

Determination of Oxidative Stress Index and Total Sialic Acid in Cattle Infested with *Hypoderma* spp.

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Abstract

Hypodermosis is a parasitic disease, which is caused by larvae of *Hypoderma lineatum* and *Hypoderma bovis* flies in cattle and causes damage on the skin. It causes economic losses due to decrease in meat and milk yield, growth deficiency and being of no use in leather processing as a result of perforation of the leather. The aim of this study was to define the oxidative-antioxidative capacity and total sialic acid levels in cattle with hypodermosis. A total of 90 cattle was used in the study, among those, 30 with hypodermosis and 60 antibody-negative healthy bovine sera were used according to ELISA test results to investigate the biochemical parameters. Biochemical analysis included total oxidant capacity (TOC), total antioxidant capacity (TAC), oxidative stress index (OSI), and total sialic acid (TSA). TAC value was found to be statistically significantly lower ($P<0.01$), TOC, OSI and TSA values were found to be higher ($P<0.05$) in cattle with hypodermosis compared to the control group. It was concluded that hypodermosis caused significant changes in sialic acid concentration and oxidative-antioxidative capacity, which are indicators of acute phase response in cattle with hypodermosis. It is thought that these obtained findings can particularly contribute to determination and diagnosis of inflammation intensity in disease.

Keywords: Cattle, Hypodermosis, Oxidative stress, Total sialic acid

Hypoderma spp. İle Enfeste Sığırlarda Oksidatif Stres İndeksi ve Total Siyalik Asit Düzeyinin Belirlenmesi

Öz

Hypodermosis, sığırlarda *Hypoderma lineatum* ve *Hypoderma bovis* tipi sineklerin larvaları tarafından oluşturulan ve deride hasara neden olan paraziter bir hastalıktır. Hayvanların et ve süt veriminde azalma, büyüme eksikliği ve derinin delinmesi sonucunda deri işlemede kullanılamaması gibi ekonomik kayıplara neden olur. Çalışmanın amacı *Hypoderma* ile enfeste sığırlarda oksidatif-antioksidatif kapasite ve total siyalik asit düzeyinin belirlenmesidir. Çalışmada, ELISA test sonuçlarına göre, 60 antikor negatif ve 30 hypodermosisli olmak üzere toplam 90 adet sığır serumu kullanıldı. Biyokimyasal olarak total oksidan kapasite (TOK), total antioksidan kapasite (TAK), oksidatif stres indeksi (OSİ) ve total siyalik asit (TSA) analizi yapıldı. Kontrol grubu ile hypodermosisli sığırlar karşılaştırıldığında istatistiksel olarak TOK, OSİ ve TSA düzeyinin arttığı ($P<0.05$), TAK düzeyinin ise azaldığı ($P<0.01$) belirlendi. Sonuç olarak, hypodermosisin akut faz yanıtın bir göstergesi olan siyalik asit konsantrasyonunda ve oksidatif-antioksidatif kapasitede önemli değişikliklere neden olduğu belirlendi. Elde edilen bu bulguların özellikle hastalıktaki inflamasyon şiddetinin belirlenmesine ve teşhisine katkıda bulunabileceği düşünülmektedir.

Anahtar sözcükler: Sığır, Hypodermosis, Oksidatif stres, Total siyalik asit

INTRODUCTION

Hypodermosis is a parasitic disease, which is created by larvae of *Hypoderma lineatum* and *Hypoderma bovis* type flies in cattle and causes damage on the skin ^[1,2]. It causes economic losses due to decrease in meat and milk yield

of animals, growth deficiency, stress-related immune depression, and being of no use in leather processing as a result of perforation of the leather ^[3,4]. Glycoproteins, glycolipids, polysaccharides, and acylated derivatives of neuraminic acid formed by condensation of sialic acid, mannosamine and phosphoenolpyruvate, are involved in



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the structure of mucoproteins [5,6]. While 85% of the sialic acid is bound to the protein part of the glycoconjugates and about 15% to the lipid, a very small amount is free. Total sialic acid (TSA) is the total of free protein and lipid-bound sialic acid [7]. Sialic acid has functions such as regulation of cellular communication, determining recognition in host-pathogen interactions [8]. It has been demonstrated that sialic acid can be an important indicator for early and accurate diagnosis, rational treatment and effective prophylactic measures in both infectious and noninfectious diseases [7,9].

While there is a balance between oxidants and antioxidants in normal organism; stress, chronic disease and infections in organism stimulate immune system and tissue damage, causing inflammatory reactions due to increase in free radical production [10].

When free radicals exceed the amount that is required for the body, they cause an oxidative damage by interacting with the molecules such as lipids, carbohydrates, proteins and nucleic acids [10,11]. The system, which functions in order to prevent the damage created by the free radicals, is defined as the antioxidant system [12]. In studies, it is reported that total oxidant capacity (TOC) and total antioxidant capacity (TAC) or oxidative stress index (OSI) change and can be used as a non-invasive marker in local and/or systemic inflammation or infections [13-16]. The aim of this study was to investigate TSA, TOC and TAC and OSI status in cattle infested by hypoderma.

