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Relationship of Oxidative Stress and Some Blood Parameters with Quarters Affected by Mastitis in Dairy Cows

T. Akkuş[1], P.F. Polat Dinçer[2], M. Ekici[3], Z. Yerlikaya[4]

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ABSTRACT

Background: This study was conducted to evaluate the relationship between oxidative stress and some blood parameters in different number of quarters affected with and without sub-clinical mastitis (SM).

Methods: A total of 50 lactating Holstein dairy cows were used in the study. The study groups were as follows; Group 1 (negative test results and no SM), Group 2 (SM in one quarter), Group 3 (SM in two quarters), Group 4 (SM in three quarters) Group 5 (SM in four quarters). Milk samples were obtained from the infected quarters for both Somatic Cell Count (SCC) measurement and bacteriological growth/bacterial identification. Blood was collected for analysis of oxidative stress parameters (total oxidative capacity and total antioxidant capacity) and some blood parameters (glucose, total cholesterol, total bilirubin, aspartate aminotransferase [AST], alanine aminotransferase [ALT]).

Result: Somatic Cell Count (SCC) was significantly lower in Group 1 than in Groups 2, 3, 4 and 5 (P<0.001). Glucose and total cholesterol levels were significantly higher in Group 5 than in Groups 1, 2, 3 and 4 (P<0.001). A significant difference was determined between all groups in respect of AST and ALT activities (P<0.001). The Total Oxidative Capacity (TOC) and oxidative stress index value (OSI) levels were significantly lower in Group 1 than in Groups 2, 3, 4 and 5 (P<0.001), while Total Antioxidant Capacity (TAC) levels were significantly higher (P<0.001). In conclusion, it was found that oxidative stress and blood-biochemistry values are significantly affected in dairy cows with sub-clinical mastitis.

Key words: Biochemical change, Cow, Sub-clinical mastitis, Total oxidant capacity, Total antioxidant capacity.

INTRODUCTION

Mastitis is one of the most important diseases in dairy farming and is closely related to oxidative stress (Abuelo *et al.,* 2015). Reactive oxygen products (ROS) are the most common oxidant substances in biological systems, which are required at physiological levels for reproductive functions, including oocyte maturation, folliculogenesis, luteolysis, ovulation and cyclic endometrial changes. However, ROS at higher than physiological levels contribute to various pathological conditions that may affect reproductive performance such as retained placenta, quarter edema, mastitis and infertility (Puppel *et al.,* 2015). ROS are enzymatpically repaired by antioxidant substances, but when the generation of free radicals exceeds the defence capacity of antioxidants, oxidative stress develops. Oxidative stress damages the macro components such as DNA, lipids and proteins, thereby disrupting cell structure, especially immune cells. Furthermore, it is known that excessive oxidative stress can result in pathological disorders of tissues and organs. These pathological disorders develop with impairments of especially the heart, skeletal muscle, liver and blood cell functions that require high energy and immunological deficiencies (Kuhn *et al.,* 2018). Antioxidant and oxidant indices are biomarkers which have been recently used for the detection of sub-clinical mastitis. Polymorphonuclear leukocytes (PMNs), which are the dominant cell type in mammary tissues, and secretions during inflammation show their bactericidal effects through a respiratory burst that produces ROS and reactive nitrogen species (RNS). Therefore, an increase in the number and activity of PMNs in milk resulting from mastitis will be associated with changes in the oxidative markers in milk (Amiri *et al.,* 2020). Serum or plasma concentrations of different oxidants/antioxidants can be measured individually in laboratories, but these measurements are time-consuming, expensive and require complex techniques. Since the individual measurement of different oxidant/antioxidant molecules is not practical, the total oxidant/antioxidant capacity of a sample is measured

[1]Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Harran University, Sanliurfa-Turkey.
[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Dokuz Eylül University, Izmir-Turkey.
[3]Department of Physiology, Faculty of Veterinary Medicine, Cumhuriyet University, Sivas-Turkey.
[4]Department of Microbiology, Faculty of Veterinary Medicine, Firat University, Elazig-Turkey.

Corresponding Author: T. Akkuş, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Harran University, Sanliurfa-Turkey. Email: tugraakkus08@hotmail.com

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and this is known as the total antioxidant capacity (TAC), and total oxidant capacity (TOC) (Erel, 2005; Erel, 2004). The diagnosis of mastitis can be made by clinical examination of udder and milk, and the chemical, physical, cellular and microbiological examination of milk. In addition, the biochemical changes caused by this disease in blood and body fluids can be determined in various laboratory tests (Kitchen *et al.,* 1980). Enzymes (aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase) found in the milk and blood of infected animals are considered biomarkers of udder health because the levels increase during inflammation in animals with mastitis. Increased levels of these enzymes in milk and blood serum may be associated with tissue damage in the udder parenchyma (Kalantari *et al.,* 2013). Examining the concentration of bilirubin, albumin, glucose and cholesterol in the blood serum, as well as the activity of AST, ALT enzymes, and the appropriate markers for liver function both allow an overall assessment of liver status and provide information about the production potential of dairy cows (Djokovic *et al.,* 2013).

