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Effect of Vitamin C at Different Doses on the Oxidant/Antioxidant System of Ewes During Late Pregnancy, Early and Late Lactation and Their Lambs

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ABSTRACT

Background: The development of connective tissue in the fetus during the ruminant gestation period increases the need for vitamin C, which is then further increased by the course of pregnancy and lactation. As pregnancy and lactation develop, free radicals and MDA produced as a result of oxidative stress interact with functional groups in the cell, resulting in cell damage. The oxidant/antioxidant balance at this time can be calculated using the total antioxidant status, total oxidant status and oxidative status index values. This study aimed to investigate the effect of two different doses of vitamin C on oxidative stress indicators in late gestation, early and late lactation ewes and their offspring.

Methods: In the study, 48 ewes of the German Meat Merino and Akkaraman breeds that were developed by crossbreeding were employed. After breeding, four groups were formed from pregnant and non-pregnant ewes. Negative Pregnancy Control group was formed from non-pregnant ewes. Positive Pregnancy Control, Positive Pregnancy Practice 1 and Positive Pregnancy Practice 2 groups were formed from pregnant ewes. After the 90th day of pregnancy, ewes in the Negative Pregnancy Control and Positive Pregnancy Control groups received saline (0.9% NaCl, 2.5 ml) injections every week concurrently with the other pregnant groups until delivery. From the 90^{th} day of pregnancy until birth, sheep participating in Positive Pregnancy Practices 1 and 2 received weekly injections of vitamin C (625 mg/CA, 2.5 ml and 1250 mg/CA, 5 ml, respectively).

Result: In the study, no statistically significant difference was found in serum MDA, TAS, TOS and OSI levels between late pregnancy, lactation and born lambs. While none of the pregnant ewes in this study aborted, vitamin C injection significantly decreased postnatal lamb mortality. This showed that vitamin C helped pregnant ewes feel less stressed throughout pregnancy and helped lower postnatal lamb mortality. In pregnant ewes, AO-effective vitamin C supplementation has been shown to have possible beneficial effects on both maternal and offspring health, whereby the number of stillbirths was reduced by vitamin C supplementation, especially in Positive Pregnancy Practice 2 lambs.

Key words: Antioxidant, Ewe, Lactation, Oxidant, Pregnant, Vitamin C.

INTRODUCTION

An increase in free radicals (Arslan and Tufan, 2010) and a decrease in antioxidants (AO) are caused by the immune system being suppressed during late pregnancy and labor (Golf and Horst, 1997). Oxidative stress (OS) is caused by an increase in reactive oxygen species (ROS) and AO consumption during lactation (Kolb and Seehawer, 2000). Reduced AO levels and elevated ROS levels have been seen in dairy cows (Bernabucci *et al*. 2005) and Syrian goats (Celi, 2010) during late gestation and lactation. According to reports, lipid peroxidation (LPO) plays a role in the etiology of many neonatal disorders and neonates, particularly preterm infants, are particularly vulnerable to oxidative damage caused by free radicals (Biondi *et al*. 2005). One of the AOs that shields cells from OS's harmful effects and scavenge ROS and nitrogen species is vitamin C (Halliwell, 1996). The development of connective tissue in the fetus as the pregnancy progresses in ruminants increases the need for vitamin C (Chattopadhyay *et al*. 1972). In some studies, it has been suggested that vitamin C reduces OS by showing a protective effect in fetal growth retardation, reducing fetal deaths and mutagenesis caused by ROS (Chen *et al*. 2006; Cederberg *et al*. 2011; Gupta *et al*. 2017).

Cells high in polyunsaturated fatty acids, such as erythrocytes, are subject to peroxidation events brought on by free radicals produced during oxidative stress. Cell

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damage is brought on by malondialdehyde (MDA), a product of lipid peroxidation (Bianchi *et al*. 1997). Oxidative stress can be monitored by various biomarkers and measurement of MDA, which has both mutagenic and carcinogenic qualities, is crucial to determining the state of LPO (Draper and Hadley, 1990), the level of cellular damage and the level of OS (Bernabucci *et al*. 2005). Oxidative stress can be monitored by various biomarkers. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative status index (OSI) values can be used to determine the oxidant/antioxidant balance. There are insufficient studies on how OS affects sheep reproductive physiology. In the light of these considerations, the aim of this study was to investigate the effect of two different doses of vitamin C on OS indicators in late gestation, early and late lactation ewes and their offspring.

