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## Assessment of oxidative stress, trace elements, serum biochemistry, and hormones levels in weaned calves with dermatophytosis

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**ABSTRACT:** In this study, it was aimed to evaluate oxidative stress, serum biochemistry, trace elements, minerals, and testosterone and thyroid hormone levels in weaned calves with dermatophytosis. A total of 28 weaned Holstein calves were used in the study, including 6-8 months old, 14 with dermatophytosis (7 males, 7 females) and 14 healthy (7 males, 7 females). The animals were grouped as the diseased and healthy animals, 14 animals in each group as well as the male diseased and the male healthy animals were grouped as 7 animals in each group for the comparison of testosterone levels. The blood analyses were performed using ELISA kits and biochemistry automatic analyzer. There was a significant difference between the diseased and healthy groups for NO (nitric oxide) ( $P<0.05$ ), TOS (total oxidative stress) ( $P<0.001$ ), TAC (total antioxidant capacity) ( $P<0.01$ ). However, in comparison of the diseased and healthy groups, serum biochemistry with the exception of glucose and triglyceride, trace elements except for manganese, minerals, and thyroid hormone levels were not statistically different ( $P>0.05$ ). In comparison of the diseased and healthy animals for testosterone levels, it was not determined any difference ( $P>0.05$ ). The present study revealed that dermatophytosis could affect oxidant status in calves with dermatophytosis, and that TOC (total oxidant capacity) and NO as oxidative stress marker might be increased for fungicidal effect in the diseased animals with dermatophytosis.

**Keywords:** Calves with dermatophytosis, oxidative stress, trace elements, serum biochemistry, hormones.

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## INTRODUCTION

**D**ermatophytosis commonly occurs in humans and animals and is an infectious diseases characterized by keratinization in stratum corneum layer of skin and hair loss. This zoonotic disease is of economic importance because of difficult control, contagious, and high cost of treatment (Chermette et al., 2008; Radostits et al., 2007). Dermatophytosis is caused by *Trichophyton*, *Microsporum* and *Epi-dermophyton* species in domestic animals (Deacon, 1988; Kahn and Line, 2010; Radostits et al., 2007). It is reported that *T. verrucosum* generally causes the disease in cattle (Al-Qudah, et al., 2010; Papini et al., 2009). Factors such as keratinase enzyme, hemolytic activity, humidity, pH, fatty acids of the skin, amino acids, hormones, individual resistance and immune response have important roles in the development of disease (Hashemi and Sarasgani, 2004; Pal and Dave, 2013; Radostits et al., 2007; Schaufuss and Steller, 2003; Xavier et al., 2008).

In calves, dermatophytosis is characterized by non-pruritic lesions around the eye, ear, and dorsum, and sometimes also develops in the generalized form (Kahn and Line, 2010). There are characteristic lesions with scaling patches of hair loss, and gray-white crust formation, but sometimes these lesions have thick crusts with suppuration (Kahn and Line, 2010).

In cattle, deficiency of minerals and trace elements causes growth retardation, immune system deficiency and dermatological lesions (Kahn and Line, 2010; Radostits et al., 2007). Zinc and selenium play roles in numerous metabolic reactions in the body (Shafiei Neek et al., 2011). Zinc deficiency may lead to the development of dermatophytosis, chronic infection and expansion of lesion area (Szczepanik and Wilkolek, 2004).

In recent years, the effect of reactive oxygen species (ROS) on the body defense system has become important in farm animals (Castillo et al., 2003). Reactive oxygen species are produced as by-products due to cellular metabolism in low concentrations for numerous physiological processes including activation of transcription factors, cell immunity, and cellular defense against microorganisms (Miller et al., 1993; Zhang et al., 2016). Reactive oxygen species are increased during diseases and pathological changes in the organism and oxidative stress occurs because of deficiency of antioxidants and increase of oxidants (Lykkesfeldt and Svendsen, 2007; Roth, 1997). In bovine animals oxidative stress has been reported in various diseases caused by pneumonia, enteritis, sepsis,

mastitis (Atakisi et al., 2010; Erkilic et al., 2016; Lykkesfeldt and Svendsen, 2007; Schott et al., 2014). In recent years, oxidative status has been investigated to enlighten the pathogenesis of dermatophytosis in animals and humans (Beigh et al., 2014; Karapehliyan et al., 2007; Kurutas and Ozturk, 2016).

