

## Lipid Peroxidation and Thiol/Disulfide Homeostasis in Cattle with Trichophytosis

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### Abstract

The aim of this study was to determine thiol/disulfide homeostasis in cattle with trichophytosis and to determine the changes in malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px=GPx) levels due to the disease. 15 cattle with trichophytosis and 15 healthy cattle in the control group constituted the material of the study. Blood samples were taken from the jugular vein of the animals in tubes with and without anticoagulant. MDA, CAT, SOD, total thiol, native thiol analyses were performed in serum and GSH-Px analyses were performed in plasma. It was determined that total thiol, native thiol and native thiol/total thiol levels among biochemical parameters in cattle with trichophytosis decreased statistically significantly. It was determined that disulfide/native thiol and disulfide/total thiol levels increased statistically compared to the control group. Although the disulfide level increased compared to the control group, it was not statistically significant. In addition, it was determined that MDA level increased compared to the control group, CAT, SOD and GSH-Px levels decreased statistically significantly compared to the control group. In conclusion, the findings obtained from the study showed that trichophytosis causes oxidative stress in cattle, and the use of oxidative stress markers, especially thiol/disulfide homeostasis markers, would contribute to the pathogenesis of trichophytosis.

**Key Words:** Cattle, oxidative stress, thiol/disulfide homeostasis, trichophytosis

### Trikofitozisli Sığırlarda Lipid Peroksidasyonu ve Tiyo/Disülfid Homeostazı

#### Öz

Amacımız trikofitozisli sığırlarda tiyo/disülfid homeostazisini ortaya koymak ve hastalığa bağlı olarak oluşan malondialdehit (MDA), katalaz (CAT), süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px= GPx) seviyesindeki değişimleri belirlemektir. Trikofitozisli 15 ve kontrol grubunu oluşturan 15 adet sağlıklı sığır çalışmanın materyalini oluşturdu. Hayvanların Vena jugularis'inden antikoagulanlı ve antikoagulanlı tüplere kan örnekleri alındı. Serumda MDA, CAT, SOD, total tiyo, natif tiyo, plazmada ise GSH-Px analizleri yapıldı. Trikofitozisli sığırlarda total tiyo, natif tiyo ve natif tiyo/total tiyo düzeylerinin istatistiksel olarak anlamlı düzeyde azaldığı belirlendi. Disülfid/natif tiyo ve disülfid/total tiyo düzeylerinin ise kontrol grubuna göre istatistiksel olarak arttığı tespit edildi. Disülfid düzeyi kontrol grubuna göre artmakla beraber istatistiksel olarak anlamsızdı. Bunun yanı sıra MDA düzeyinin kontrol grubuna göre arttığı, CAT, SOD ve GSH-Px düzeylerinin ise kontrol grubuna göre istatistiksel olarak anlamlı düzeyde azaldığı belirlendi. Sonuç olarak çalışmadan elde edilen bulgular trikofitozisin sığırlarda oksidatif strese neden olduğu, oksidatif stres belirteçlerinden özellikle de tiyo/disülfid homeostazis belirteçlerinin kullanımının trikofitozisin patogeneze katkısı sağlayacağı kanısına varıldı.

**Anahtar Kelimeler:** Oksidatif stres, sığır, tiyo/disülfid homeostazı, trikofitozis

## INTRODUCTION

Trichophytosis is a skin disease that is characterized by dandruff and keratinized crusting of the skin caused by fungi, causing losses such as growth slowdown, live weight loss, and deterioration of skin quality (1). These factors, which are found all over the world, cause infections in humans and animals, and generally epidermophyton species are effective in humans, microsporum species in carnivores, and trichophyton species in horses, pigs and ruminants (2). The disease is transmitted to healthy animals through direct contact (3). Clinical symptoms include hair loss, skin crusting, erythema and itching (4). The lesions, which are commonly found on the head and neck, are grayish in appearance, slightly raised, and round in shape. Diagnosis is made by seeing round, grayish lesions on the skin. Definitive diagnosis is made by microscopic examination of samples taken from the lesions (3).

Antioxidants are classified according to their structures as enzymes (catalase 'CAT', superoxide dismutase 'SOD', glutathione peroxidase 'GSH-Px = GPx') and non-enzymes (reduced glutathione 'GSH') or according to their cell localization (5). Oxidative stress is formed as a result of insufficiency of antioxidant mechanisms and increase of reactive oxygen species (6). Thiol is a very important antioxidant in preventing damage caused by oxidative stress and protects the cell against oxidative stress. It appears that the thiol status changes in various diseases and that thiol/disulfide homeostasis is very important in the pathogenesis of diseases. Therefore, determination of thiol/disulfide homeostasis can provide very important information about various physiological or pathological processes (7,8). For these reasons, the aim of this study is to reveal the thiol/disulfide homeostasis in cattle with trichophytosis and to determine the changes in MDA, CAT, SOD, and GSH-Px levels that occur due to the disease.

