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CATALASE ACTIVITY AND THE LEVELS OF MDA, AOPP IN SHEEPS WITH SUBCLINICAL MASTITIS

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Abstract. Subclinical mastitis is one of the major causes of economic losses in animal breeding. To determine the plasma levels of malondialdehyde (MDA), advanced oxidation protein products (AOPP) and catalase (CAT) during subclinical mastitis, twenty subclinical mastitic and ten healthy control ,total thirty Akkaraman sheep were used in the first lactation period .Plasma levels of MDA, AOPP and CAT of the subclinical control and mastitic were found to be 4.58 ± 0.59 - 4.94 ± 0.25 µmol / L; 94.03 ± 5.88 - 100.73 ± 5.09 µmol / L ve 380.70 ± 79.96 - 644.61 ± 59.84 kU / L respectively. The level of AOPP in the subclinical mastic group was slightly increased, but CAT activity was significantly increased ($p < 0.01$). As a result, oxidative stress was observed in mastitis. To increase resistances of animals, antioxidant compounds as injectable and food additives could be administered during pregnancy and followed by lactation.

Keywords: *AOPP, catalase, MDA, sheep, subclinical mastitis.*

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1. Introduction

Mastitis, one of the biggest problems of dairy producers today, causes great loss every year in the livestock economy due to the decrease of milk yield, the degradation of the quality of milk, the increase of the cost of treatment and in some cases the removal of the animal. Economic losses due to mastitis are estimated to be £ 90 million annually in England (Booth, 1989) and two billion dollars in the United States (Seykora and McDaniel, 1985). In Turkey, this loss is 400 million YTL annually (Anonymous, 2010b). 70% of the total economic losses are subclinical mastitic losses (Anonymous, 2010c).

The quality and quantity of the products obtained from the milk due to intra-mammary infection and / or inflammation are affected, which is important for the sheep sector. The most significant losses associated with mastitis and involving sheep are caused by subclinical mastitis. Subclinical mastitis is 20 to 30 times more frequent than the clinical form (Risvanlı and Kalkan, 2001), affecting breast tissue and milk composition, but none of these changes can be detected by visual and clinical examination, thus the herd prevalence is significantly higher (Alaçam, 1997). For this reason, there is a possibility of being converted into clinical mastitis.

Studies done in sheep with subclinical mastitis are limited. For this reason, plasma malondialdehyde (MDA), advanced oxidative protein products (AOPP)

levels and catalase (CAT) activities were determined in subclinical mastitic sheep and especially AOPP levels which have not been investigated in mastitis cases perviously will be presented on scientific platform .

2. Material and method

A total of 30 Akkaraman sheep, aged 3-5 years old, were raised in a farm of in Çaldıran district of Van, having the same management and feeding conditions, and in the first period of lactation. First of all, all sheep in the herd were clinically examined . Animals without general infection, no signs of acute or chronic inflammation in their mammary glands were separated .The milk of these selected sheep were evaluated by mastitis indicator paper and CMT for the diagnosis of mastitis (Megarsa et al., 2010; Housawi et al., 2008). 20 sheep with positive results with mastitis indicator paper and CMT named as mastitic group and 10 healthy sheep that gave negative results were the control group.

Blood samples of all sheep were taken in heparinized tubes from the vena jugularis , centrifuged for 10 minutes at 2500 rpm and plasma samples were separated.The activity of CAT (Goth, 1991), MDA (Yoshioka et al., 1979) and AOPP levels (Witko-Sarsat et al., 1999) were measured spectrophotometrically.

The unpaired t test was used to test the significance of the difference between the two groups (control and mastitic) independent of each parameter studied (Sümbüloğlu and Sümbüloğlu, 1998).

Results

The mean values of plasma MDA, AOPP levels and CAT activities for mastitic and healthy sheep are given in Table 1.

