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Research Article

Determination of Levels of Some Acute Phase Proteins, Tumor Necrosis Factor-α, Interleukin-1 and Interleukin-6 in Cattle with Trichophythosis

Cansu ÇAKIR ZOROĞLAN [1] Oğuz MERHAN[2*]

[1]*Kafkas University, Institute of Health Sciences, Kars, TÜRKİYE*

[2]*Kafkas University, Faculty of Veterinary, Department of Biochemistry, Kars, TÜRKİYE*

ABSTRACT

Our aim in the study was to determine the levels of some acute phase proteins (APP) and proinflammatory cytokines in cattle with trichophytosis. The animal material of the study consisted of total 30 cattle, 15 with trichophytosis and 15 healthy. The blood samples taken from the *Vena jugularis* of the cattle in the study into tubes without anticoagulant. In the obtained serum samples, haptoglobin, serum amyloid A (SAA), ceruloplasmin, albumin, total protein, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) analyzes were performed. It was determined that haptoglobin, SAA, TNF-α, IL-1, IL-6 and ceruloplasmin levels were increased in cattle with trichophytosis compared to the control group. In addition, albumin, total protein and globulin values were lower than the control group, but the difference between the groups was statistically insignificant. As a result, it is thought that trichophytosis causes changes in biochemical parameters in cattle, the use of biochemical parameters, especially APPs, will contribute to the diagnosis of trichophytosis.

Keywords: *Acute phase proteins, cattle, proinflammatory cytokine, trichophytosis.*

Trikofitozisli Sığırlarda Bazı Akut Faz Proteinleri, Tümör Nekrozis Faktör-α, İnterlökin-1 ve İnterlökin-6 Düzeylerinin Belirlenmesi

ÖZET

Çalışmadaki amacımız trikofitozisli sığırlarda bazı akut faz proteinler (AFP)'in ve proinflamatuar sitokin düzeylerinin belirlenmesidir. Çalışmanın hayvan materyalini 15 trikofitozisli ve 15 adet sağlıklı olmak üzere toplam 30 adet sığır oluşturdu. Çalışmada yer alan sığırların *Vena jugularis*'inden antikoagulansız tüplere alındı. Elde edilen serum örneklerinde haptoglobin, serum amiloid A (SAA), seruloplazmin, albümin, total protein, tümör nekrozis faktör-α (TNF-α), interlökin-1 (IL-1) ve interlökin-6 (IL-6) analizleri yapıldı. Trikofitozisli sığırlarda haptoglobin, SAA, TNF-α, IL-1, IL-6 ve seruloplazmin düzeylerinin kontrol grubuna göre yükseldiği belirlendi. Bunun yanı sıra albümin, total protein ve globulin değerlerinin ise kontrol grubuna göre düşmekle beraber gruplar arası fark istatistik olarak anlamsız olarak belirlendi. Sonuç olarak, trikofitozisin sığırlarda biyokimyasal parametrelerde değişime neden olduğu, biyokimyasal parametrelerden özellikle de AFP'lerin kullanımının trikofitozisin teşhisine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: *Akut faz protein, sığır, proinflamatuvar sitokin, trikofitozis.*

Correspondence: Oğuz MERHAN, Kafkas University, Faculty of Veterinary, Department of Biochemistry, Kars, TÜRKİYE.
oguzmerhan@hotmail.com
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Introduction

Trichophytosis is an enzootic and zoonotic skin disease characterized by hair loss, dandruff and keratinized crusting of the skin caused by fungi such as *Epidermophyton, Microsporium* and *Trichophyton*. It causes economic losses such as slowdown in animal growth, loss of live weight, deterioration of leather quality, ban on export of sick animals (Gökçe et al., 1999; Papini et al., 2009). *Trichophyton verrucosum* and *Trichophyton mentagrophytes* have been reported as the most important causative agents of the disease in cattle (Balıkçı and Gazioğlu, 2017). The disease generally shows symptoms in the form of itchy, painless, round, chalk dust/asbestos-like lesions in the head, neck and inguinal region of animals (Yılmazer and Aslan, 2010).

Acute phase proteins (APP) are known as proteins synthesized by the liver in inflammation, infection, tissue damage, neoplastic developments and some immunological diseases (Petersen et al., 2004; Murata et al., 2004; Gökçe and Bozukluhan, 2009). It is important because it is a good marker in the formation of inflammatory process and disease diagnosis, as well as being used in separating clinical diseases from subclinical and in the follow-up of treatment (Bozukluhan and Merhan, 2022; Merhan and Bozukluhan, 2022). The aim of the study is to determine the levels of some APP and proinflammatory cytokines in cattle with trichophytosis.