## MATERIAL AND METHODS

The animal material of the study was obtained from livestock farms in Digor district of Kars province, of different breeds (8 Montefon crossbreeds and 22 Simmental crossbreeds), of both genders, aged between 5-18 months, 15 with trichophytosis and 15 control animals. Blood samples taken from the jugular vein of the animals into tubes with and without anticoagulant, ethylene diamine tetraacetic acid (EDTA), were centrifuged at 3000 rpm for 15 minutes to obtain serum and plasma. The disease was diagnosed according to clinical symptoms (grayish, round, raised, chalk dust-like lesions on the head and neck), and the final diagnosis was made microscopically. Skin scrapings were taken from the lesioned areas of the cattle with a sterile scalpel and processed with 10% KOH. Preparations were examined under

the microscope and the observation of typical spores was evaluated as positive for trichophytosis.

MDA measurement was performed by the method reported by Yoshiko et al. (9), CAT, SOD, GSH-Px (Cayman Chemical Co., USA), total thiol and native thiol (Rel Assay Diagnostics, Turkey) were measured colorimetrically (Epoch, Biotek, USA) using a commercial test kit. Disulfide = (Total thiol-Native thiol)/2, Disulfide/Total Thiol (%) = (Disulfide x 100)/Total thiol, Disulfide/Native Thiol (%) = (Disulfide x 100)/Native thiol, and Native Thiol/Total Thiol (%) = (Native thiol x 100)/Total thiol was calculated with the formulas (10).

## Statistical Analysis

SPSS 20.0 package program was used to evaluate the study data. Independent Sample T-test was used to compare the groups.

## RESULTS

In animals infected with trichophytosis that underwent clinical examination, chalk-like, round lesions were detected on various parts of the animal's body, especially on the head and neck. Hyphae were found in direct microscopic examination of the lesional skin scrapings of the animals used in the study.

It was determined that MDA levels ( $P < 0.001$ ) increased statistically significantly in cattle with trichophytosis. In addition CAT, SOD, ( $P < 0.001$ ), and GSH-Px ( $P < 0.01$ ) levels decreased statistically significantly compared to the control group (Table 1). It was determined that the biochemical parameters total thiol, native thiol ( $P < 0.001$ ) and native thiol/total thiol ( $P < 0.01$ ) levels in cattle with trichophytosis decreased statistically significantly compared to the control group. It was determined that disulfide/native thiol and disulfide/total thiol ( $P < 0.01$ ) levels increased statistically compared to the control group. Although disulfide level increased, it was statistically insignificant ( $P > 0.05$ ) (Table 2).

**Table 1.** Means and standard errors of lipid peroxidation (MDA) and some antioxidant (CAT, SOD, and GSH-Px) parameters in clinically healthy and trichophytosis cattle

Parameters	Control	Infected	P
Malondialdehyde ( $\mu\text{mol/L}$ )	2.93 $\pm$ 0.11	7.31 $\pm$ 0.29	$P < 0.001$
Catalase (nmol/min/mL)	31.50 $\pm$ 1.55	15.73 $\pm$ 0.90	$P < 0.001$
Superoxide Dismutase (U/mL)	226.78 $\pm$ 6.14	107.59 $\pm$ 3.71	$P < 0.001$
Glutathione Peroxidase (nmol/min/mL)	0.40 $\pm$ 0.04	0.24 $\pm$ 0.02	$P < 0.01$

**Table 2.** Means and standard errors of thiol/disulfide homeostasis parameters in clinically healthy and trichophytosis cattle

Parameters	Control	Infected	P
Total Thiol ( $\mu\text{mol/L}$ )	476.15 $\pm$ 7.01	418.45 $\pm$ 6.65	$P < 0.001$
Natif Thiol ( $\mu\text{mol/L}$ )	378.67 $\pm$ 6.65	297.94 $\pm$ 4.81	$P < 0.001$
Disulfide ( $\mu\text{mol/L}$ )	48.74 $\pm$ 4.88	60.26 $\pm$ 4.17	NS
Disulfide/Native Thiol (%)	13.17 $\pm$ 1.50	20.57 $\pm$ 1.78	$P < 0.01$
Disulfide/Total Thiol (%)	10.12 $\pm$ 0.93	14.28 $\pm$ 0.85	$P < 0.01$
Native Thiol/Total Thiol (%)	79.77 $\pm$ 1.86	71.44 $\pm$ 1.69	$P < 0.01$

NS: Non Significant

## DISCUSSION AND CONCLUSION

In the study, in animals with trichophytosis that underwent clinical examination, round lesions with a chalk appearance were detected in various parts of the body, especially in the head and neck region (11-13).