The aim of this study was to reveal the relationship between oxidant-antioxidant status and some blood parameters in different number of quarters affected with and without sub-clinical mastitis in dairy cows.

MATERIALS AND METHODS

Selection of animals and test protocol

This study was conducted on 50 Holstein breed dairy cows, aged 2-5 years, selected by a random sampling method (a total of 200 quarter milk samples). All the cows had the same nutrition of grass silage, corn silage, and hay, together with concentrated feed based on factory feed and barley, and were managed under the same conditions on a privately owned dairy farm in Eyyübiye District of Şanlıurfa province, south-east Turkey and the study was carried out in March-April 2021. The cows included in the study were those that had a normal previous calving and had not received any treatment for the last three months according to the farm records. First, milk samples were obtained following the asepsis/antisepsis rules and CMT was performed (Jackson and Cockcroft, 2002). The groups were formed according to the quarters with mastitis. According to the test results, 5 study groups were created; Group 1 were cows negative in all 4 quarters and the cows in Groups 2-5 had sub-clinical mastitis in one, two, three or four quarters, respectively. Each study group consisted of 10 animals. All cows in the study were in the mid-lactation period (100-200 days following birth). None of the cows had been given antibiotic treatment in the last month, and quarters appeared normal on clinical examination but had CMT positivity. Milk samples were taken from the infected quarters of the animals in each group for SCC measurement and bacterial culture/bacteria identification. Blood samples were taken and after centrifugation at 3000 rpm for 10 min, the serum was removed for analysis of oxidative stress parameters (TOC & TAC) and some blood parameters (ALT, AST, Glucose, Total Cholesterol, Total Bilirubin).

California mastitis test (CMT) and somatic cell count (SCC)

Milk samples taken from each of the four quarters were transferred into the test container with four separate compartments. For the CMT, an equal amount of milk was mixed with the test solution containing bromocresol purple and anionic detergent (2 ml milk and 2 ml test solution). By rotating the test plate slowly, alterations such as color changes or gel formation were evaluated. Milk SCC was determined using an automatic somatic cell counter (Lactoscan SCC®, Nova Zagora, Bulgaria) on a private dairy farm. According to the CMT and SCC results, 10 cows were CMT negative (mixed liquid, no sediment) and SCC<400,000/ml, and 40 cows were CMT +1 positive (with marked sediment, no gel form) and SCC>400,000/ml (Ruegg and Reinemann, 2002).

Milk and blood samples

Quarters were wiped with 70% alcohol. After removing few strips of milk to remove saprophyte microorganisms, a 10 ml milk sample was taken from each quarter into falcon tubes. The milk samples taken were sent to Firat University, Faculty of Veterinary Medicine, Department of Microbiology, under cold chain conditions for the bacteria isolation to be performed. Blood samples of 10 ml taken from vena jugularis into tubes containing clotting activator were transported to the laboratory in ice within 30 min, then centrifuged at 3000 g for 10 minutes, and the serum obtained was stored at -80 °C until TAC-TOC measurement and biochemistry analyses were performed.

Bacteria isolation and identification

In the laboratory, cultures were prepared by adding 0.1 ml of the milk samples to the general and selective media (blood agar, MacConkey agar and Edward's medium). The cultures were incubated at 37°C for 24-48 hours under aerobic conditions, then were evaluated in respect of colony growth during incubation. Macroscopic examination of the growing colonies in the culture environments was made and the colonies were purified and classified according to the MALDI-TOF MS system (Matrix-mediated laser desorption ionization-flight time mass spectrometry database v2.0, bioMerieux, France) (Koneman *et al.,* 1997).

Some blood parameters, determination of total antioxidant capacity (TAC), total oxidant capacity (TOC) and oxidative stress index value (OSI)

Glucose, T-cholesterol, BUN, T-bilirubin, AST and ALT activities were measured using an Arkray Spotchem EZ SP-4430 dry system biochemistry analyzer (Koka-Shi®, Shiga, Japan) (Dülgeroglu, 2018). Serum TAC levels were measured using a commercial kit (Rel Assay Diagnostics kit, Mega Medicine®, Gaziantep, Turkey) at 660 nm spectrophotometrically (Molecular Device SpectraMax M5 Plate Reader®, Pleasanton, California, United States) (Erel, 2004). Serum TOC levels were determined using a commercial kit (Rel Assay Diagnostics kit, Mega Medicine, Gaziantep, Turkey) at 530 nm spectrophotometrically

(Molecular Device SpectraMax M5 Plate Reader, Pleasanton, California, United States) (Erel, 2005). The ratio of TOC level to TAC level was defined as the Oxidative Stress Index (OSI) (Kaya *et al.,* 2016).