In humans, pathogenic fungi and yeasts are reported to be affected by steroids, and this has become special area of interest in clinical research (Brasch, 1997; Clemons et al., 1988). An in vitro study has demonstrated that the growth of *T. rubrum* and *E. floccosum* is suppressed by androgenic hormones but *T. mentagrophytes* and *M. canis* is least responsive to most hormones (Brasch and Flader, 1996). In addition, testosterone levels in patients with dermatophytosis caused by *E. floccosum* decreased compared to healthy subjects but testosterone levels were not different between the patients with *T. rubrum* and healthy subjects (Brasch and Flader, 1996). However, to the best knowledge of authors, the role of androgenic hormones is not known in male calves with dermatophytosis.

Deficiency of minerals and trace elements leads to immune system deficiency, dermatological lesions and dermatophytosis. In addition, oxidative status has been shown in the pathogenesis of infectious diseases and the role of androgenic hormones is not known in male calves with dermatophytosis. In this study, it was aimed to evaluate oxidative stress, serum biochemistry, trace elements, minerals, and testosterone and thyroid hormone levels in calves with dermatophytosis.

## MATERIALS AND METHODS

### Animals

This study was carried out on total 28 weaned Holstein calves, 6-8 month old, 14 with dermatophytosis (7 males and 7 females) and 14 healthy weaned calves (7 males and 7 females). Each group consisted of 14 weaned calves. Group I was the female and male diseased calves; Group II was the female and male healthy calves. All calves in the study were from one farm and all animals were kept under similar management conditions and were not kept overcrowded (including 96 calves in herd). All calves were healthy at weaning and throughout the study period except for dermatophytosis. Clinical examinations were performed by the same clinician (KS) and took skin scraping and hair samples randomly from the 14 calves of 45 calves with suspected dermatophytosis.

### Microbiological analysis

Skin scrapings and hair samples (in calves showed skin lesions) were in part processed for microscopy by use of 10-20% potassium hydroxide (KOH) and after 30 min examined under 400X magnification of the light microscope. Rest of the samples was seeded on the Sabouraud Dextrose agar (SDA, OXOID) supplemented with chloramphenicol (0.05 mg/mL), and plates were incubated at 25°C and 37°C for a period of 1-4 weeks and examined on a daily basis as noted by studies (Larone, 1995; Quinn et al., 1999). The isolated fungal colonies were stained with lactophenol blue. The macro- and microscopic characteristics of isolates were detected as *Trichophyton* sp. (Larone, 1995; Robert et al., 2008).

### Blood sampling

Blood samples were obtained from the calves by venipuncture of *vena jugularis* to vacutainer tubes. The blood samples were centrifuged at 3,000 rpm for 10 min and the serum samples were allocated to Eppendorf tubes and stored at -20 °C until analyses.

### Total antioxidant capacity (TAC) analysis

Determination of TAC levels was performed using a novel automated colorimetric measurement method (Erel, 2004). The assay has finest quality precision values, lower than 3%. The results were indicated as mmolTrolox Equivalents/L for serum.

### Total oxidant capacity (TOC) analysis

Determination of TOC levels was performed using a novel automated measurement method (Erel, 2005). The results were indicated as mmol H<sub>2</sub>O<sub>2</sub> Equivalents/L for serum.

### Plasma total nitric oxide (NO) analysis

The plasma total NO level measurement was performed by colorimetric method using NO detection kit (Enzo Life Science).

### Serum biochemistry analyses

Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total protein, albumin, glucose, urea, creatinine, total bilirubin, triglyceride (TG), total cholesterol, low density lipoprotein cholesterol (LDL-C), calcium (Ca), magnesium (Mg), retinol and  $\beta$ -carotene levels were determined by colorimetric method by a biochemistry auto analyzer using commercial test kits (Beckman Coulter, AU5800, USA).

### Serum trace elements analyses

The serum copper (Cu), zinc (Zn), iron (Fe) were measured by flame atomic absorption (flame-AA) spectroscopy (Perkin Elmer AAS 800). Selenium (Se) was measured by hydride system atomic absorption (hydride-AA) spectroscopy. Manganese (Mn) levels were measured using atomic absorption spectrometer-graphite furnace system (graphite furnace-AAS).

### Hormone analyses

Testosterone hormone, thyroid stimulating hormone (TSH), free triiodothyronine (fT<sub>3</sub>), and free thyroxin (fT<sub>4</sub>) levels were analyzed by chemiluminescence method (Beckman Coulter DXI 800, USA).

