Association of antioxidant administrations to udder health and milk quality in Awassi ewes

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Abstract

This study aimed to investigate the effect of antioxidant administration on udder health in Awassi ewes. The animals were divided into two groups as control (C; n = 25) and treatment groups (T; n = 25). Group T was treated with vitamins (E and B1) and selenium (Se) on day 0 of the study. In groups, blood samples were collected on day 0 (1st measurement time) and day 14 (2nd measurement time) of the study, and milk samples were taken on day 14. In blood samples, total antioxidant status (TAS), total oxidant status (TOS), and selected biochemical indices were analysed. In milk samples, TAS, TOS, somatic cell count (SCC) analyses and pathogen isolation including fungi and bacteria were performed. In addition, oxidative stress index (OSI) was calculated for both milk and blood samples. In Group T, TAS level increased from 1st t to 2nd measurements ($P < 0.01$). At the time of 2nd measurement, aspartate aminotransferase (AST) and cholesterol levels decreased in Group T compared to Group C ($P < 0.01$). In Group T, cholesterol decreased at 2nd measurement compared to 1st measurement ($P < 0.01$). It was found that milk TAS increased in Group T ($P < 0.01$). However, milk SCC, TOS and OSI levels decreased in Group T ($P < 0.01$). Fungal isolation rate was significantly lower in milk samples collected from Group T compared to Group C ($P < 0.01$). Antioxidant treatment positively affected udder health indicators in terms of milk SCC, fungal organism growth, and oxidative stress.

Ovine, oxidative stress, ruminants, supplementation

Although sheep milk production makes only about 2% of total cow milk yield, dairy sheep represent an important sector of agricultural activities in many areas (Pazzola et al. 2018), and efforts are made to keep this limited production at the optimum level in order to meet the demand for sheep milk. Therefore, udder health in ewes is of critical importance as it affects the milk quality (Pazzola et al. 2018) as well as quantity (Paithane and Khodke 2017). Many studies have been conducted to increase milk quality by improving udder health (Schukken et al. 2003; Yang and Li 2015; Vrdoljak et al. 2020), and the vast majority of researches have focused on mastitis control in dairy herds (Menzies and Ramanoon 2001; Gelasakis et al. 2015; Libera et al. 2021). Moreover, it is possible to keep udder health and milk quality at the optimum level with various management strategies performed in healthy sheep without mastitis. It is known that one of the important strategies effective for udder health is antioxidant administration (Sharma et al. 2007; Celi and Chauhan 2013). Vitamin, mineral substances and trace elements are known as antioxidant substances (Yang and Li 2015).

Oxidant substances can be produced in the body during normal metabolic functioning, and if their level exceeds the neutralizing capacity of antioxidant substances, oxidative stress occurs (Puppel et al. 2015; Rossi and Dell'Anno 2024). As a result of oxidative stress, damage may occur to components of the cell such as lipids, proteins, and DNA, and as a result, serious problems occur in the function, activity, and immunological structure

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of the cells (Martins et al. 2021; Archana and Jyotsana 2024). In this case, susceptibility to many diseases, especially immune system-related diseases such as mastitis, may increase (Novac and Andrei 2020).

Antioxidants protect cells from the harmful effects of oxidative stress (Celi and Chauhan 2013; Kurt and Eşki 2021). Mammary epithelial cells are more sensitive to oxidative stress as they produce a large amount of oxidant substances due to their high metabolism (Yang and Li 2015). So, impaired udder health is associated with decreased antioxidant levels and increased oxidant levels (Yang and Li 2015). Antioxidants improve the function of the defence system in immune cells (Lykkesfeldt and Svendsen 2007; Yang and Li 2015), protect the mammary gland against infections, and increase milk quality by reducing the somatic cell counts (SCC) (Yang and Li 2015). On the other hand, milk antioxidant levels and the presence of bacterial and fungal organisms can also affect milk quality (Novac and Andrei 2020). It is also thought that antioxidant administrations can directly increase antioxidant levels in milk and may be useful in the fight against fungi and bacteria due to their immunomodulatory effects (Stobiecka et al. 2022).