Statistical analysis

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS for Windows; version 22.0) software. The conformity of variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were stated as mean ± standard error of mean values for normally distributed variables. Data showing normal distribution were evaluated using the One-way ANOVA test. If there was a significant difference between the groups, the comparison was performed using the post-hoc Scheffe test. The homogeneity of the variances was determined with the Levene test. A value of $P<0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

California mastitis test (CMT) results

The CMT results of the study groups are presented in Table 1. All the quarters of the 10 animals in Group 1 were CMT and bacteriology negative.

In Groups 2-5, the number of positives CMT were according to scheduling 1-4 quarters respectively. In milk samples taken from CMT positive quarters, aerobic bacteria were isolated in 8 (80%) in Group 2, in 14 (70%) in Group 3, in 23 (76.6%) in Group 4, and in 32 (80%) in Group 5. When evaluated as a total, aerobic bacteria isolation was achieved in 77 (77%) of 100 CMT positive quarters of 40 lactating cows.

Bacteria isolated from cultivated milk

The bacteria isolated from the milk sample cultures according to the groups are presented in Table 2. The micro-organisms isolated from 77 milk samples were as follows: *Staphylococcus aureus* in 35 (45.4%), *Streptococcus agalactiae* in 15 (19.4%), *Streptococcus dysgalactiae* in 10 (12.9%), *Micrococcus spp.* in 4 (5.1%), *Coagulase Negative Staphylococcus* (CNS) in 3 (3.8%), *Corynebacterium bovis* in 3 (3.8%), Escherichia coli in 3 (3.8%), *Bacillus spp.* in 1 (1.2%), *Arcanobacterium pyogenes* in 1 (1.2%), *Pasteurella multocida* in 1 (1.2%) and *Citrobacter diversus* in 1 (1.2%). In a previous study, the rates of micro-organisms isolated and identified from 125 CMT positive quarter milk samples were as follows: 47.3% *Staph. aureus*, 16.3% *C. pyogenes*, 8.2% E. coli, 6.5% *Candida albicans*, 6% *S. agalactiae* and 15.7% other micro-organisms (Ates *et al.,* 1991). In other studies, Staph. aureus has been reported to be the main pathogen most frequently isolated in sub-clinically infected

Table 1: Determination of cows with sub-clinical mastitis.

Groups	Number of cows examined	CMT (-)		CMT (+)		Number of quarters	CMT (-) quarters		CMT (+) quarters		Isolated	quarters
	n	n	%	n	%	n	n	%	n	%	n	%
Group 1	10	10	20	-	-	40	40	20	-	-	-	-
Group 2	10	-	-	10	20	40	30	15	10	5	8	80
Group 3	10	-	-	10	20	40	20	10	20	10	14	70
Group 4	10	-	-	10	20	40	10	5	30	15	23	76.6
Group 5	10	-	-	10	20	40	-	-	40	20	32	80
Total	50	10	20	40	80	200	100	50	100	50	77	77

Table 2: Bacteria species isolated with MALDI-TOF MS (Matrix-mediated laser desorption ionization-flight time mass spectrometry database v2.0, bioMerieux, France).

Isolated Bacteria	Group 1	Group 2	Group 3	Group 4	Group 5	Total	% Isolation	Front right	Front left	Rear right	Rear Left
Staphylococcus aureus	-	4	7	10	14	35	45.4	9	10	7	9
Streptococcus agalactiae	-	2	3	4	6	15	19.4	4	3	4	4
Streptococcus dysgalactiae	-	1	1	3	5	10	12.9	3	2	3	2
Micrococcus spp.	-	-	1	2	1	4	5.1	-	2	2	-
KNS	-	1	2	-		3	3.8	1	-	-	2
Corynebacterium bovis	-	-	-	1	2	3	3.8	1	1	1	-
Escherichia coli	-	-	-	2	1	3	3.8	-	2	-	1
Bacillus spp.	-	-	-	-	1	1	1.2	-	1	-	-
Arcanobacterium pyogenes	-	-	-	-	1	1	1,2	-	-	-	1
Pasteurella multocida	-	-	-	1		1	1,2	-	1	-	-
Citrobacter diversus	-	-	-	-	1	1	1,2	-	1	-	-
Total	-	8	14	23	32	77	100	18	23	17	19

quarters in Belgium and other regions and countries (Sampimon *et al*. 2005; Pitkale *et al.* 2004; Wilson *et al*. 1997). Similar to the findings of previous research, the present study showed that *Staph. aureus* appears to be the most commonly isolated bacteria in milk with sub-clinical mastitis. When other factors were considered, although the rates varied, the bacteria isolated were similar to those in other studies. Changes in the prevalence of Staph. aureus in the herd can be explained by differences between herds regarding post-milking teat dipping and dry-term udder treatment (Hogan et al., 1987). Studies have reported that the prevalence of Str agalactiae varies between 8.5% (Wilson *et al*., 1997), 0.1% (Pitkale *et al*., 2004) and 1-2% (Andersen *et al*., 2003). In the present study, this rate was found to be 19.4%. This situation, which is contrary to the literature, was thought to be due to the fact that the mammary health control program was not fully implemented on the farm where the study was conducted. In addition, coliform bacteria, which are generally included in short-term acute clinical mastitis cases, have been reported to have a low prevalence in parallel with the current study (Hoblet *et al*., 1991).

