**Scholars Journal of Agriculture and Veterinary Sciences** OPEN ACCESS
Abbreviated Key Title: Sch J Agric Vet Sci
ISSN 2348–8883 (Print) | ISSN 2348–1854 (Online)
Journal homepage: www.saspublishers.com

# Investigation of GSH, Ceruloplasmin, and Vitamin A, C and E Levels in Sheep with Sub-Clinical Mastitis

Baris ERTUNC[1], Nihat MERT[2], Semih YASAR[3*], Handan MERT[2]

[1]Van Yuzuncu Yil University, Institute of Health Science, Department of Biochemistry, Van, Turkey
[2]Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Biochemistry, Van, Turkey
[3]Van Yuzuncu Yil University, Vocational High School of Ozalp, Department of Medical Laboratory Technics, Van, Turkey

***Corresponding author:** Semih YASAR
| **Received:** 04.02.2019 | **Accepted:** 15.02.2019 | **Published:** 25.02.2019
**DOI:** 10.36347/sjavs.2019.v06i02.001

**Abstract** **Original Research Article**

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 ($p \leq 0.05$), vitamin A ($p \leq 0.05$), vitamin C ($p \leq 0.05$) and vitamin E ($p \leq 0.01$) 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.



## INTRODUCTION

Mastitis is a disease that surfaces with inflammation and causes reduced milk quality and yield [1], earlier removal of the diseased animals from the herd [2], and significant amounts of economic losses [3]. Mastitis may also influence human health if unprocessed raw milk and dairy products are consumed [4].

Short-life reactive atoms or molecules which contain an unpaired $e^-$ in their outmost orbitals are called free radicals, and compounds that mitigate their effects on the metabolism are called antioxidants. While free radicals are actually normal products of the metabolism, they may still cause genetic mutations, molecular alterations, cell destruction, and aging. The most commonly encountered free radicals are superoxide ($O_2$), hydrogen ($H^+$), nitrogen oxide (NO), peroxide radical ($HO_2$), hydroxyl (OH), and nitrogen dioxide ($NO_2$) [5].

Antioxidants are elements that are able to eliminate these antioxidants and take a role in the prevention of radical material production, antioxidant defense mechanisms, removal of free radicals, blocking secondary chain reactions, increasing the antioxidant material capacity, and cell restoration. Ceruloplasmin, Vitamins A and C, superoxide dismutase, tocopherol 1, glutathione peroxidase, glutathione, glucose, and uric acid are substances that display antioxidant properties [5].

Ceruloplasmin is an extracellular antioxidant and neutralizes superoxide radicals and may bind free oxygen radicals to its own structure and break them down, suppressing their functions temporarily [6].

While vitamin A is not a particularly strong antioxidant itself, it takes many roles within the immune system [7]. Deficiency of vitamin A causes development problems in ductal and alveolar epithelia of breasts and causes reduced immunity against mastitis, and may result in increased severity of the disease [8].

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 inflation 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-1: Average blood GSH, ceruloplasmin, and vitamin A, C, and E levels of healthy sheep and sheep with mastitis**

| Parameters | n | Control group (X±SEM) | n | Sheep with mastitis (X±SEM) |
|---|---|---|---|---|
| GSH mg/dl | 10 | 19.61 ± 2.95 | 20 | 15.43 ± 1.47[a] |
| Ceruloplasmin mg/dl | 10 | 16.54 ± 0.14 | 20 | 17.13 ± 0.11 |
| Vitamin A mg/L | 10 | 0.44 ± 0.03 | 20 | 0.39 ± 0.04[a] |
| Vitamin E µg/dl | 10 | 290.1 ± 14.3 | 20 | 254.3 ± 11.8[b] |
| Vitamin C µg/ml | 10 | 6.75 ± 0.15 | 20 | 5.91 ± 0.17[a] |

[a] $p \leq 0.05$; [b] $p \leq 0.01$

## 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/lt, while in the control group the average level was 0.39±0.04 mg/lt. 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