Material and Methods

The study was carried out with the approval of the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK) Ethics Committee numbered 2021/023. The animal material of the study consists of cattle of different breeds (8 montofon crosses and 22 simental crosses), both sexes (8 females and 22 males), and aged between 5-18 months, obtained from livestock farms in Kars province Digor district. A total of 30 cattle, 15 with trichophytosis and 15 healthy cattle in the control group, were formed. The diagnosis of the disease was made according to clinical and microscopic findings. The samples taken from the *Vena jugularis* of the cows in the study into tubes without anticoagulant were centrifuged at 3000 rpm for 15 minutes and their serums were obtained. Serums were stored at -20°C until analysis.

Ceruloplasmin was measured by the Colombo and Richterich (1964) chemical method, haptoglobin (Cat. No:TP-801), serum amyloid A (SAA, Cat. No:TP-802) (Tridelta Phase, Ireland) and TNF-α (Cat. No:E0019Bo), IL-1 (Cat. No:E0197Bo), IL-6 (Cat. No:E0001Bo, BT LAB, China) were measured with the ELISA kit, and albumin, total protein (Biolabo, France) was measured using a commercial test kit. Globulin value was calculated by subtracting albumin values from total protein.

Statistical Analysis: Independent Sample T-test was used to compare the groups in the statistical package for social sciences (SPSS) 20.0 package program.

Results

Haptoglobin, SAA, TNF-α, IL-1, IL-6 ($P<0.001$) and ceruloplasmin ($P<0.05$) levels were found to be statistically higher in cattle with trichophytosis compared to the control group. Although albumin, total protein and globulin values were lower than the control group, the difference between the groups was statistically insignificant ($P>0.05$) (Table 1). In the clinical examination of animals with trichophytosis, round, chalk dust/asbestos-like lesions were detected in the head, neck and inguinal region. When the samples taken from cattle were examined microscopically, it was determined that all of the factors were *Trichopyton verrucosum*.

Discussion

Trichophytosis is an enzootic and zoonotic skin disease in calves and young cattle characterized by hair loss, dandruff and keratinized crusting of the skin caused by fungi such as *Epidermophyton, Microsporium* and *Trichophyton*. It causes economic losses such as slowdown in animal growth, loss of live weight, deterioration of leather quality, ban on export of sick animals (Özkanlar

Table 1. Some acute phase proteins, tumor necrosis factor-α, interleukin-1 and interleukin-6 parameters in clinically healthy cattle with trichophytosis. Data are presented as mean±standard error (X±SEM).

Parameters	Control	Infected	P
Haptoglobin (g/L)	0.079±0.003	0.362±0.022	0.0001
Serum Amyloid A (µg/mL)	11.93±0.90	91.80±6.46	0.0001
Ceruloplasmin (mg/dL)	11.63±0.48	17.48±2.05	0.010
Albumin (g/L)	2.97±0.05	2.95±0.06	NS
Total Protein (g/L)	6.89±0.15	6.86±0.13	NS
Globulin (g/dL)	3.92±0.16	3.91±0.13	NS
TNF-α (pg/mL)	77.26±11.47	165.34±5.29	0.0001
IL-1 (pg/mL)	38.44±4.53	124.75±10.70	0.0001
IL-6 (pg/mL)	88.73±5.96	154.41±5.52	0.0001

NS: Non Significant

et al., 2009; Papini et al., 2009; Bozukluhan, 2014). As reported in the studies (Kabu and Sayın, 2016; Constable et al., 2017), in the clinical examination of animals with trichophytosis, round, chalk dust/asbestos-like lesions were detected in the head, neck and inguinal region. When the lesions were examined, it was determined that the causative agent was *Trichopyton verrucosum*.

Fungi produce different proteolytic enzymes and metabolic products, especially keratinases (Muhsin et al., 1997; Schaufuss and Steller, 2003). Following the infection, the organism reacts to the metabolic products that pass from the skin to the bloodstream (Atakisi et al., 2006; Apaydin Yildirim, 2020). As a result of this reaction, APP is produced from the liver. APP levels can change in conditions such as inflammation or infection, stress (Murata et al., 2004; Merhan and Bozukluhan, 2022).

Along with the activation of mononuclear cells such as monocytes and granulocytes in the inflammatory region, cytokines such as TNF-α, IL-1 and IL-6 are released. Cytokines are substances in peptide or glycoprotein structure that initiate and regulate inflammation as well as immunity (Ceciliani et al., 2002; Merhan and Bozukluhan, 2022). In a study conducted on buffaloes infected with *Fasciola gigantica*, it was reported that IL-6 and IL-8 levels were increased (Molina, 2005). In another study conducted in Anatolian buffaloes with trichophytosis, they reported that the cytokine level was higher than the control group (Kabu and Sayın, 2016). In the study, it was determined that the proinflammatory cytokine level was higher in cattle with trichophytosis compared to the control group. This increase may be due to the inflammatory response to trichophytosis.