Some blood parameters

The blood biochemistry values are presented in Table 3. Glucose and total cholesterol were significantly higher in Group 5 than in Groups 1, 2, 3 and 4 (P<0.001). There was no significant difference between Groups 1, 2, 3 and 4 (P>0.05). Total bilirubin was significantly lower in Group 1 compared to Groups 2, 3, 4 and 5 (P<0.001). It was significantly lower in Group 2 than in Groups 4 and 5 (P<0.001). It was significantly lower in Group 3 than in Group 5 (P<0.001). There was no significant difference between Groups 2 and 3, Groups 3 and 4, and Groups 4 or 5 (P>0.05). In this study, the relationship was evaluated between blood-biochemistry values such as glucose, T-cholesterol, T-bilirubin, AST and ALT, measured to assess liver function. Previous studies have reported that activities of AST and ALT enzymes increase in accordance with the elevation in CMT score (Qayyum *et al.,* 2018). In another study, milk and blood AST and ALT activities were higher in camels with sub-clinical mastitis compared to healthy animals (Ali *et al.,* 2016). In the current study, the increase in the values measured from the cows with sub-clinical mastitis was seen to be consistent with the data found in literature. When the effect of the number of infected quarters on the blood-biochemistry values is examined, glucose and T-cholesterol levels were determined to be lowest in Group 1 and highest in Group 5. While there was no statistically significant difference in these measurements between Groups 1, 2, 3, and 4, the difference between these groups and Group 5 was statistically significant, suggesting that the liver metabolic load may have increased due to the number of affected quarters. Similarly, T-bilirubin, AST and ALT were measured at the lowest activity in Group 1 and at the highest level in Group 5. The difference in the total bilirubin levels between Group 1 and the other groups (with sub-clinical mastitis) was found to be increasingly significant. The AST and ALT activities were significantly different in all the groups as the number of infected quarters increased. With the increase in the number of infected quarters, so the activities of liver enzyme parameters increased significantly (Ali

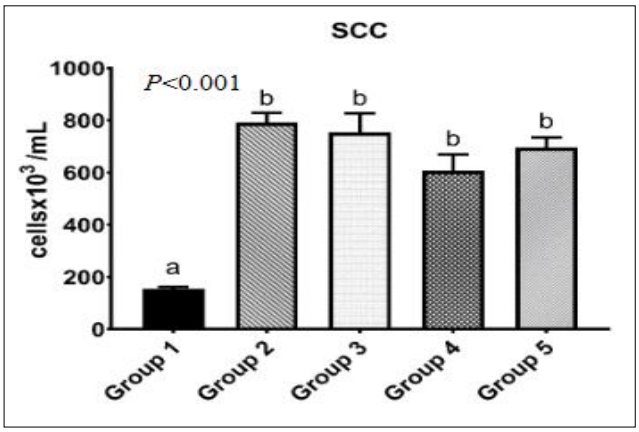

Fig 1: The number of somatic cell count (SCC) in the groups. ***: P<0.001. The values in the column chart represent the mean ± SEM.

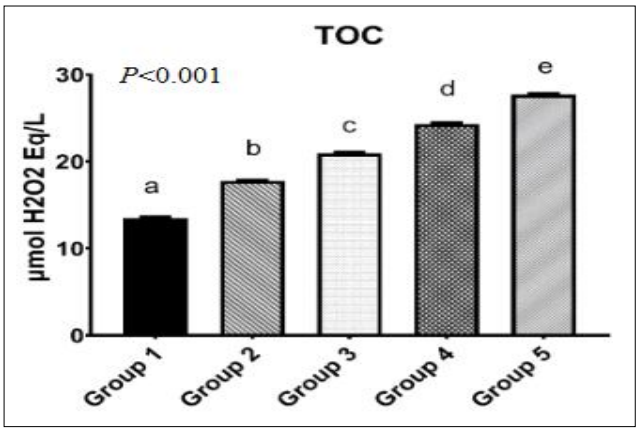

Fig 2: Total oxidant capacity (TOC) in the groups. ***: P<0.001. The values in the column chart represent the mean ± SEM.