### Statistical analysis

The comparison of the data between the diseased group and the healthy group was performed (SPSS, Version 11.5 Microsoft, Chicago, IL, USA) by using independent samples t test and Mann-Whitney *U* tests in 95% confidence interval. The significance degree between two groups was determined to be  $P < 0.05$ . Data were expressed as mean  $\pm$  standard error of the mean (SEM) and as median (minimum - maximum).

## RESULTS

Oxidative stress markers such as TOC, TAC and NO levels in the weaned calves with dermatophytosis and in the healthy calves are given in Table 1. Total NO, TOC levels in calves with dermatophytosis were significantly increased compared to the healthy calves. TAC levels were significantly decreased in calves with dermatophytosis compared to the healthy calves.

The levels of trace elements and minerals are given in Table 2. The levels of these parameters except for manganese were not significantly different between the diseased and healthy calves. However, manganese levels were significantly lower than those of the healthy calves. Other elements (Cu, Se, Zn, Fe, Ca, Mg) were not found to be statistically important.

The levels of serum biochemistry parameters and hormones such as testosterone, TSH, fT<sub>3</sub>, and fT<sub>4</sub> are given in Table 3. The levels of these parameters were not significantly different between the diseased and the healthy calves. The serum glucose and TG levels were significantly increased in the diseased animals over those in the healthy animals.

**Table 1.** Levels of total oxidant capacity, total antioxidant capacity and nitric oxide in calves with dermatophytosis and in the healthy calves

Parameter	Group I (n=14)	Group II (n=14)
Total NO ( $\mu\text{mol/L}$ )	70.09 $\pm$ 3.40 <sup>a</sup>	50.96 $\pm$ 3.54 <sup>b</sup>
TAC (mmolTrolox Equiv./L)	2.13 (1.78-2.61) <sup>a</sup>	2.86 (2.11-3.12) <sup>b</sup>
TOC ( $\mu\text{mol H}_2\text{O}_2$ Equiv./L)	4.41 $\pm$ 0.07 <sup>a</sup>	3.54 $\pm$ 0.09 <sup>b</sup>

Group I: Calves with dermatophytosis; Group II: Healthy calves; NO: Nitric oxide; TAC: Total antioxidant capacity; TOC: Total oxidant capacity. The total NO, and TOC values were given as mean  $\pm$  SEM for each group including 14 weaned calves, and the TAC values were given as median (minimum - maximum) for each group including 14 weaned calves.

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

**Table 2.** Levels of trace elements (Cu, Zn, Se, Fe, and Mn) and minerals (Ca and Mg) in calves with dermatophytosis and in the healthy calves

Parameter	Group I (n=14)	Group II (n=14)
Cu (ppm)	0.43 $\pm$ 0.02	0.45 $\pm$ 0.01
Zn (ppm)	0.77 $\pm$ 0.06	0.71 $\pm$ 0.04
Se (ppb)	13.77 $\pm$ 1.31	15.70 $\pm$ 0.88
Fe (ppm)	0.50 $\pm$ 0.03	0.48 $\pm$ 0.02
Ca (mg/dL)	9.5 (6.46-15.15)	9.15 (3.41-9.68)
Mg (mg/dL)	4.39 $\pm$ 0.17	4.19 $\pm$ 0.20
Mn (ppm)	1.88 $\pm$ 0.13 <sup>a</sup>	3.05 $\pm$ 0.17 <sup>b</sup>

Group I: Calves with dermatophytosis; Group II: Healthy calves. The Cu, Zn, Se, Fe, Mg and Mn values were given as mean  $\pm$  SEM for each group including 14 weaned calves, and the Ca values were given as median (minimum - maximum) for each group including 14 weaned calves.

