This study was carried out to investigate the effects of subclinical mastitis, which has great importance in terms of economic losses in animal husbandry, on the glutathione, ceruloplasmin, and vitamin A, E, and C levels of the sheep during their first lactation phase. A total of 30 Akkaraman sheep were used as the live material of the study. 20 of these were animals diagnosed with subclinical mastitis with mastitis-indicator papers, CMT, and mastitis diagnosis by their milk; while 10 the sheep were reserved as the healthy control group. The metabolic levels of glutathione, ceruloplasmin, and vitamin A, E, and C levels were measured in blood plasma of the sheep, and were found to be 15.43±1.47 to 19.61±2.95 mg/dl, 16.54±0.14 to 17.13±0.11 mg/dl, 0.39±0.04 to 0.44±0.03 mg/lt, 254.3±11.8 to 290.1±14.3 µg/dl, and 5.91±0.17 to 6.75±0.15 µg/ml respectively. There were statistically significant reductions in plasma GSH ($p$≤0.05), vitamin A ($p$≤0.05), vitamin C ($p$≤0.05) and vitamin E ($p$≤0.01) levels in the sheep afflicted by subclinical mastitis compared to the control group. Their ceruloplasmin levels were also slightly decreased. These decreased levels indicate an increased oxidative stress, and as a result, supporting the weakened immune systems of the sheep with antioxidants, either through injection or as a feed additive during the pregnancy and lactation periods, may reduce oxidative stress and increase the resistance of the animals to mastitis infection.

**Keywords:** Ceruloplasmin, GSH, sheep, subclinical mastitis, vitamins.
The primary function of vitamin E is to act as the strongest antioxidant agent. Vitamin E prevents the oxidation of unsaturated fatty acids within the membranes, protecting them from any potential degradation [9].

Vitamin C (ascorbic acid) is a keto-lactone with the closed formula of C_6H_8O_6. It is particularly abundant in green herbs and fruits, and in citrus fruits. It is effortlessly absorbed small intestines. It’s resistant to freezing but is vulnerable to heating [6]. Vitamin C takes part in oxidation and reduction processes as an antioxidant, and in some cases, is used as co-factor for iron and copper [10].

This paper aims to investigate the blood GSH, ceruloplasmin, and vitamin A, C, and E levels of sheep with mastitis.

**Materials and Methods**

The live specimens for this study consisted of 30 animals raised under the same conditions in a farm located in the Toprakseven village of Çaldıran district of Van. The animals were of 3 to 5 years old, and all were in their initial lactation phase. The animals were first clinically diagnosed, and those without findings of chronic or acute inflammation in their udders or any other generic infection symptoms were separated. Those remained were then subjected to mastitis test using mastitis indicator papers and California mastitis test (CMT) [11]. 10 of the animals which returned negative on the CMT and mastitis indicator paper tests were selected as the control group, while 20 which returned positive results were selected as the test group.

A mastitis indicator paper can be used to determine the change of acidity in the milk which may occur in case of an infection. The milk of the sheep which were tested using these papers was also subjected to the CMT. Blood samples were also collected from the jugular veins of the sheep into 10 ml tubes with and without anticoagulants (heparin). The blood samples were then tested for GSH [12, 13], ceruloplasmin [14], and Vitamins A, E [15], and C [16] analyzes.

**Results**

The average blood GSH, ceruloplasmin, and vitamin A, C, and E levels of healthy sheep and sheep with mastitis are given in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>Control group (X±SEM)</th>
<th>n</th>
<th>Sheep with mastitis (X±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH mg/dl</td>
<td>10</td>
<td>19.61 ± 2.95</td>
<td>20</td>
<td>15.43 ± 1.47^a</td>
</tr>
<tr>
<td>Ceruloplasmin mg/dl</td>
<td>10</td>
<td>16.54 ± 0.14</td>
<td>20</td>
<td>17.13 ± 0.11</td>
</tr>
<tr>
<td>Vitamin A mg/L</td>
<td>10</td>
<td>0.44 ± 0.03</td>
<td>20</td>
<td>0.39 ± 0.04^b</td>
</tr>
<tr>
<td>Vitamin E µg/dl</td>
<td>10</td>
<td>290.1 ± 14.3</td>
<td>20</td>
<td>254.3 ± 11.8^b</td>
</tr>
<tr>
<td>Vitamin C µg/ml</td>
<td>10</td>
<td>6.75 ± 0.15</td>
<td>20</td>
<td>5.91 ± 0.17^c</td>
</tr>
</tbody>
</table>

^a p≤0.05; ^b p≤0.01

**Discussion and Conclusion**

Sheep-raising has a significant place in the animal husbandry sector all around the world. Sheep may be raised for their meat, milk, and fleece, and is traditionally bred in small, family-type businesses. Sheep are usually preferred due to their low nutrition and care costs compared to other animals [17].

There are not many studies on literature conducted on mastitis. Most of the existing mastitis studies are on the cows, and this is why most of the studies attributed in this paper are studies them.

The studies on cows report lower GSH levels in animals with mastitis compared to control groups [18, 19, 20, 21, 22]. In another study conducted on sheep with mastitis, MDA levels were reported to be higher than normal [17].

In the present study, the average GSH level of healthy sheep is measured as 19.61±2.95 mg/dl, while it was found to be 15.43±1.47 mg/dl for the sheep with mastitis. The difference is statistically significant (p<0.05). This result parallels the findings of the studies in the literature.

The ceruloplasmin levels of the control group and sheep with mastitis in our study were found to be 16.54±0.14 and 17.13±0.11 mg/dl respectively, which is also concordant with the findings of similar studies conducted on cows with mastitis [21, 23]. Ceruloplasmin usually increases during inflammatory cases, like the case is in our study; the difference between control and test group in this case, however, was found to be statistically insignificant.

Numerous literature studies have revealed that vitamin A level decreases in cows with mastitis [24-26]. That being said, there are a few studies which report increased Vitamin A levels in mastitis groups compared to controls [21, 27]. In our study, the average vitamin A level for healthy sheep was found as 0.44±0.03 mg/l, while in the control group the average level was 0.39±0.04 mg/l. The difference between the groups was found to be statistically relevant with p≤0.05. The
reduction in the vitamin A level is mostly in parallel to the findings of the literature studies.

The studies conducted in cows with mastitis report reduced vitamin E levels in the animals with mastitis, compared to control groups [22, 25, 28, 29, 30, 31]. Some of these studies report the difference to be statistically relevant, while others do not. One study reports partially increased vitamin E levels for the mastitis group compared to the control [27]. In our study, the control group’s average vitamin E levels were determined as 290.1±14.3 µg/dl, while the mastitis group had an average vitamin E level of 254.3±11.8 µg/dl. This reduction in the mastitis group was found to have a statistical significance level of p≤0.01. This reduction may have occurred due to some of the vitamin E reserves being used against the lipid peroxidation that takes place as a result of the inflammation.

Studies conducted on cows with mastitis report a lowered amount of vitamin C levels, similar to the vitamins A and E, and explain that sub-clinical mastitis reduces the antioxidant concentrations in the blood [21, 32]. In the present study, vitamin C levels of the control and mastitis groups were found to be 6.75±0.15 and 5.91±0.17 µg/ml respectively, and the difference between the groups was found to be statistically relevant with a significance level of p≤0.05.

In our study and in other results, subclinical mastitis disturbs the antioxidant balance of the organism. Such effects cause deficiencies in living conditions such as milk yield, reproduction and chances of survival. To prevent this, preventive medicine and early diagnosis are of great importance.

REFERENCES