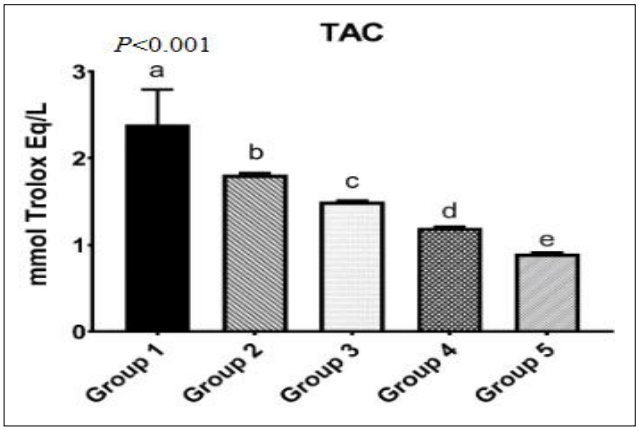

Fig 3: Total antioxidant capacity (TAC) in the groups. ***: P<0.001. The values in the column chart represent the mean ± SEM.

Table 3: Blood biochemical parameters in the groups.

Groups	Glucose (mg/dL)	T-Cholesterol (mg/dL)	T-Bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)
Group 1 (Control)	47.80±1.51[a]	148.30±8.17[a]	0.38±0.03[a]	32.24±0.99[a]	13.42±0.37[a]
Group 2 (One infectedquarter)	45.10±1.61[a]	165.40±5.55[a]	0.70±0.02[b]	44.03±0.57[b]	17.57±0.35[b]
Group 3 (Two infectedquarters)	46.80±1.57[a]	184.40±6.59[a]	0.76±0.04[bc]	50.84±0.37[c]	20.93±0.22[c]
Group 4 (Three infected quarters)	53.90±3.48[a]	187.20±12.96[a]	0.86±0.03[cd]	55.67±0.40[d]	24.42±0.19[d]
Group 5 (Four infectedquarters)	66.20±1.96[b]	245.20±16.14[b]	0.99±0.04[d]	61.17±0.50[e]	27.33±0.22[e]
P value (One-way ANOVA)	<0.001	<0.001	<0.001	<0.001	<0.001

[a,b,c,d]: Different letters in the same column represent a statistically significant difference. ANOVA: Analysis of variance. Collected data are represented by the mean and SEM (standart error of mean). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, T-Cholesterol: Total cholesterol, T-Bilirubin: Total bilirubin.

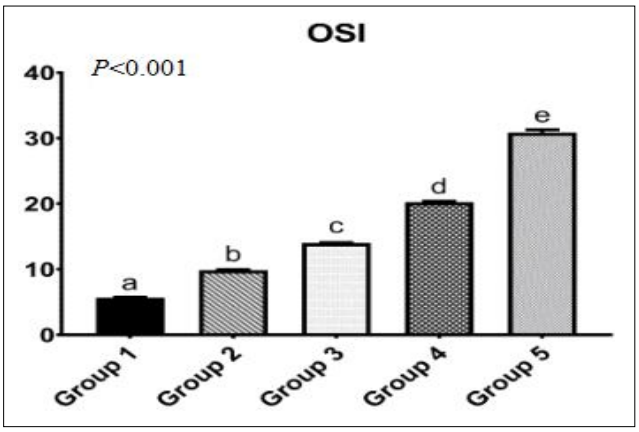

Fig 4: Oxidative stress index (OSI) value in the groups (arbitrary unit). ***: P<0.001. The values in the column chart represent the mean ± SEM.

et al., 2016). This increase was thought to be related to microcirculation permeability as a result of tissue damage caused by free radicals in the quarter. It was thought that evaluations of sub-clinical mastitis should be made not only on an animal basis but also in terms of infected quarters.

Findings related to somatic cell count (SCC)

Ten cows in each group; from all CMT negative quarters in Group 1, 10 CMT positive in Group 2, 20 CMT positive in Group 3, 30 CMT positive in Group 4, and 40 CMT positive quarters in Group 5 SCC levels were established by taking the average of milk samples. When the SCC and CMT results were compared, the average SCC in the quarters in Group 1, which was CMT negative, was 155.05 cells/ml, 792.08 cells/ml in Group 2, 754.55 cells/ml in Group 3, 605.84 cells/ml in Group 4, and 695.33 cells/ml in Group 5 quarters. The difference in mean SCC values between Group 1 and the other groups was statistically significant (P<0.001), and no significant difference was observed between the sub-clinical mastitis groups (P>0.001) (Fig 1).

Total oxidant capacity (TOC), total antioxidant capacity (TAC) and oxidative stress index (OSI) measurements

Total Oxidative Capacity (TOC) and oxidative stress index value (OSI) levels were significantly lower in Group 1 than in Groups 2, 3, 4 and 5 (P<0.001), while Total Antioxidant Capacity (TAC) levels were significantly higher (P<0.001), (Fig 2, Fig 3, Fig 4).