MATERIAL and METHODS

The research work was carried out with the approval of the Institutional Ethics Committee of Kafkas University, Faculty of Veterinary Medicine (KAU 2014/011). 90 cattle of Brown Swiss breed, aged between 2 and 5 years, were used in the study. These animals were obtained from dairy farms in Kars, Turkey. These farms were visited during September and November of 2016. The animals in control group were received an antiparasitic drug (Ivomec F; Novakim, Turkey, 200 µg/kg/bw) to remove any presence of internal parasite before use. Routine clinical examinations (fever, number of breaths, pulse, native examination of the feces, etc.) were carried out for each animal. Blood samples obtained from *jugular veins* of animals were collected into plain tubes, centrifuged at 3000 rpm for 15 min and obtained sera were stored at -20°C until analyzed. The presence of Hypoderma antibodies in serum samples was investigated

by an ELISA kit (IDEXX Bovine Hypodermosis Antibody Test Kit-P06110). The test was performed according to manufacturer's instructions, and results were measured spectrophotometrically (Epoch, Biotek, USA). According to ELISA test results 60 hypodermosis infested and 30 antibody-negative healthy bovine sera were used to investigate the biochemical parameters.

The levels of TOC and TAC were measured using commercially available Rel Assay diagnostic kits (Gaziantep, Turkey) via microplate reader (Epoch, BioTek, USA). Briefly, hydrogen peroxide and trolox were used as standards to calculate for TOC and TAC, respectively. OSI which is the indicator of the degree of oxidative stress was calculated using (Arbitrary Unit) = [TOC (µmol H₂O₂ equivalent/L)/10xTAC (mmol Trolox equivalent/L)] formula [17]. TSA concentrations were determined according to the spectrophotometric method developed by Sydow [18] (Epoch, Biotek, USA).

Statistical Analysis

SPSS [19] for Windows 20.0 was used for the statistical analyses. The distribution of the data obtained from the groups were shown as normal distribution according to the Kolmogorov-Smirnov test. Therefore, Student's t-test was then used to compare the differences of the values observed in group with infested with those observed in control group.

RESULTS

Clinical examination showed that the general status of both control and infested animals were in good health and no parasite eggs were found in the feces of the animals.

In this study, there were significant increases in the concentrations of TOC (P<0.05), OSI (P<0.05) and TSA (P<0.05) levels in the *Hypoderma spp.* infested group compared to the control group. However, TAC (P<0.01) values were significantly lower in infested group than in the control group (Table 1).

DISCUSSION

Hypodermosis is a parasitic disease, which causes economic losses such as affect weight gain, welfare, bovine immune defense mechanisms and the leather industry [20,21]. Larval secretions of *Hypoderma spp.* are comprised of three main components (hypodermins A, B and C) with potent anti-inflammatory and immunosuppressive effects in primo-

Table 1. Total oxidant & antioxidant capacity and total sialic acid levels in control and *Hypoderma spp.* infested groups

Parameters	Control	Infested	P
TAC (mmol Trolox Eq/L)	1.18±0.08	0.92±0.05	P<0.01
TOC (µmol H ₂ O ₂ Eq/L)	29.64±1.46	38.02±2.82	P<0.05
OSI (Arbitrary Unit)	3.03±0.33	4.89±0.52	P<0.05
TSA (mg/dL)	64.65±2.00	70.94±1.61	P<0.05

infested cattle. In addition, the migrating fly larvae trigger serious allergic reactions and inflammation in cattle. Acute phase response occurs depending on cellular and humoral response during migration of the parasite larva in tissues of animals [22-26] and concentration of sialic acid of which function is found in acute phase response increases depending on the damage occurred in cell membrane during infestation [27]. TSA values increase considerably in infectious, tumoral and metabolic diseases [9]. It has been reported that serum TSA value increases in some diseases such as theileriosis, anaplasmosis [28], echinococcosis [9], foot and mouth disease [29], and leptospirosis [30]. In this study, it was also specified that TSA value increased considerably in cattle with hypodermosis than healthy animals. Increased TSA value in cattle with hypodermosis can be explained by tissue damage which was caused during the migrating fly larva.

Although there are many methods for determining oxidative stress, these methods are complex and expensive methods that require long time and effort, and measuring the oxidant/antioxidant molecules individually allows only the evaluation of the molecule being measured. Therefore, it has been reported that TAC and/or TOC measurement is more beneficial rather than measuring oxidants or antioxidant individually in order to specify oxidant/antioxidant balance [31,32].

It has been reported that oxidative stress develops in viral, bacterial and parasitic diseases such as foot and mouth disease [29], brucellosis [15], ascaridiosis [33] and sheep-poxvirus infection [34]. It has been stated that parasitic infections cause damage in cells and tissues depending on increase of the free radicals in host cells [35]. The studies have reported in cattle infested with hypoderma, it has been stated that oxidative stress develops [36,37]. In this study, it was also specified that TOC value increased and TAC value decreased in infested cattle in which oxidative stress developed in parallel to the study conducted. The reason of increase in TOC can be explained by the fact that parasitic infections cause free radical related damage in cells and tissues of the host [35]. It was also specified in the study that OSI value increased considerably compared to the control group. OSI, which is defined as the ratio of TOC value to TAC value, is an indicator of oxidative stress value and OSI value reflects oxidative condition in animals infected with hypodermosis.

In conclusion, significant changes in sialic acid concentration and oxidative-antioxidative capacity, which are an indicator of acute phase response, occur in cattle with hypodermosis. It is thought that these obtained findings can particularly contribute to determination and diagnosis of inflammation intensity in disease.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration between all

authors. KB, OM and GTT: Designed the experimental procedures. KB, OM and NA: Conducted the research work. OM, NA: Helped in laboratory analysis. OM, GGT, KB: Prepared tables, revised and submitted the manuscript. All authors read and approved the final manuscript.

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