Secreted proinflammatory cytokines are transported to the liver through the blood and stimulate the production of APP. Haptoglobin, SAA and ceruloplasmin are important APPs in cattle (Petersen et al., 2004; Merhan and Bozukluhan, 2022). Haptoglobin, which is found at very low levels in the serum of healthy ruminants, increases significantly in its concentration following tissue damage and inflammation (Petersen et al., 2004; Tothova et al., 2014). Haptoglobin, an important APP in cattle, has been reported to increase in diseases such as hypodermosis, pneumonia, enteritis, peritonitis, endocarditis, and traumatic reticuloperitonitis (Bozukluhan and Gökçe, 2007; Merhan et al., 2016; Merhan et al., 2017a; Bozukluhan et al., 2021).

Serum amyloid A is a positive APP used in determining the prognosis and severity of inflammation in complex with high-density lipoprotein (HDL) (Witkowska-Pilaszewicz et al., 2019; Merhan and Bozukluhan, 2022). SAA, which can increase over 1000 times in the circulation in inflammatory conditions, has functions such as preventing antibody formation by lymphocytes, inducing collagenase, and increasing leukocyte adhesion to endothelial cells (Petersen et al., 2004; Murata et al., 2004). The levels of SAA increases in bacterial (Merhan et al., 2017b), viral (Merhan et al., 2017c), and parasitic infections (Bozukluhan et al., 2017). In addition, serum levels increase in ketosis (Brodzki et al., 2021), after operations (Bozukluhan and Gökçe, 2007), and in fasting for more than 3 days (Katoh et al., 2002).

Ceruloplasmin, which is of moderate importance in cattle, has a molecular weight of about 151 kDa and a half-life of 5-7 days (Hellman and Gitlin, 2002). Ceruloplasmin, which has oxidase activity for many polyamine and polyphenol substrates, is an oxido-reductase and plays a role in the activity of ferroxidase, which is necessary for the oxidation of Fe^{+2} to Fe^{+3} (Hellman and Gitlin 2002; Merhan and Bozukluhan, 2022). In a study conducted with dogs with different diseases, they reported that APP concentration increased significantly in dogs with skin problems (Ulutaş et al., 2007). Studies on trichophytosis in cattle are limited in number, and in a study they conducted in Anatolian buffaloes infected with trichophytosis, they reported that haptoglobin and SAA levels increased statistically significant (Kabu and Sayın, 2016).

In other studies, it was reported that haptoglobin, SAA and ceruloplasmin levels increased before the treatment and decreased after the treatment when compared with the control group (Balıkçı and Gazioğlu, 2017; Şeliman, 2018). In the study, it is thought that haptoglobin, SAA and ceruloplasmin concentrations increased compared to the control group, and this increase is probably related to tissue damage and inflammation due to trichophytosis.

Biochemical changes in the blood are used in the diagnosis of many diseases. Studies have reported that trichophytosis causes changes in the blood biochemistry of animals (Atakisi et al., 2006; Arslan et al., 2007; Karapehlivan et al., 2007). Atakisi et al., (2006) reported that the liver enzymes gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), alanine amino transferase (ALT), aspartate amino transferase (AST), and adenosine deaminase activities increased in cattle with trichophytosis compared to the healthy group. Arslan et al. (2007) reported that there was no change in blood urea nitrogen (BUN), creatinine levels, AST and ALT activities in cattle with trichophytosis. In addition, Kumar and Khurana (2002) reported in another study that AST and ALT enzyme activity was not statistically significant when compared to the healthy group. In a study by Kabu and Koca (2018), they reported that serum ALT and AST activities increased, BUN and creatinine levels decreased compared to the control group in buffaloes with trichophytosis. There was no statistical difference between the groups in GGT activity, levels of total protein, albumin, and total bilirubin. In studies conducted in ruminants with trichophytosis (Karapehlivan et al., 2007; Yıldırım et al., 2010), they reported that there was no significant difference between the groups in total protein and albumin levels. In the study, it was determined that albumin, total protein and globulin levels decreased when compared to the control group, but this decrease was not statistically significant.

Conclusion

As a result, it is thought that trichophytosis causes changes in biochemical parameters in cattle, the use of biochemical parameters, especially APPs, will contribute to the diagnosis of trichophytosis and more detailed studies should be done 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.

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