Studies on oxidative stress are among current topics and many scientific studies have been conducted in this area (Abuelo *et al*., 2013). Oxidative stress is defined as an imbalance in favor of oxidants between oxidant and antioxidant substances (Puppel *et al*., 2015). When free radicals and antioxidant substances encounter each other in the body, antioxidants prevent damage by inhibiting target molecules or by delaying oxidation thereby protecting the organism against tissue damage (Puppel *et al*., 2015). Antioxidants have been found to have an antilipolytic effect, but free radicals disrupt the insulin mechanism, thereby stimulating lipolysis. In the case of oxidative stress, lipolysis is constantly active, and liver metabolic load increases in this case (Abuelo *et al*., 2016). Tissue damage caused by intramammary inflammation results in the generation of reactive oxygen species (ROS), increasing the incidence of oxidative stress in mammary tissue, which can lead to increased permeability of microcirculatory arteries due to free radical injury. In a previous study investigating the oxidant/antioxidant status in cows with sub-clinical mastitis, it was emphasized that MDA and NO levels were higher than that of healthy control groups, and TAC and GSH levels were lower, indicating the contribution of udder-related oxidative stress and potential oxidative damage (Saleh *et al.,* 2022; Sadek *et al.,* 2017). Similarly, it has been reported that MDA and H_2O_2 (Hydrogen peroxide) levels are increased and TAC levels are decreased in blood and milk serum of cows with sub-clinical mastitis compared to the control group (Nediæ *et al.,* 2019). Moreover, TAC and MDA concentrations have been reported to increase significantly over time in untreated cows with sub-clinical mastitis (Tabatabaee *et al.,* 2021). Higher SCCs have also been strongly correlated with higher MDA levels (Yakan *et al.,* 2021). Total antioxidant capacity can be used to provide a simple understanding of the antioxidant status in the body (Farghali *et al.,* 2021; Abdel-Saeed and Salem, 2019). The importance of TAC and TOC levels in monitoring oxidative stress-related diseases such as mastitis and the transition period in dairy cows has been emphasized (Amiri *et al.,* 2020; Kurt *et al.,* 2019; Turk *et al.,* 2017; Andrei *et al.,* 2016; Aydilek *et al.,* 2014; Atakisi *et al.,* 2010; Mandebvu *et al.,* 2003). In previous studies, milk with sub-clinical mastitis has

been compared with cow and goat milk samples without sub-clinical mastitis and milk with mastitis has been reported to have higher TOC levels and lower TAC levels (Silanikove *et al.,* 2014; Atakisi *et al.,* 2010). In another study, the TAC level was high in low-SCC milk and low in high-SCC milk in the samples taken according to the density of the somatic cells (Nediæ *et al.,* 2019; Andrei *et al.,* 2016). In our study, the serum TOC-TAC level was measured according to the number of infected quarters and the increase in TAC level and decrease in TOC level in animals with sub-clinical mastitis were consistent with the literature data (Silanikove *et al.,* 2014; Atakisi *et al.,* 2010). The use of antioxidants increases as a result of the effect of free radicals that occur due to inflammation, which occurs in mastitis cases, and as a result, the levels of antioxidants decrease. In mammary gland inflammation, more oxygen is used depending on the activity of phagocytic cells that have migrated of phagocytic cells to the site of inflammation. The oxidative stress parameter measurements were determined to have a strong relationship with the number of infected quarters.

CONCLUSION

In conclusion, it was found that oxidative stress and some blood parameters are significantly affected by infected quarters in cases with sub-clinical mastitis. *Staph. aureus* was determined to be the main cause of sub-clinical mastitis in the dairy cows in this study, in line with data from other countries. Therefore, udder health control programs should be expanded in dairy cattle enterprises. It can be considered that this study will be of guidance for future studies with greater numbers of animals and examinations at every stage of lactation, especially after birth.

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Conflict of interest statement

None of the authors of this article has any conflict of interest.

REFERENCES

Abdel-Saeed, H. and Salem, N.Y. (2019). Evaluation of total antioxidant capacity, malondialdehyde, catalase, proteins, zinc, copper and IgE response in ovine verminous pneumonia. International Journal of Veterinary Science. 8: 255-258.

Abuelo, A., Alves-Nores, V., Hernandez, J., Muino, R., Benedito, J.L., Castillo, C. (2016). Effect of parenteral antioxidant supplementation during the dry period on postpartum glucose tolerance in dairy cows. Journal of Veterinary Internal Medicine. 30: 892-898. DOI: 10.1111/jvim.13922.

Abuelo, A., Hernandez, J., Benedito, J.L., Castillo, C. (2015). The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. Journal of Animal Physiology and Animal Nutrition. 99: 1003-1016. DOI: 10.1111/jpn.12273.

Abuelo, A., Hernandez, J., Benedito, L., Castillo, C. (2013). Oxidative stress index (OSI) as a new tool to assess redox status in dairy cattle during the transition period. Animal. 7: 1374-1378. DOI: 10.1017/S1751731113000396.

Ali, F., Hussain, R., Qayyum, A., Gul, S.T., Iqbal, Z., Hassan, M.F. (2016). Milk somatic cell counts and some hemato-biochemical changes in sub-clinical mastitic dromedary she-camels (*Camelus dromedarius*). Pakistan Veterinary Journal. 36: 405-408.