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

**Table 3.** Levels of serum biochemistry, and thyroid hormones in calves with dermatophytosis and in the healthy calves

Parameter	Group I (n=14)	Group II (n=14)
Triglyceride (mg/dL)	27.71 $\pm$ 2.88 <sup>a</sup>	19.00 $\pm$ 1.99 <sup>b</sup>
Total cholesterol (mg/dL)	82.28 $\pm$ 5.74	74.84 $\pm$ 4.33
LDL-C (mg/dL)	38.07 $\pm$ 3.21	35.46 $\pm$ 2.53
Urea (mg/dL)	14.28 $\pm$ 1.08	15.53 $\pm$ 1.35
Creatinine (mg/dL)	0.7 (0.6-0.9)	0.8 (0.6-0.9)
Total bilirubin (mg/dL)	0.18 $\pm$ 0.005	0.19 $\pm$ 0.008
Glucose (mg/dL)	70.64 $\pm$ 2.76 <sup>a</sup>	62.84 $\pm$ 3.46 <sup>b</sup>
Total protein (g/dL)	6.65 (6.30-8.60)	6.70 (4.50-8.10)
Albumin (g/dL)	2.80 $\pm$ 0.07	2.81 $\pm$ 0.10
AST (U/L)	87 $\pm$ 4.59	94.46 $\pm$ 5.46
GGT (U/L)	18.85 $\pm$ 0.98	17.07 $\pm$ 1.04
ALP (U/L)	133.50 (89-222)	102.0 (50-222)
Retinol (mg/dL)	26.19 (17.56-41.04)	23.34 (16.64-37.50)
$\beta$ -carotene (mg/dL)	12.77 $\pm$ 1.18	12.85 $\pm$ 1.45
TSH uIU/mL	0.18 (0.0-9.0)	0.14 (0.0-2.05)
Free triiodothyronine (fT <sub>3</sub> ) pg/mL	2.78 (2.39-4.07)	3.19 (1.66-3.88)
Free thyroxine (fT <sub>4</sub> ) ng/dL	0.79 $\pm$ 0.04	0.83 $\pm$ 0.05

Group I: Calves with dermatophytosis; Group II: Healthy calves. The triglyceride, total cholesterol, LDL-C, urea, total bilirubin, glucose, albumin, AST, GGT,  $\beta$ -carotene, and free thyroxine values were given as mean  $\pm$  SEM for each group including 14 weaned calves. The creatinine, total protein, ALP, retinol, TSH, and free triiodothyronine values were given as median (minimum - maximum) for each group including 14 weaned calves.

Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

**Table 4.** Serum testosterone levels in the calves with dermatophytosis and in the healthy calves

Parameter	Group I (n=7)	Group II (n=7)
Testosterone (ng/dL)	1.64 ± 0.24	3.66±0.96

Group I: Calves with dermatophytosis; Group II: Healthy calves. The testosterone values were given as mean ± SEM for each group including 7 male weaned calves.

## DISCUSSION

Zoophilic dermatophytes induced infections are acute or chronic and highly inflammatory. Keratinocytes, after infected with a zoophilic dermatophyte, express pro-inflammatory genes and secrete cytokines to contribute recruitment of inflammatory cells in the skin, tissue remodeling and wound healing (Martinez-Rossi et al., 2017). Experimental dermatophyte infection in mice has showed that dermal inflammation and histopathologically macrophages, dendritic cells, neutrophils are present. Inflammation also results in cytokine over expression such as transforming growth factor- $\beta$ , interleukin-1 $\beta$ , and IL-6 (Cambier et al., 2014). The pathogens are cleared in the body by ROS produced by phagocyte cells (Mittal et al., 2014). In addition, during inflammation, macrophages and neutrophils produce NO for microbicidal effect (Mizokami et al., 2016). Comply with these studies, in the present study, significant increase in TOC and NO levels was found in calves with dermatophytosis compared to the healthy calves. This suggested that TOC and NO as oxidative stress marker might be increased for fungicidal effect in the groups with dermatophytosis.

Oxidative stress as revealed by high MDA levels and lower SOD and catalase levels has been reported in dogs with dermatophytosis (Beigh et al., 2014). Similarly, in calves with dermatophytosis, oxidative stress through high MDA and NO, and low antioxidant GSH has been reported (Karapehlivan et al., 2007). In addition, significant oxidative/nitrosative stress revealed by increased MDA, NO and 3-NT levels have been demonstrated in patients with pityriasis versicolor (Kurutas and Ozturk, 2016). Similarly, in the present study, oxidative stress was found in calves with dermatophytosis via increased TOC and NO, and decreased TAC levels. This study suggested that oxidative stress might mediate the fungicidal activity in calves with dermatophytosis.