Considering the above information, it is hypothesized that antioxidant status may have an effect on udder health and milk quality. Therefore, the present study aimed to investigate the effect of antioxidant administration on milk oxidative stress, SCC, and bacterial and fungal isolates in ewes. Furthermore, alkaline phosphatase (ALP), alanine aminotransfrease (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), cholesterol and glucose were analysed to evaluate the health status of the animals.

Materials and Methods

Animals and management

The present study was approved by the Ethics Committee of Ceyhan Veterinary Faculty, Cukurova University, Adana, Türkiye (approval number: 09/02 and 05.08.2021), and was conducted on a total of 50 female Awassi ewes during the late lactation period in a commercial sheep farm. The ewes had a similar body condition score (2.5), milk yield (1.5–2 l), age (2) and parity (1). They were clinically healthy and did not experience any problems in terms of udder health. Before the study, clinical examination of the mammary gland of each animal was performed. Animals with clinically healthy mammary glands and negative California mastitis test were included in the study. The animals were managed with the semi-extensive sheep farming system.

Groups and study design

The ewes were randomly divided into two groups; a control group (C; n = 25) and a treatment group (T; n = 25). Group C did not receive any treatment. Group T were treated intramuscularly with injections of 2 ml of solution (E-sevil, Vilsan®, Ankara, Türkiye) containing selenium (Se), vitamins E and B1 (each ml contained 1 mg of sodium selenite, 60 mg of Vitamin E, 40 mg of Vitamin B1) on day 0 (at the beginning of the study). The study protocol was created according to the routine practices of the sheep farm. The study design and measured indicators are presented in Fig. 1.

Blood and milk samples

The milk and blood samples were collected by a veterinarian before morning milking and feeding. In C and T groups, blood samples were collected from the jugular vein into sterile serum tubes with clot activator (Hema and Tube®, Sant Agostino, Ferrara, Italy) on days 0 and 14 of the study. On the 14th day, milk samples were equally taken from two udder lobes of each ewe into a sterile tube (10 ml; two samples per ewes; Isolab®, Eschau, Unterfranken, Germany) under aseptic conditions according to the previously described method (Kurt and Eşki 2021). One of the two milk samples taken from each ewe was transported to the laboratory under cold chain (at 4 °C) for microbiological and SCC analyses. Blood and other milk samples were centrifuged at 1,500 × *g* for 10 min and 4,000 × *g* for 15 min, respectively. Finally, serums were harvested and stored at −20 °C until analyses. In blood samples, serum total antioxidant status (TAS), total oxidant status (TOS), and some biochemical indicators (ALP, ALT, AST, GGT, cholesterol and glucose) were analysed. In milk samples, TAS, TOS, SCC, and microbiological analyses (fungal and bacterial isolation) were performed.

Biochemical blood analyses

Blood serum ALP, ALT, AST, GGT, cholesterol and glucose levels were analysed by electrochemiluminescence immunoassay (ECLIA) method using commercial kits (Roche/Hitachi, Cobas®, Mannheim, Germany).

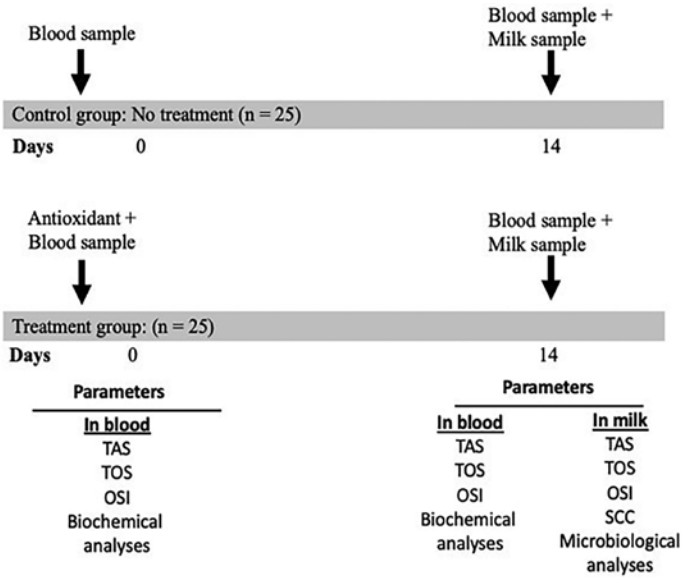

Fig 1. Experimental study design and measured indicators.