1. Philpot WN, Nickerson SC. Mastitis: Counter attack. 1st edition, Babsob Bros Co, Illinois, USA. 1991.
2. Han HR, Pak SI, Kang SW, Jong WS, Youn CJ. Capsular polysaccharide typing of domestic mastitis-causing staphylococcus aureus strains and its potential exploration of bovine mastitis vaccine developmen. I. Capsular polysaccharide typing, isolation and purification of the strains. J Vet Sci. 2000; 1: 53–60.
3. Mert N, Tayar M, Ogan M, Muftuoglu A, Yavuz M. A study on the biochemical changes caused by subclinic mastitis milk in dairy cows. Uludag Univ J Fac Vet Med. 1992; 11: 13-18.
4. Nickerson SC. Immunological aspects of mammary involution. J Dairy Sci. 1989; 72: 1665-1678.
5. Dündar Y, Aslan R. Antioxidant stress and antioxidants in medicine, Afyon Kocatepe University Press Afyon, Turkey; 2000.
6. Akkuş İ. Free Radicals and Physiopathological Effects. 1st Edn, Mimoza Press, Konya, Turkey. 1995.
7. Weiss WP, Hogan JS, Todhunter DA, Smith KL. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J Dairy Sci. 1997; 80: 1728-1737.
8. Nockels CF. Antioxidants improve cattle immunity following stress. Animal Feed Science and Technology. 1996 Oct 1;62(1):59-68.
9. Mert N, Bildik A, Ertekin A, Dede S. Biochemistry, YYU Faculty of Veterinary Medicine Press, Van, Turkey. 1999.
10. Champe PC, Harvey RA. Biochemistry. Tokullugil A, Dirican M, Ulukaya E, (editors). 2st Edn, Nobel Tip Bookshops Ltd. Comp, Istanbul, Turkey. 1997.
11. Megersa B, Tadesse C, Abunna F, Regassa A, Mekibib B, Debela E. Occurrence of mastitis and associated risk factors in lactating goats under pastoral management in Borana, Southern Ethiopia. Trop Anim Health Prod. 2010; 42: 1249-1255.
12. Beutler E, Dubon O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61: 882-888.
13. Rizzi R, Caroli A, Bolla P, Acciaioli A, Pagnacco G. Variability of reduced glutathione levels in Massese ewes and its effect on daily milk production. Journal of Dairy Research. 1988 Aug;55(3):345-53.
14. Uskoković V, Wu V. Calcium phosphate as a key material for socially responsible tissue engineering. Materials. 2016;9(6):434.
15. Miller KW, Yang CS. An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α-tocopherol, and various carotenoids. Analytical biochemistry. 1985 Feb 15;145(1):21-6.
16. Omaye ST. Ascorbic acid analysis. II. Determination after derivatisation with 2.2. dinitrophenylhidrazine. Selected methods for determination of ascorbic acid in animal cells tissues and fluids. Methods in enzymology. 1979;62:7-8.
17. Zengin K, Mert H, Mert N. Catalase activity and the levels of MDA, AOPP in sheeps with subclinical mastitis. Research in: Agricultural & Veterinary Sciences. 2017;1(1):5-11.
18. Deveci H, Apaydin AM, Kalkan C, Ocal H. Breast Diseases in Pets. 1st Edition. Firat University Press, Elazig, Turkey. 1994.
19. Simsek H, Aksakal M. Effect of vitamin E on lipid peroxidation and some antioxidants in blood and milk in cows with subclinical mastitis. Ankara Univ Vet Fak Derg. 2005; 52: 71-76.
20. Erisir M, Kandemir FM, Yuksel M. The Effect of Subclinical Mastitis on MDA, GSH Levels and

GSH-Px, CAT Activities in Cow Milk. F Ü Sag Bil Vet Derg. 2011; 25: 67-70.
21. Musal B, Ulutas PA, Turkyilmaz S. Blood vitamin C, vitamin A, β-carotene, ceruloplasmin, and glutathione and malondialdehyde concentrations in cows with subclinical mastitis treated with intramammary antibiotics. Revue Med Vet. 2007; 158: 633-640.
22. Atroshi F, Parantainen J Sankari S, Osterman T. Prostaglandins and glutathione peroxidase in bovine mastitis. Res Vet Sci. 1986; 40: 361-366.
23. Chassagne M, Barnouin J, Chacornac JP. Biological predictors for early clinical mastitis occurrence in Holstein cows under field conditions in France. Preventive veterinary medicine. 1998 Apr 16;35(1):29-38.
24. Salmanoglu B, Pamukcu T, Yarim GF. Adenosine deaminase, vitamin A and beta-carotene levels in milk and blood in the administration of intranasal levamisole in dairy cows with subclinical mastitis. Ankara Univ Vet Fak Derg. 2002; 49: 17-21.
25. Simsek H, Aksakal M. The effect of vitamin E on plasma vitamin A, beta-carotene, glutathione peroxidase, reduced glutathione and milk vitamin A levels in cows with subclinical mastitis. Firat Univ Sag Bil Derg. 2006; 20: 199-203.
26. Chew BP, Hollen LL, Hillers JK, Herlugson ML. Relationship between vitamin A and β-carotene in blood plasma and milk and mastitis in Holsteins. J Dairy Sci. 1982; 65: 2111-2118.
27. Kaya I, Guven A. Determination of Vitamin A, β-Carotene and Vitamin E Levels in Cows with Mastitis. Kafkas Univ Vet Fak Derg. 2008; 14: 57-61.
28. Braun U, Forrer R, Fürer W, Lutz H. Selenium and vitamin E in blood sera of cows from farms with increased incidence of disease. The Veterinary Record. 1991 Jun;128(23):543-7.
29. Erskine RJ, Eberhart RJ, Hutchinson LJ, Spencer SB. Herd management and prevalence of mastitis in dairy herds with high and low somatic cell counts. Journal of the American Veterinary Medical Association. 1987 Jun;190(11):1411-6.
30. Ndiweni N, Field TR, Williams MR, Booth JM, Finch JM. Studies on the incidence of clinical mastitis and blood levels of vitamin E and selenium in dairy herds in England. The Veterinary record. 1991 Aug;129(5):86-8.
31. Colitti M, Stradaioli G, Stefanon B. Effect of α-tocopherol deprivation on the involution of mammary gland in sheep. Journal of dairy science. 2000 Feb 1;83(2):345-50.
32. Kleczkowski M, Kluciński W, Shaktur A, Sikora J. Concentration of ascorbic acid in the blood of cows with subclinical mastitis. Polish journal of veterinary sciences. 2005;8(2):121-5.