Amiri, P., Rad, A.H.F., Heidarpour, M., Azizzadeh, M., Khoramian, B. (2020). Diagnostic accuracy of milk oxidation markers for detection of subclinical mastitis in early lactation dairy cows. Comparative Clinical Pathology. 29: 95-101.

Andersen, H.J., Pedersen, L.H., Aarestrup, F.M. Chrie´l, M. (2003). Evaluation of the surveillance program of Streptococcus agalactiae in Danish dairy herds. Journal of Dairy Science. 86: 1233-1239. DOI: 10.3168/jds.S0022-0302(03)73707-2.

Andrei, S., Matei, S., Rugina, D., Bogdan, L., Ştefanuţ, C. (2016). Interrelationships between the content of oxidative markers, antioxidative status, and somatic cell count in cow's milk. Czech Journal of Animal Science. 61: 407-413. DOI: 10.17221/70/2015-CJAS.

Atakisi, O., Oral, H., Atakisi, E., Merhan, O., Pancarci, S.M., Ozcana, A., Kaya, S. (2010). Subclinical mastitis causes alterations in nitric oxide, total oxidant and antioxidant capacity in cow milk. Research Journal of Veterinary Sciences. 89: 10-13. DOI: 10.1016/j.rvsc.2010.01.008

Ates, M., Erganis, O., Çorlu, M., Serpek, B. (1991). Microbial flora and LDH activity of milk samples obtained from cows with mastitis in Konya region. Turkish Journal of Veterinary and Animal Sciences. 16: 19-29.

Aydilek, N., Varisli, Ö., Selek, Ş., Korkmaz, Ö., Atli, M.O., Taskin, A. (2014). The effect of estrous cycle on oxidant and antioxidant parameters in dairy cows. Kafkas University Journal of Veterinary Faculty. 20: 703-709. DOI: 10.9775/kvfd.2014.10947.

Djokovic, R., Samanc, H., Jovanovic, M., Fratic, N., Doskovic, V., Stanimirovic, Z. (2013). Relationships among blood indicators of hepatic functions and lipid content in the liver during transitional period in high-yielding dairy cows. Acta Scientiae Veterinariae. 41: 1-6.

Dülgeroglu, Y. (2018). Verification of some biochemistry tests that to be analyzed in Spotchem EZ SP-4430 dry chemistry system. Turkish Bulletin of Hygiene and Experimental Biology. 75: 127-134. DOI: 10.5505/TurkHijyen.2018.49344.

Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry. 37: 277-285. DOI: 10.1016/j.clinbiochem.2003.11.015.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry. 38: 1103-1111. DOI: 10.1016/j.clinbiochem.2005.08.008.

Farghali, H.A., AbdElKader, N.A., AbuBakr, H.O., Ramadan, E.S., Khattab, M.S., Salem, N.Y., Emam, I.A. (2021). Corneal ulcer in dogs and cats: novel clinical application of regenerative therapy using subconjunctival injection of autologous platelet-rich plasma. Frontiers in Veterinary Science. 8: 123. DOI: 10.3389/fvets.2021.641265.

Hoblet, K.H., Schnitkey, G.D., Arbaugh, D., Hogan, J.S., Smith, K.L., Schoenberger, P.S., Bowman, G.L. (1991). Costs associated with selected preventive practices and with episodes of clinical mastitis in nine herds with low somatic cell counts. Journal of the American Veterinary Medical Association. 199: 190-196.

Hogan, J.S., Pankey, J.W., Duthie, A.H. (1987). Growth inhibition of mastitis pathogens by long-chain fatty acids. Journal of Dairy Science. 70: 927-934.

Jackson, P. and Cockcroft, P. (2002). Clinical examination of farm animals. Blackwell Science Ltd Oxford, UK. pp. 154-166.

Kalantari, A., Safi, S., Foroushani, A.R. (2013). Milk lactate dehydrogenase and alkaline phosphatases biomarkers in detection of bovine subclinical mastitis. Annals of Biological Research. 4: 302-307.

Kaya, I., Kükürt, A., Karapehlivan, M. (2016). Investigation of serum oxidative stress index and paraoxonase activity levels in colostrum period of dairy cows. International Journal of Livestock Research. 6: 1-7. DOI: 10.5455/ijlr.20160308022801.

Kitchen, B.J., Middleton, G., Durward, I.G., Andrew, R.J., Salmon, M.C. (1980). Mastitis diagnostic tests to estimate mammary gland epithelial cell damage. Journal of Dairy Science. 63: 978-983. DOI: 10.3168/jds.S0022-0302(80)83035-9.

Koneman, E.M., Allen, S.D., Dovell, V.R., Sommers, H.M., Winn, W.C. (1997). Color Atlas and Textbook of Diagnostic Microbiology. 5th Edition, Philadelphia, JP Lippincott Comp. pp. 1-5.