MATERIALS AND METHODS

This study was supported by Dicle University Scientific Research Coordination Office with the project number 17/012 and conducted in the Department of Biochemistry, Faculty of Veterinary Medicine, Health Sciences between 2017-2019. German Meat Merino and Akkaraman crossbred sheep obtained from Bahri Dağdaş International Agricultural Research Institute were used in the study. Forty-eight clinically healthy Central Anatolian Merino ewes with an average live weight of 60±4.5 kg, older than 2 years and having given birth at least once were used. The nutrients of non-pregnant, pregnant and lactating ewes and their lambs were prepared following NRC standards. Sponges impregnated with Medroxyprogesterone acetate were applied intravaginally for 14 days to the ewes, which were examined with an ultrasound device before the study. Estrus synchronization was performed by PMSG injection within 24 hours after the sponges were removed. A search ram (5 ewes+1 ram) was introduced between the ewes 12 hours after the injection and the ewes were mated. Four groups of pregnant and non-pregnant ewes were formed by ultrasound examination on the 45th day after breeding. Negative Pregnancy Control (NPC, n=8) was formed from non-pregnant ewes. A total of 4 groups were formed from pregnant ewes as Positive Pregnancy Control (PPC, n=13), Positive Pregnancy Practice 1 (PPP1, n=13) and Positive Pregnancy Practice 2 (PPP2, n=14) (Table 1).

NPC and PPC ewes were injected with saline (0.9% NaCl, i.m. 2.5 ml) every week after the 90th day of gestation until delivery simultaneously with the other pregnant groups. Ewes in PPP1 and PPP2 received injections of [250 mg/ml (625 mg/CA) 2.5 ml] and [250 mg/ml (1250 mg/CA) 5 ml] every week, respectively, from the 90th day of pregnancy until birth. Blood samples were taken from ewes on day 0, 105th and 135th days of gestation and 15th and 75th days of lactation before the start of the study. The formation of lamb groups was determined according to the groups of ewes giving birth and blood samples were taken from the vena jugularis into vacuum gel tubes during the 1st and 4th weeks after birth. Blood samples were centrifuged at 3000 rpm for 10 min and serum was separated. Serum separated for oxidative parameters and MDA levels were stored at × 80°C until analysis. TAS and TOS levels were measured using an AU5800 Beckman Coulter autoanalyzer and serum MDA levels were measured using a spectrophotometer (UV-1601 UV-Visible Spectrophotometer, Shimadzu, Japan). Determination of serum MDA levels was performed by the thiobarbituric acid reaction method and TAS and TOS measurements were performed using Rel Assay Diagnostics commercial kits (Yagi, 1984). The conformity of the data to normal distribution was analyzed using the Kolmogorov-Smirnov test. A two-way analysis of variance was used to compare the groups in repeated samples. Duncan’s test was used for multiple comparisons between treatment groups and the dependent t-test was used for comparisons between measurement times. The results were presented as mean±standard error of the mean (SEM) and the significance of the difference between the groups was accepted as $p<0.05$. SPSS 17.0 statistical program was used for data analysis.

RESULTS AND DISCUSSION

This study evaluated the effects of different doses of vitamin C, an important AO, on oxidant/antioxidant status during late pregnancy and early and late lactation in ewes. Additionally, the same parameters were examined in 50 lambs who were born from these ewes. During the study, no adverse clinical signs were observed in pregnant ewes. However, a total of 66 lambs were born from all three groups. Lamb birth and mortality data of all three groups are given in Table 2. No statistically significant difference was found in MDA, TAS, TOS and OSI parameters when comparisons were made within and between groups of sheep ($p>0.05$) (Table 1). However, there were significant differences in intra-group comparisons, although not statistically significant. In the PPC group, MDA levels decreased throughout pregnancy, the lowest MDA level was found on day 135 of pregnancy and the highest MDA level was found on day 75 of lactation. In the PPP1 group, the lowest MDA level was measured on the 105th day of pregnancy, whereas in the PPP2 group, unlike the other groups, MDA levels increased during pregnancy until delivery and reached the highest level on the 75th day of lactation. There was a numerical increase in TAS levels in PPP1 and PPP2 groups on day 135 of pregnancy compared to day 0. While TOS levels showed a partial increase towards the end of pregnancy in PPP1, they started to decrease on day 135 of pregnancy in PPP2 and reached the lowest level on day 75 of lactation.