Trace elements such as Cu, Zn, Se, Mn and Fe have cofactor roles in antioxidant enzymes. Several studies have demonstrated that trace elements have important roles in antioxidant enzymes expression and activi-

ties. For example, a selenium, zinc, copper, iron, and manganese deficient diet has been demonstrated to cause a significant decrease in GSH-Px, Cu,Zn-SOD, catalase and GSH-Px, and Mn-SOD activities (Gong and Xiao, 2018; Malecki and Greger, 1996; Prohaska and Brokate, 2001; Toyoda et al., 1989). The deficiencies of trace elements may be caused by dietary imbalances or diseases. In calves with dermatophytosis, trace elements such as whole blood Se, serum Zn, and Cu levels have been significantly decreased in line with significant reduction in antioxidant defense systems including GSH-Px activities and glutathione levels by attributing to possible dermatophyte consumption (Al-Qudah et al., 2010). In other studies, significant decrease in serum zinc levels without changes in blood leukocytes levels (Nisbet et al., 2006), significant decrease in serum Mn and Zn levels and increase in Cu levels in bovine dermatophytosis (Paksoy et al., 2013), and significant decrease in serum Fe in cattle with dermatophytosis (Yildirim et al., 2010) have been reported. In contrast to the results of those studies, in the present study, serum Cu, Zn, Se, Fe levels were not different statistically in the group with dermatophytosis compared to the healthy control group. The serum Mn levels were significantly decreased in calves with dermatophytosis than in the healthy calves in line with the result of Paksoy et al. (2013). The serum Ca and Mg minerals between the group with dermatophytosis and the healthy group were not different statistically. This study did not found any significant difference except Mn in serum trace elements and serum minerals between the diseased group and the healthy group. Similarly, the serum Zn and Cu levels were not statistically different between young cattle with dermatophytosis and the healthy young cattle (Aslan et al., 2010). In addition, a recent study in patients with tinea pedis has revealed that zinc and selenium levels are significantly lower on the lesion site than those on the healthy site, but Cu levels are significantly higher on the lesion site than those on the healthy site. In addition, positive correlation between the lesional area Cu and the lesional area 8-iso-PGF<sub>2 $\alpha$</sub>  (lipid peroxidation product) has been demonstrated (Miraloglu et al., 2016). It is thought that evaluating oxidant status and trace ele-

ments on the lesional site can provide better knowledge in elucidating the dermatophytosis pathogenesis.

Significant decrease in the serum Zn and vitamin A levels has been reported in calves with dermatophytosis (Pasa and Kiral, 2009). Several studies have reported that the levels of vitamin A may be changed in infection conditions (Bendich, 1993; Chew, 1987; Or et al., 2002). Zinc has the effects on vitamin A metabolism such as absorption, transport and utilization through protein synthesis, and Zn-dependent dehydrogenase enzyme (Christian and West, 1998). These studies have determined the important association between zinc and vitamin A. In the present study, contrary to the findings of Pasa and Kiral (2009), retinol and  $\beta$ -carotene levels were also in normal ranges in line with normal serum Zn levels in calves with dermatophytosis.

In addition, the present study found no statistical difference in the serum biochemical parameters such as triglyceride, total cholesterol, LDL, urea, creatinine, total bilirubin, glucose, total protein, albumin, TSH,  $fT_3$ ,  $fT_4$ , AST, GGT, ALP in calves with dermatophytosis compared to healthy calves. This revealed that the calves with dermatophytosis may have normal organ functions. However, in a study (Atakisi et al., 2006) evaluated serum adenosine deaminase and liver function tests in dermatophytic cattle, increased adenosine deaminase, GGT, ALT, AST, and LDH levels have been found and it was thought to be associated with possible liver damage due to the toxic

metabolic products of the fungi.

Androgenic hormones can affect fungal growth in male patients with dermatophytosis (Hashemi and Sarasgani, 2004). Serum testosterone levels have been reported to significantly decrease in patients with dermatophytosis caused by *E. floccosum* (Hashemi and Sarasgani, 2004). In contrast to *E. floccosum*, *T. mentagrophytes* and *M. canis* are less susceptible to the androgenic hormones (Brasch and Flader, 1996). In the present study, testosterone levels were not significantly different between male calves with dermatophytosis and the healthy male calves but the male calves with dermatophytosis had non-significant reduction of testosterone levels compared to the male healthy calves.

## CONCLUSIONS

The present study revealed that dermatophytosis might affect oxidant status in calves with dermatophytosis and TOC and NO as oxidative stress marker might be increased for fungicidal effect in the groups with dermatophytosis. In addition, serum biochemistry parameters including thyroid and testosterone hormones with the exception of glucose and triglyceride, trace elements except for Mn and minerals were found to be in normal ranges. However, future studies with larger sample sizes are needed to be conducted for changes in testosterone levels.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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