TAS: Total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index; SCC: somatic cell count.

Determination of milk somatic cell counts

Milk SCC was determined using an automatic somatic cell counting device (DCC®, DeLaval International AB, Tumba, Sweden) and a commercial counting cassette (Delaval Cell Counter Cassettes: 92865881). All procedures of the analysis were performed according to the manufacturer's instructions.

Microbiological examination in milk samples

Fungi, Gram-negative and Gram-positive bacteria were identified by standard procedures according to the guidelines of the National Mastitis Council (Hogan et al. 1999). As described in the previous study (Kurt and Eşki 2021), blood agar (Oxoid, CM0055) and MacConkey agar (Oxoid, CM0007, Hampshire, UK) were used for bacteriological isolation, while sabouraud Dextrose agar (Merck, 105438, Darmstadt, Germany) was used for fungal isolation.

Total antioxidant status and total oxidant status analyses

Milk and blood serum TAS and TOS levels were measured colorimetrically using Enzyme Linked Immunosorbent Assay (ELISA, Stat Fax 2100, Awareness Technology, Ramsey, USA) device and commercial kits (TAS and TOS: Rel Assay Diagnostics®, Gaziantep, Türkiye). Measurements of these indicators were performed according to the manufacturer's instructions.

Calculation of oxidative stress index

The oxidative stress index (OSI) value was defined as the ratio of TOS to TAS. Firstly, the resulting unit of TAS (mmol Trolox eq/l) was converted to µmol Trolox eq/l, and the OSI value was calculated with the following formula: OSI (arbitrary unit) = TOS (µmol H_2O_2 Eq/l) / TAS (mmol Trolox Eq/l) × 100, as described by a previous study (Kurt and Eşki 2021).

Statistical analysis

Statistical analysis of the obtained data was performed using the SPSS (Version: 16.0; IBM, USA) package program. Normality test was performed using Kolmogorov-Smirnov test (ANOVA). Since all parameters showed normal distribution, they were analysed with the parametric One-Way ANOVA test. In the study, the relationship between the groups was analysed with independent *t*-test. Paired samples *t*-test was used to determine the changes between the 1st and 2nd measurements. In addition, the relationship between the measurements was determined with the Pearson's correlation coefficient. $P < 0.05$ was accepted as the significance level, and results were given as mean ± standard error of the mean.

Results

Blood

At the time of 1st measurement, blood TAS, TOS and OSI values were found to be similar between Group C and Group T ($P > 0.05$). At the time of the 2nd measurement, no difference was observed in terms of TAS, TOS, and OSI in Group T compared to Group C ($P > 0.05$). In Group C, no difference was found between the 1st and 2nd measurements in terms of TAS, TOS and OSI values ($P > 0.05$). In Group T, TAS levels increased significantly from the 1st to the 2nd measurement ($P < 0.01$), while the TOS and OSI levels did not change ($P > 0.05$) (Table 1).

At the time of the 1st measurement, ALP, ALT, AST, GGT, cholesterol and glucose values were similar between Group C and Group T ($P > 0.05$). At the time of the 2nd measurement, AST and cholesterol levels decreased significantly in Group T compared to Group C ($P < 0.01$), but no difference was observed in terms of ALP, ALT, GGT and glucose ($P > 0.05$). In Group C, ALP, ALT, AST, GGT, cholesterol and glucose values were similar

Table 1. Oxidative stress indicators determined in the control (C) and treatment (T) groups.

Indicator	Group	1st measurement (Day 0) Mean ± SEM	2nd measurement (Day 14) Mean ± SEM
TAS (mmol/l)	Group C (n = 25)	1.21 ± 0.03	1.21 ± 0.02
	Group T (n = 25)	1.15 ± 0.02^{A}	1.33 ± 0.02^{B}
TOS (μmol)	Group C (n = 25)	19.05 ± 0.87	18.50 ± 0.72
	Group T (n = 25)	19.04 ± 1.08	20.97 ± 1.19
OSI	Group C (n = 25)	1.60 ± 0.08	1.56 ± 0.07
	Group T (n = 25)	1.67 ± 0.09	1.59 ± 0.08

$^{A, B}$ Different uppercase superscripts in the same row represent statistical significance ($P < 0.01$)

TAS: Total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index

Table 2. Biochemical indicators determined in the control (C) and treatment (T) groups.

