GSH-Px activity was significantly higher in the early postpartum period, according to Sayıner et al.[52], whereas Pilarczyk et al.[48] suggested that the highest GSH-Px activity in the initial stage of lactation may be an indicative of higher oxidative stress in this period compared to other stages. Vitamin E administration during the prepartum period was found to significantly boost GSH-Px levels at calving in the current study ($p < 0.05$). Khan et al.[34] reported that vitamin E administration increased GSH-Px levels, and Vani et al.[61] reported that vitamin E increased GSH-Px activity in the cell, reducing free radicals and thus helping to protect the cell from oxidative damage. These findings, which were discovered by Khan et al.[34] and Vani et al.[61], reveal that vitamin E administration increases the body's defenses against oxidative stress.

The oxidant-antioxidant balance is known to be related to all key activities in the liver, and liver cells are destroyed during oxidative stress[41,51]. During the peripartum period, dairy cows' liver functions deteriorate with increased inflammation and oxidative stress.[14,59]. Glutamic-Oxaloacetic Transaminase (GOT), Gamma-Glutamyl Transferase (GGT), and Glutamic Pyruvic Transaminase (GPT) concentrations in the blood are good indicators of liver damage and typically indicate the presence of liver injury.[22,50] GOT, GGT, and GPT levels beyond a certain threshold are linked to liver cell destruction (lysis and necrosis).[10,33] Vitamin E administration has been shown to improve liver function in transitional cows.[12] The concentrations of GOT and GGT increased after calving, according to the current study. The increase in GOT levels was statistically significant ($p<0.05$).

In cows treated with vitamin E, the increase in GGT concentration was lower, but not statistically significant ($p>0.05$). For preterm postpartum cows without clinical illness, postpartum GOT and GGT values were generally within the predicted range.[10,25,45] Vitamin E administration resulted in a substantial reduction in GPT levels ($p<0.05$). This finding suggests that vitamin E administration may help sustain GPT levels in cows while also reducing the negative effects of oxidative stress on the liver produced by calving.

It is known that glucose concentrations increase in the prepartum period and decrease after calving.[7,32,53] A negative energy balance and a change to milk production are linked to this decline.[16] In this study, it was discovered that in the group given vitamin E, glucose levels reduced significantly during the partum and postpartum periods ($p<0.05$). These findings were consistent with those of Omur et al.[44], and Avcı and Kızıl,[5] who linked the reduced glucose levels to the administration of minerals involved in carbohydrate metabolism. However, decreased glucose levels with vitamin E administration in the current study indicates that this response cannot be attributed solely to mineral application.

Higher concentrations of triglyceride have been reported during prepartum period and these levels decreased after calving.[20,53] This decrease has been attributed to the mammary glands' use of triglycerides for milk fat production during lactation.[26] However, Chandra et al.[17] found that triglyceride levels were higher before calving in dairy cows and they decreased with calving, and Zn+Vit E application had no effect on these levels. In the current study, triglyceride levels decreased with calving in both groups, but this decrease was significantly ($p<0.05$) less in the vitamin E group.

Under normal conditions, cortisol levels in cows are close to 5ng/ml, but can range between 10-20 ng/ml, however, plasma cortisol levels increase by 20-30% during controlled stress. In cows, an increase occurs 3-4 times during birth; in metabolic disorders such as hypocalcemia, this increase can reach 5-7 times.[29] The prenatal increases in plasma cortisol concentrations observed in this study were similar to those previously reported.[28] While Khan et al.[34] found that vitamin E administration reduced cortisol levels in cows, and Gupta et al.[27] found that a single dose of Evit+Se administration in cows 3 weeks before calving reduced cortisol levels at the time of calving, vitamin E administration had no effect on cortisol levels in the current study. Differences in care, nutrition, and management between trials could explain the differences between the studies.

It has been reported that GSHPX and MDA levels affect reproductive performance in cows; in the summer, when MDA levels are high and GSH-Px levels are low, the period between calving and conception is lengthened, and the number of inseminations increases.[21] Vitamin E administration shortened the calving-first estrus, calving-first insemination, and calving-conception periods compared to the control group in the present study, albeit the difference was not statistically significant ($p>0.05$). From this perspective, it is considered that vitamin E administration during the peripartum period decreases oxidative stress in the postpartum phase, hence improving reproductive parameters. Similarly, Moghimi-Kandelousi et al.[39] found that vitamin E shortens the calving-first estrus and calving-conception periods. Evit+Se improved these values, according to Pontes et al.[47]and Bayril et al.[8]. High amounts of Vitamin E, on the other hand, did not shorten the calving-first estrus time but did shorten the calving-conception time, according to Arechiga et al.[4]. However, increased vitamin C and E doses did not improve reproductive indicators, according to González-Maldonado et al.[24].

CONCLUSIONS

As a result, a single dose of 3000 mg of vitamin E given to dairy cows approximately 10 days before calving increased GSH and GSH-Px activity, which helps protect the cell from oxidative damage and may thus be effective in reducing birth stress. GPT readings were found to be lower, suggesting that vitamin E administration could help protect the liver. It also increased reproductive performance by reducing the time between calving-first estrus, calving -first insemination, and calving-conception, however the difference was not statistically significant ($p>0.05$) when compared to the control group. New studies on more animals are needed to completely understand the effects of vitamin E administration on reproductive performance.

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