Kuhn, M.J., Mavangira, V., Gandy, J.C., Sordillo, L.M. (2018). Production of 15-F(2t) isoprostane as an assessment of oxidative stress in dairy cows at different stages of lactation. Journal of Dairy Science. 101: 9287-9295. DOI: 10.3168/jds.2018-14669.

Kurt, S., Salar, S., Küplülü, Þ., Baþtan, A. (2019). Effects of prepartally supplements of vitamins and trace elements on total antioxidant and oxidant status and udder health parameters on day 14 postpartum in holstein-friesian cows. Kocatepe Veterinary Journal. 12: 322-328. DOI: 10.30607/kvj.568217.

Mandebvu, P., Castillo, J.B., Steckley, D.J., Evans, E. (2003). Total antioxidant capacity: A tool for evaluating the nutritional status of dairy heifers and cows. Canadian Journal of Animal Science. 83: 605-608. DOI: 10.4141/A03-027.

Nedić, S., Vakanjac, S., Samardžija, M., Borozan, S. (2019). Paraoxonase 1 in bovine milk and blood as a marker of subclinical mastitis caused by *Staphylococcus aureus*. Research in Veterinary Science. 125: 323-332. DOI: 10.1016/j.rvsc.2019.07.016.

Pitkala, A., Haveri, M., Pyorala, S., Myllys, V., Honkanen-Buzalski, T. (2004). Bovine mastitis in Finland 2001-Prevalence, distribution of bacteria and antimicrobial resistance. Journal of Dairy Science. 87: 2433-2441. DOI: 10.3168/jds.S0022-0302(04)73366-4.

Puppel, K., Kapusta, A., Kuczynska, B. (2015). The etiology of oxidative stress in the various species of animals, a review. Journal of the Science of Food and Agriculture. 95: 2179-2184. DOI: 10.1002/jsfa.7015.

Qayyum, A., Khan, J.A., Hussain, R., Ahmad, T.I., Zahoor, I., Ahmad, M., Mubeen, M. (2018). Correlations of blood serum and milk biochemical profiles with subclinical mastitis in cholistani cattle. Pakistan Journal of Agricultural Sciences. 55: 959-964. DOI: 10.21162/PAKJAS/18.6682.

Ruegg, P.L. and Reinemann, D.J. (2002). Milk quality and mastitis tests. The Bovine Practitioner. 36: 41-54. DOI: 10.21423/bovine-vol36no1p41-54.

Sadek, K., Saleh, E., Ayoub, M. (2017). Selective, reliable blood and milk bio-markers for diagnosing clinical and subclinical bovine mastitis. Tropical Animal Health and Production. 49: 431-437.

Saleh, N., Allam, T.S., Omran, A., Abdelfattah, A.M. (2022). Evaluation of changes in hemato-biochemical, ınflammatory and oxidative stress ýndices as reliable diagnostic biomarkers for subclinical mastitis in cows. Alexandria Journal of Veterinary Sciences. 72: 23-34. DOI: 10.5455/ajvs.140786.

Sampimon, O., Sol, J., Kock, P. (2005). Changes in bulk milk somatic cell count and distribution of mastitis pathogens over the past 50 years in The Netherlands. In: Proceedings Fourth IDF International Mastitis Conference, Maastricht, The Netherlands. p. 963-968.

Silanikove, N., Merin, U., Shapiro, F., Leitner, G. (2014). Subclinical mastitis in goats is associated with upregulation of nitric oxide-derived oxidative stress that causes reduction of milk antioxidative properties and impairment of its quality. International Journal of Dairy Science. 97: 3449-3455. DOI: 10.3168/jds.2013-7334.

Tabatabaee, N., Heidarpour, M., Khoramian, B. (2021). Milk metabolites, proteins and oxidative stress markers in dairy cows suffering from *Staphylococcus aureus* subclinical mastitis with or without a spontaneous cure. Journal of Dairy Science. 88: 326-329. DOI: 10.1017/S0022029921000613.

Turk, R., Koledic, M., Macesic, N., Benic, M., Dobranic, V., Duricic, D., Roncada, P. (2017). The role of oxidative stress and inflammatory response in the pathogenesis of mastitis in dairy cows. Mljekarstvo. 67: 91-101. DOI: 10.15567/mljekarstvo.2017.0201.

Yakan, A., Özkan, H., Çamdeviren, B., Kaya, U., Karaaslan, İ., Dalkiran, S. (2021). Expression patterns of major genes in fatty acid synthesis, inflammation, oxidative stress pathways from colostrum to milk in Damascus goats. Scientific Reports. 11: 9448. DOI: 10.1038/s41598-021-88976-0.

Wilson, D.J., Ruben, G.N., Das, H.H. (1997). Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects on somatic cell count and milk production. Journal of Dairy Science. 80: 2592-2598. DOI: 10.3168/jds.S0022-0302(97)76215-5.