Tissue damage and inflammation in the organism activate many cells. Among the activated cells, phagocytic cells, which play an important role in body defense, cause oxidative stress due to excessive oxygen consumption while performing this task (14). In case of oxidative stress, excessively produced free radicals damage cell compounds (15). Studies have shown that bacterial/viral diseases such as brucellosis (16), sheeppox in sheep (17), hypodermosis (18,19), and pneumonia (20), it has been reported that the oxidant-antioxidant balance is disrupted and oxidative stress occurs. In study on cattle with trichophytosis, found a significant increase in MDA levels (15). Additionally, Bayyit and Merhan (21) reported in another study they conducted in cattle with dystocia that the MDA level was higher in cattle with dystocia. In this study, in parallel with the above studies, it was determined that the oxidant-antioxidant balance was disrupted and as a result, the MDA concentration increased when the control group and the trichophytosis group were compared. The reason for this is; It is thought that it may occur due to lipid peroxidation caused by stress in animals with trichophytosis and/or free radicals formed by phagocytes, which have an important role in host defense.

Antioxidants are classified according to their structures as enzymes/non-enzymes or according to their cell localization. The most important enzymatic defense systems against oxygen radicals are CAT, SOD, and GSH-Px (22). Important antioxidant enzymes such as CAT, SOD and GSH-Px neutralize free radicals (23). Kataria et al. (24) reported in a study conducted on cattle with brucellosis that serum CAT, SOD, glutathione reductase, monoamine oxidase, and peroxidase activities increased significantly in cattle with brucellosis. In another study, a significant increase in CAT, SOD, and GSH-Px activities was reported in cattle with trichophytosis (15). In a study conducted in dogs with trichophytosis, low SOD, and CAT activities were reported (25). In another study conducted in sheep and goats infected with *Haemonchus contortus*, it was reported that MDA increased and SOD which one of the antioxidant markers, decreased (26). Additionally, two different studies conducted in calves with trichophytosis reported that antioxidant activity decreased (27,28). Similarly, in this study, decreased antioxidant levels (SOD, CAT and GSH-Px) in cattle with trichophytosis may be due to the defense mechanism against lipid peroxidation.

Thiol/disulfide homeostasis has functions, antioxidant defense, apoptosis, regulation of enzyme functions, etc. Alteration of thiol/disulfide homeostasis plays a role in the pathogenesis of many diseases, especially chronic diseases (29). Thiols, which regulate intracellular redox status, are the first antioxidants consumed in the oxidative environment. In addition, plasma values are a good indicator of tissue redox potentials (30). Thiols, which are reported to be formed by dendritic cells in the skin, have an important role in oxidative stress and disease pathogenesis (29). In human medicine, changes in thiol-disulfide concentrations have been reported in many inflammatory diseases (31,32). In veterinary medicine, it has been reported that there is a decrease in total

thiol and native thiol levels during dehorning of calves with hot cautery, and there is no significant difference in disulfide level (33). Also conducted in sheep with toxoplasmosis, it was reported that total thiol and native thiol levels were significantly lower, and disulfide, disulfide/native thiol and disulfide/total thiol were higher (34). In the study, it was determined that total thiol, native thiol, and native thiol/total thiol levels decreased statistically significantly compared to the control group. It was determined that disulfide/native thiol and disulfide/total thiol levels increased statistically compared to the control group. Although the disulfide level increased, it was statistically insignificant. The levels of thiol/disulfide homeostasis parameters in the study are compatible with the above studies, and we believe that this may be due to the formation of oxidative stress and the organism's consumption of thiols to combat oxidative stress against the increasing oxidative stress level.

As a result, the findings obtained from the study indicate that trichophytosis causes oxidative stress in cattle, and it is thought that the use of oxidative stress markers, especially thiol/disulfide homeostasis markers, will contribute to the pathogenesis of trichophytosis and more detailed studies should be conducted on this subject.

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This study was summarised by the first author's Master Thesis.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

OM took part in the study planning and LB sample collection. The writing of the study and final checks were carried out with the contributions of all authors.

## ETHICAL STATEMENT

This study was started after receiving the ethics committee approval of Kafkas University Animal Experiments Local Ethics Committee dated 24.03.2023 and coded 2023/029.

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