Table 1.Plasma MDA, AOPP levels and catalase activities of healthy and mastitic sheep

Parameters	**n**	**Control X±SEM**	**n**	**Mastitic Sheep X±SEM**
MDA (µmol/L)	18	4.58± 0.59	10	4.94±0.25
AOPP (µmol/L)	20	94.03±5.88	10	100.73±5.09
Catalase (kU/L)	20	380.70±79.96	10	644.61±59.84*

* $p<0.01$

In mastitic sheep, plasma catalase activity was significantly higher than the control group and statistical significance was found ($p <0.01$),but there were no significant differences between the levels of MDA and AOPP in comparison to the control group ($p> 0.05$)

3. Discussion

The leukocyte and epithelial cells pass through the milk when mastitis agents infect the mammary duct (Ergun and Mert, 1984) Depending on the amount of neutrophils coming into the inflamed breast lobe , there is a 10% reduction in the level of milk oxygen compared to the normal tissue in relation to

an increase in the use of oxygen in the tissue (Mayer et al., 1988). Neutrophils try to destroy the bacterial metabolites of oxygen (O^{2-} and H_2O_2) by oxidative methods (Aksakal et al., 1997). Phagocytosis is associated with the "respiratory burst" name reaction, which results in the formation of superoxide radicals and other oxygen metabolites by increasing oxygen and glucose consumption in the body. The resulting radicals are used to kill phagocytized microorganisms or to induce intracellular bactericidal reactions. These oxidant molecules, which constitute important defense molecules against foreign substances and infectious agents when they are at a certain level, are formed on certain levels or if antioxidants are inadequate, they can harm the proteins or lipids, carbohydrates, nucleic acids and enzymes which are building elements of the cells or organism (Şimşek and Aksakal, 2005).

In parallel with the increase of free radicals, the level of malondialdehyde, the end product of lipid peroxidation, increases. MDA a product of oxidative lipid degeneration produced during arachidonic acid enzyme oxygenation, causes cross-linking and polymerisation of membrane components (Bird and Draper, 1984). This changes the internal membrane properties such as deformation, ion transport, enzyme activity and aggregation of cell surface components (Akkus, 1995, Arıkan et al., 2001, Deveci, 2007). In the mammary gland, phagocytic cells migrate to the place of inflammation and, depending on their activity, use more oxygen, resulting in end-stage lipid peroxidation. Thus, the level of MDA increases (Mayer et al., 1988).

Musal et al. (2007) examined blood levels of vitamin C, vitamin A, β-carotene, glutathione, ceruloplasmin, and malondialdehyde in the cows of the subclinical mastitis Holstein Frisian before intramammary treatment (day 0) and after 14 and 21 days of treatment They found no significant difference in MDA levels in repeated samples of individual groups. Ranjan et al. (2005) investigated the erythrocyte MDA levels in clinical and subclinical mastitis cows. There was a significant difference ($p < 0.05$) between the clinical mastitic animals and the control group with respect to MDA levels, but not with subclinical mastitis. They argued that this increase in the level of erythrocyte lipid peroxidation, which is not observed in subclinical mastitis cows but in clinical mastitis patients, may be due to an excessive increase of ROS production in the inflamed mammary gland and thus a shift of prooxidant / antioxidant balance to oxidative damage in clinical mastitis. In this study, plasma MDA level was 4.94 μmol / L in subclinical mastitic sheep and 4.58 ± 0.59 μmol / L in healthy sheep and differences were statistically insignificant ($p > 0.05$) .

Oxidative modification of proteins by reactive derivatives plays a role in the etiology or progression of a number of disorders and diseases (Çakatay and Kayalı, 2004). AOPP is the terminal product of proteins that are exposed to free radicals and is responsible for the reaction between plasma proteins and chlorine oxidants regulated by a neutrophil enzyme, myeloperoxidase (Noyan et al., 2006). AOPP is related to various diseases in humans like; (Witko-Sarsat et al., 1998), diabetes mellitus (Kalousová et al., 2002), diabetic nephropathy (Shi et al., 2008), coronary artery diseases (Kaneda et al., 2002) and obesity(Atabek et al., 2006). Chronic accumulation of AOPP has been shown to stimulate inflammatory events

in the diabetic kidney (Shi et al., 2008) and chronic renal insufficiency (Witko-Sarsat et al., 1998) and may be a byproduct of neutrophil activation during infections.