Indicator	Group	1st measurement (Day 0) Mean ± SEM	2nd measurement (Day 14) Mean ± SEM
ALP (U/l)	Group C (n = 25)	51.00 ± 1.46	53.24 ± 1.00
	Group T (n = 25)	49.88 ± 1.58	51.76 ± 2.51
ALT (U/l)	Group C (n = 25)	21.40 ± 0.84	24.00 ± 1.23
	Group T (n = 25)	21.92 ± 0.88	22.88 ± 0.94
AST (U/l)	Group C (n = 25)	74.08 ± 1.53	76.36 ± 1.28^{a}
	Group T (n = 25)	72.60 ± 2.11	71.32 ± 2.06^{b}
GGT (U/l)	Group C (n = 25)	38.16 ± 1.67	36.96 ± 1.34
	Group T (n = 25)	39.48 ± 1.44	39.64 ± 1.91
Cholesterol (mg/dl)	Group C (n = 25)	83.08 ± 2.69	85.32 ± 3.25^{a}
	Group T (n = 25)	88.36 ± 2.36^{A}	72.52 ± 1.68$^{B, b}$
Glucose (mg/dl)	Group C (n = 25)	45.88 ± 2.42	48.04 ± 2.24
	Group T (n = 25)	43.84 ± 1.54	45.64 ± 1.40

$^{A, B, a, b}$ Different uppercase superscripts in the same row and different lowercase superscripts in the same column represent statistical significance ($P < 0.01$)

ALP: Alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase, GGT: gamma-glutamyl transferase

between the 1st and the 2nd measurement ($P > 0.05$). In Group T, cholesterol decreased significantly at the 2nd measurement compared to the 1st ($P < 0.01$), while ALP, ALT, AST, GGT and glucose values did not change ($P > 0.05$) (Table 2).

Milk

The TAS in milk was found to be increased in Group T compared to Group C ($P < 0.01$). However, milk SCC, TOS and OSI values decreased in Group T compared to Group C ($P < 0.01$) (Table 3).

No bacterial species could be isolated from the milk samples collected from both Group C and Group T. However, the fungal isolation rate was significantly lower in milk samples collected from Group T compared to Group C ($P < 0.01$) (Table 4).

Representative images of the microbiological analyses of this study are presented in Fig. 2 (Plate I).

Table 3. Milk somatic cell counts and oxidative stress indicators in milk serum samples obtained from the control (C) and treatment (T) groups.

Indicator	Group C (n = 25) Mean ± SEM	Group T (n = 25) Mean ± SEM
SCC	653.20 ± 24.68^{A}	487.80 ± 46.45^{B}
TAS	0.95 ± 0.02^{A}	1.16 ± 0.01^{B}
TOS	22.00 ± 0.59^{A}	17.78 ± 0.81
OSI	2.36 ± 0.09^{A}	1.54 ± 0.07^{B}

$^{A, B}$ Different uppercase superscripts in the same row represent statistical significance ($P < 0.01$)

SCC: Somatic cell count; TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index

Table 4. Microbiological analysis results of milk samples in the control (C) and treatment (T) groups.

Indicator	Group C (n = 25) Mean ± SEM	Group T (n = 25) Mean ± SEM
Fungi	1.80 ± 0.08^{A}	1.28 ± 0.09^{B}

$^{A, B}$ Different uppercase superscripts in the same row represent statistical significance ($P < 0.01$)

Discussion

As a current approach, the presented study suggested that the antioxidant status may affect the udder health and milk quality in Awassi ewes. To confirm this assumption, two groups of healthy ewes in the same lactation period were created. Finally, antioxidant treatment was administered to one group. The difference of TAS, TOS, OSI and selected biochemical indicators between the groups, and the effect of antioxidant administrations on udder health were investigated. The primary reason for biochemical measurements including ALP, ALT, AST, GGT and cholesterol was to assess liver function and the health status of the animals, as liver function can affect milk evaluation (Mordak et al. 2020) and results of other targeted measurements. At the 1st measurement time, these values were similar in both groups. However, at the 2nd measurement time, AST and cholesterol values decreased in Group T compared to Group C. We think that this is due to antioxidant therapy because it is known that antioxidant substances can have an improving effect on liver function (Bouwstra et al. 2008).