The results of the analysis of MDA, TAS, TOS and OSI parameters in the born lambs are given in Table 3. Measurements were performed in each group at postnatal weeks 1 and 4. When in-group and between-group comparisons were made, no statistically significant difference was found in MDA, TAS, TOS and OSI parameters ($p>0.05$). In intra-group comparisons, there was a statistically insignificant numerical decrease in MDA levels with the growth of lambs in the PPP1 and PPP2 groups. The most significant decrease in MDA level was observed in PPP2 group.

Table 1: MDA, TAS, TOS and OSI values in sheep at different stages of pregnancy and lactation. ($\overline{X}$±SEM).

Parameter	Days	NPC (n:8)	PPC (n:13)	PPP1 (n:13)	PPP2 (n:14)
MDA	day 0	1.90±0.20	1.90±0.16	1.94±0.17	1.68 ±0.14
	105th day	1.67±0.14	1.81±0.11	1.76±0.11	1.82±0.10
	135th day	1.66±0.18	1.65±0.15	1.85±0.15	1.97±0.13
	15th day of lactation	1.93±0.22	1.94±0.18	1.91±0.19	1.93±0.16
	75th day of lactation	1.72±0.15	2.17±0.12	1.82±0.12	2.02±0.10
TAS	day 0	0.89±0.05	0.95±0.05	0.76±0.05	0.77±0.05
	105th day	1.00±0.04	1.03±0.04	1.11±0.04	1.04±0.04
	135th day	1.05±0.04	1.05±0.04	1.07±0.04	1.08±0.03
	15th day of lactation	1.02±0.04	0.99±0.04	1.04±0.04	1.05±0.03
	75th day of lactation	1.00±0.02	1.00±0.02	1,02±0,02	0.99±0.02
TOS	day 0	7.11±0.23	7.12±0.23	6.88±0.23	7.21±0.20
	105th day	7.03±0.07	7.14±0.07	6.99±0.07	7.12±0.06
	135th day	7.08±0.04	7.04±0.04	7.03±0.04	7.15±0.04
	15th day of lactation	7.07±0.05	7.07±0.05	7.05±0.05	7.07±0.04
	75th day of lactation	6.98±0.05	7.07±0.05	7.02±0.05	7.00±0.04
OSI	day 0	0.80±0.50	0.75±0.10	0.91±1.40	0.93±0.50
	105th day	0.70±0.30	0.69±0.10	0.63±0.10	0.69±0.20
	135th day	0.67±0.10	0.67±0.00	0.66±0.10	0.66±0.00
	15th day of lactation	0.69±0.10	0.71±0.30	0.68±0.10	0.68±0.10
	75th day of lactation	0.70±0.30	0.71±0.30	0.69±0.00	0.71±0.20

No statistically significant difference was found in MDA, TAS, TOS and OSI parameters in the comparisons between and within groups ($p>0.05$).NPC= Negative pregnancy control, non-pregnant ewes, 2.5 ml 0.9% NaCl.PPC= Positive pregnancy control, pregnant ewes, (2.5 ml %0.9 NaCl). PPP1= Positive pregnancy practise 1, pregnant ewes (2.5 ml vitamin C). PPP2= Positive pregnancy practise 2, pregnant ewes (5 ml vitamin C).

Table 2: Lamb birth and death data of pregnant ewes.

	PPC	PPP1	PPP2
Normal number of births	21	22	23
Number of postnatal lamb deaths	7	2	1
Diarrhea-related deaths	1	3	2

PPC= Lambs of positive pregnancy control ewes, (2.5 ml %0.9 NaCl). PPP1= Lambs of positive pregnancy practise 1 ewes (2.5 ml vitamin C). PPP2= Lambs of positive pregnancy practise 2 ewes (5 ml vitamin C).