In recent years there has been an increase in protein oxidation in various diseases in experimental studies (Yılmaz et al., 2010; Kilic and Yildirim, 2008) and the human field (Conrad et al., 2000; Telci et al., 2000, Lim et al., 2002) There is an upward trend in the number of researches. However, as there is no study of AOPP levels in mastitis animals, the number of studies on AOPP levels in veterinary medicine is also low.

In this study, AOPP levels were found to be 100.73 ± 5.09 μmol / L in mastitic sheep and 94.03 ± 5.88 μmol / L in healthy sheep ($p \geq 0.05$). Although not statistically significant, there is a slight increase in AOPP levels compared to healthy animals. In animals, mastitis-induced inflammatory events may result in an increase in AOPP levels due to increased free radicals initiating protein oxidation and oxidant / antioxidant balance shifting towards oxidative damage. The small increase in AOPP level may indicate that oxidative stress has begun, that the disease may have just begun because it is in the first period of lactation, at which stage the antioxidants found in the organism are effective and sufficient. The AOPP levels in mastitic sheep have already been made for the first time in this research. For this reason, further study of AOPP levels in the field of veterinary medicine is needed.

The defense system created by living organisms to the damage caused by ROS uses enzymes, which are expressed as antioxidant enzymes, to remove these species by converting them into less toxic substances. Catalase, an antioxidant enzyme, has different catalytic activity in various tissues, catalyses the conversion of hydrogen peroxidation to water and oxygen. While enzyme activity is high in the liver and kidney, in the connective tissue is very low. CAT is a somatic oxidant protectant. The affinity of catalase to H_2O_2 is higher than that of glutathione peroxidase, and CAT is abundant in erythrocytes (Karabulut et al., 2002, Agar et al., 1986). Exposure to oxidative stress induces the transcriptions of several genes including hemoxygenase, superoxide dismutase, catalase, glutathione peroxidase which protects cells from oxidative damage (Demple and Amabile-Cuevas, 1991). While acute increases in ROS production forms short-term CAT activation, chronic imbalance of ROS production causes both CAT activation and expression increase (Johnson, 2002).

Simsek and Aksakal (2005) found that erythrocyte catalase levels in the subclinical mastitic cows were 48.06 ± 4.77 kU / gr protein before treatment, 50.32 ± 6.35 kU / gr protein after treatment and 52.19 ± 6.26 kU / gr protein in healthy cows. Catalase activity was found to be significant ($p < 0.05$) between healthy and mastitic groups, and not significant between mastitic and treatment groups; The use of catalase activity due to the effects of free radicals resulting from inflammation in mastitis infections reduced levels but the post-administration of the increase is insignificant and may be a function of the duration of application , dosage and individual metabolic differences in animals.

Limited number of studies have been found on catalase activity in mastitic sheep. In this study, plasma catalase activity in subclinical mastitic sheep was found to be 644.61 ± 59.84 kU / L higher than control (380.70 ± 79.96 kU / L) and statistical importance was dedected ($p < 0.01$). Increased catalase activity in mastitis animals can be interpreted as an increase in the production of antioxidant enzymes in order to compensate for the oxidative stress that is initiated by the antioxidant enzyme system.

4. Conclusion

As a result, the diagnosis of subclinical mastitis becomes important when the economic losses caused by mastitis are taken into account and since the symptoms can not be determined by visual and clinical examination, the prevalence of the herd is considerably high. In the present study, there was a slight increase in plasma AOPP levels in subclinical mastitis sheeps examined, with an increase in statistical significance in catalase activity. These changes emphasize the fact that mastitic oxidative stress is observed in the organism and that in order to increase the antioxidant capacity of the animals against the presence of oxidants during the inflammatory events, the supplementing of certain antioxidant substances during pregnancy and subsequent lactation periods, in the form of injections or feed additives could be affected. It can be stated that these preventions may also affect the resistance to mastitis infection in a positive way.

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