Many researchers have reported that antioxidant therapy reduces the negative effects of oxidant substances and is an effective method of combating oxidative stress (Lykkesfeldt and Svendsen 2007; Celi 2010; Celi and Chauhan 2013). Therefore, it is expected that antioxidant administrations decrease OSI values by increasing the antioxidant capacity of the blood (Lykkesfeldt and Svendsen 2007; Abuelo et al. 2013; Celi and Chauhan 2013). However, in the presented study, antioxidant therapy had no effect on blood TAS, TOS and OSI values in Group T compared to Group C. We thought that the reason for this might be the administration dose and/or therapy frequency of the antioxidant substances. On the other hand, antioxidant administration increased the TAS level at the 2nd measurement compared to the 1st measurement in Group T. Thus, it appears that antioxidant therapy improved the antioxidant capacity in Group T, although antioxidant treatment did not affect the blood TAS, TOS, and OSI values.

It is known that the SCC value is a typical indicator of milk quality and udder health in dairy animals (Schukken et al. 2003; Yang and Li 2015). The milk secretion of sheep is usually apocrine and the acceptable SCC value has not been definitively determined (Paape et al. 2007; Albenzio et al. 2019). A previous study reported that the SCC value of hygienic sheep milk should be below 700,000 cells/ml (Sevi et al. 1999). In this study, SCC findings in both groups were within acceptable limits. Another study reported that the SCC values of good quality and average quality milk were below 500,000 cells/ml and between 500,000 and 1,000,000 cells/ml, respectively (Albenzio et al. 2019).

According to this information, it is understood that the milk samples in Group T were of good quality, whereas the milk samples in Group C were of average quality. We also performed some microbiological and oxidative stress analyses as well as SCC in milk samples for a more comprehensive study. It was observed that TOS, OSI, and SCC values decreased significantly, and the TAS value increased in Group T compared to Group C. It was thought that this situation may have resulted from the administration of antioxidants. In addition, previous studies have reported that antioxidant administrations have an effective role in reducing SCC in dairy animals (Alhussien and Dang 2018).

According to the above information, it was an expected result that while the TAS value increased, the SCC, TOS, and OSI values decreased in milk samples of Group T. However, there is a contradictory situation, because the fact that antioxidant treatment did not affect blood oxidative stress indicators (TAS, TOS, OSI) cannot be explained with this information. It was thought that this might be due to the fact that the udder tissue can use antioxidant substances more actively or they can be more effective in the udder tissue. A previous study on sheep and goats found that a diet containing phenol, an antioxidant substance, increased phenols in blood by 10% in the fifth week of the study, while increasing phenol concentrations in milk by 32%. Thus, it is understood that the antioxidant substance passes into milk more than into blood (Leparmarai et al. 2019). Another study conducted on heifers indicated that α-tocopherol concentrations in blood may be lower in the last few weeks before parturition, and this may be related to the transfer of vitamin E to the mammary gland (Bouwstra et al. 2008). These findings support our idea. However, we think that this relationship needs a comprehensive explanation.

On the other hand, no bacterial isolation was obtained in either group, while the fungal isolation rate was lower in Group T than in Group C. However, the antifungal effect of antioxidants is fully unknown. This situation can be explained by two hypotheses. One is that the improving effect of TAS on the immune system may have limited the growth of fungi in milk. The other is that this effect may be related to the decreased OSI level in milk (Abuelo et al. 2015), reflecting a better redox balance which can inhibit fungal proliferation (Zhao and Wang 2020; Kafkas et al. 2021).