Gür *et al*. (2011) reported that MDA levels were higher in pregnant ewes compared to non-pregnant ones; similarly, Aydýn *et al*. (2010) reported that levels of some AOs and MDA level increased in ewes during pregnancy compared to pre-pregnancy and that OS occurred especially in the middle stages of pregnancy. Castillo *et al*. (2005), TOS related MDA, which is known as an indicator of OS, increased in early lactation compared to late gestation. It was reported that plasma glutathione peroxidase (GSH-Px) activity associated with TAS, an indirect indicator of oxidative stress, showed higher values 4 weeks after calving compared to 2 weeks before calving (Wullepit *et al*. 2009). In separate studies conducted on Sakız and Awassi ewes, it was reported that plasma GSH-Px activity was higher and plasma CAT activity was lower at the end of pregnancy compared to non-pregnant ewes (Öztabak *et al*. 2005; Erisir *et al*. 2009). However, Castillo *et al*. (2006) reported that mean MDA and TAS concentrations were not significantly different between 10, 6, 2 and 1 week before calving and 1, 2 weeks after calving and late lactation in dairy cows. Öztabak *et al*. (2005) reported that LPO levels in the 4th and 5th months of pregnancy in Sakız ewes were not different from non-pregnant ewes. Similarly, Erisir *et al*. (2009) reported that there was no significant difference in MDA level between early and late pregnancy in Awassi ewes compared to non-pregnant ewes.

Aydın and Köse (2015) reported that MDA serum concentrations in Saanen goats decreased significantly during pregnancy and lactation compared to pre-pregnancy values and reached the lowest value in the 3rd month of pregnancy, while AO levels reached the highest value in the same periods. Karapehlivan *et al*. (2013) found that TAS levels were lower in early lactation goats compared to late lactation goats, while TOS and OSI levels were significantly higher. The same researchers reported that there were significant differences in MDA levels between early and late lactation periods of goats (Karapehlivan *et al*. 2013).

The findings obtained in studies conducted on humans also show difference. Arıkan *et al*. (2001) reported that serum MDA concentration in late pregnancy was statistically higher and Yüksel *et al*. (2015) reported that it was lower, while Shilina *et al*. (1999) reported that there was no significant change in serum MDA levels despite an increase in AO levels during pregnancy.

In the present study, no statistically significant difference was detected in serum MDA, TAS, TOS and OSI levels in late

Table 3: MDA, TAS, TOS and OSI values of born lambs ($\overline{X}$±SEM).

Parameter	Post-natal	PPC (n:13)	PPP1 (n:17)	PPP2 (n:20)
MDA	1.week	2.52±0.20	2.56±0.17	2.98±0.17
	4.week	2.57±0.19	2.45±0.17	2.50±0.16
TAS	1.week	1.19±0.03	1.11±0.02	1.11±0.02
	4.week	1.16±0.03	1.10±0.02	1.15±0.02
TOS	1.week	7.00±0.05	6.99±0.03	6.93±0.03
	4.week	6.92±0.05	6.88±0.04	6.86±0.03
OSI	1.week	0.59±0.30	0.63±0.18	0.62±0.16
	4.week	0.60±0.27	0.63±0.23	0.60±0.20

There is no statistical difference in the evaluation made between lambs born (p>0.05).PPC= Lambs of positive pregnancy control, (2.5 ml % 0.9 NaCl).PPP1= Lambs of positive pregnancy practise 1, (2.5 ml vitamin C). PPP2= Lambs of positive pregnancy practise 2, (5 ml vitamin C).

gestation and lactation periods of ewes and lambs born to these mothers. In this regard, it was observed that the results were consistent with the values reported in the literature by several researchers (Shilina *et al*. 1999; Öztabak *et al*. 2005; Castillo *et al*. 2006; Erisir *et al*. 2009). While no abortion occurred in any of the pregnant ewes in the study, mortality was observed at different rates in lambs born with or without vitamin C supplementation. When lamb mortality was examined, it was determined that vitamin C injection during pregnancy significantly reduced the number of postnatal lamb deaths and a similar situation was observed in fish fed with adequate levels of vitamin C by (Kop, 2019), however, deaths due to diarrhea were close to each other. In this instance, it may be claimed that vitamin C helped both the mother and the fetus have better pregnancies by reducing stress during the ewes' pregnancies. The fact that postnatal lamb deaths were lower in pregnant ewes given vitamin C indicates that may have contributed positively as an antioxidant.

CONCLUSION

The number of stillbirths decreased with vitamin C supplementation, especially in PPP2 group lambs, indicating that it will significantly contribute to animal health and the economy. AO-effective vitamin C supplementation in pregnant ewes has potential beneficial effects on both maternal and offspring health. The statistics acquired show that more thorough research is required to create fresh methods to lower lamb mortality and financial losses.

Conflict of interest

The authors declare no conflicts of interest.

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