Although these findings support our idea, this situation needs to be investigated thoroughly for a definitive result. Since all animals in this study were randomly selected from clinically and mammary gland-healthy animals, milk samples were thought to have similar values, and therefore, milk samples were not examined at the beginning of the study. In fact, the similarity of the analysis results of blood indicators on day 0 between the groups supports this assumption. Because the main aim was to determine the effect of antioxidant administration on milk indicators, milk samples were analysed on the 14th day after administration. However, we believe that examining milk samples at the beginning of the study in future studies would make the results more detailed.

In conclusion, this study investigated the relationship between antioxidant therapy and udder health indicators in Awassi ewes. Antioxidant therapy did not affect blood oxidative stress indicators. However, antioxidant treatment positively affected udder health indicators in terms of milk SCC count, fungal organism growth, and antioxidant content. Yet, we believe that the relationship between antioxidant therapy and fungal isolates in milk needs to be investigated comprehensively by further studies in ewes.

Conflict of interest

The authors declare that there is no conflict of interests.

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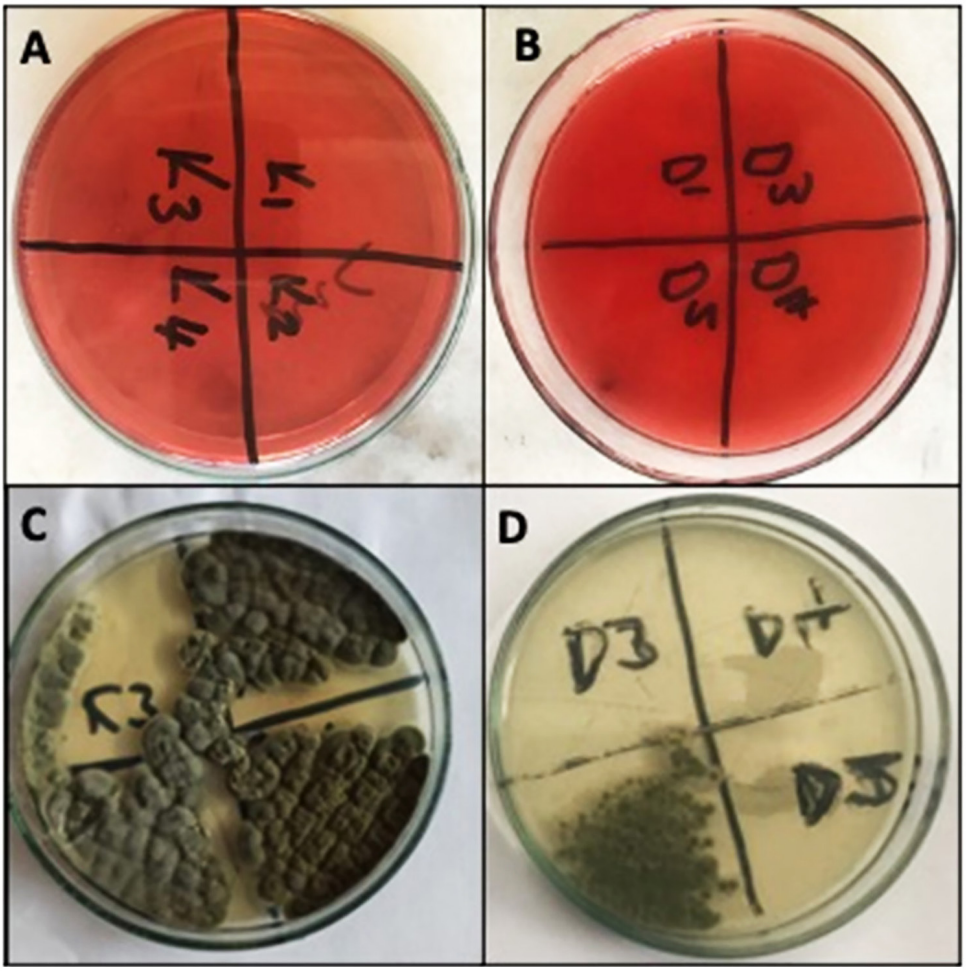

Fig 2. Representative images of fungal and bacterial isolates obtained in this study. Bacterial isolation in the control Group C (A) and the treatment Group T (B); fungal isolation in Group C (C) and Group T (D).