Kafkas Univ Vet Fak Derg
x (x): xxx-xxx, 2020
DOI: 10.9775/kvfd.2020.24071

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Article in Press

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

Oğuz MERHAN [1,a] Gencay Taşkın TAŞÇI [2,b] Kadir BOZUKLUHAN [3,c] Nilgün AYDIN [2,d]

[1] University of Kafkas, Faculty of Veterinary Medicine, Department of Biochemistry, TR-36100 Kars - TURKEY
[2] University of Kafkas, Faculty of Veterinary Medicine, Department of Parasitology, TR-36100 Kars - TURKEY
[3] University of Kafkas, Kars School of Higher Vocational Education, TR-36100 Kars - TURKEY
ORCIDS: [a] 0000-0002-3399-0667; [b] 0000-0002-8590-1101; [c] 0000-0003-4929-5156; [d] 0000-0002-0571-7882

Article ID: KVFD-2020-24071 Received: 13.02.2020 Accepted: 20.06.2020 Published Online: 28.06.2020

How to Cite This Article

Merhan O, Taşçı GT, Bozukluhan K, Aydın N: Determination of oxidative stress index and total sialic acid in cattle infested with *Hypoderma* spp.. *Kafkas Univ Vet Fak Derg,* 2020 *(Article in Press).* DOI: 10.9775/kvfd.2020.24071

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

Correspondence
+90 474 2426836/5145
oguzmerhan@hotmail.com

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 H_2O_2 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 infected 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_2O_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 larva 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 individiually in order to specify oxidant/antioxidant balance [31,32].

It has been reported that oxidative stress developes 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 developes [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 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 GGT: 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.

REFERENCES

1. Özkutlu Z, Sevgili M: Seroprevalence of hypodermatosis in cattle in the province of Sanliurfa (Turkey). *Acta Parasitol Turcica*, 29, 275-279, 2005.

2. Constable PD, Hinchcliff KW, Done SH, Grünberg W: Diseases of the skin, eye, conjunctiva, and external ear. **In,** Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. 11th ed., 1626-1628, Elsevier, China, 2017.

3. Boulard C: Durably controlling bovine hypodermosis. *Vet Res*, 33, 455-464, 2002. DOI: 10.1051/vetres:2002032

4. Hassan M, Khan MN, Abubakar M, Waheed HM, Iqbal Z, Hussain M: Bovine hypodermosis-a global aspect. *Trop Anim Health Prod*, 42, 1615-1625, 2010. DOI: 10.1007/s11250-010-9634-y

5. Schauer R, Kamerling JP: Exploration of the sialic acid world. *Adv Carbohydr Chem Biochem*, 75, 1-213, 2018. DOI: 10.1016/bs.accb.2018.09.001

6. Traving C, Schauer R: Structure, function and metabolism of sialic acids. *Cell Mol Life Sci*, 54, 1330-1349, 1998. DOI: 10.1007/s000180050258

7. Merhan O, Özcan A: Investigation of serum ceruloplasmin and total sialic acid levels in geese. *Kafkas Univ Vet Fak Derg*, 10, 139-142, 2004.

8. Pearce OMT, Laubli H: Sialic acids in cancer biology and immunity. *Glycobiology*, 26, 111-128, 2016. DOI: 10.1093/glycob/cwv097

9. Yarım GF, Umur Ş, Açıcı M, Beyhan YE: Serum sialic acid levels in cattles with cystic echinococcosis. *Ankara Univ Vet Fak Derg*, 57, 61-63, 2010. DOI: 10.1501/Vetfak_0000002311

10. Karabulut H, Gülay MŞ: Serbest radikaller. *MAKU Sag Bil Enst Derg*, 4, 50-59, 2016.

11. Tabakoğlu E, Durgut R: Veteriner hekimlikte oksidatif stres ve bazı önemli hastalıklarda oksidatif stresin etkileri. *AVKAE Derg*, 3, 69-75, 2013.

12. Süleyman H, Gül V, Erhan E: Oksidatif stres ve doku hasarı. *Erzincan Tıp Derg*, 1, 1-4, 2018.

13. Bozukluhan K, Atakisi E, Atakisi O: Nitric oxide levels, total anti-oxidant and oxidant and capacity in cattle with foot-and-mouth-disease. *Kafkas Univ Vet Fak Derg*, 19, 179-181, 2013. DOI: 10.9775/kvfd.2012.7244

14. Celi P, Gabai G: Oxidant/antioxidant balance in animal nutrition and health: The role of protein oxidation. *Front Vet Sci*, 2, 1-13, 2015. DOI: 10.3389/fvets.2015.00048

15. Merhan O, Bozukluhan K, Kuru M, Büyük F, Özden Ö, Kükürt A: Investigation of oxidative stress index and lipid profile in cattle with Brucellosis. *Kafkas Univ Vet Fak Derg*, 23, 933-937, 2017. DOI: 10.9775/kvfd.2017.18004

16. Aydoğdu U, Coşkun A, Başbuğ O, Ağaoğlu ZT: Evaluation of total oxidant-antioxidant status and oxidative stress index in dogs with parvoviral enteritis. *F Ü Sağ Bil Vet Derg,* 32, 161-164, 2018.

17. Karababa F, Yesilova Y, Turan E, Selek S, Altun H, Selek S: Impact of depressive symptoms on oxidative stress in patients with psoriasis. *Redox Rep*, 18, 51-55, 2013. DOI: 10.1179/1351000213Y.0000000039

18. Sydow G: A simplifield quick method for determination of sialic acid in serum. *Biomed Biochim Acta*, 44, 1721-1723, 1985.

19. SPSS: IBM SPSS Statistics for Windows, Version 20.0. Armonk, New York, 2011.

20. Chabaudie N, Villejoubert C, Boulard C: The response of cattle vaccinated with Hypodermin A to a natural infestation of *Hypoderma bovis* and *Hypoderma lineatum*. *Int J Parasitol*, 21, 859-862, 1991. DOI: 10.1016/0020-7519(91)90155-Z

21. Taşcı S, Değer S, Akgül Y: Hypodermosis in Van and around. *Y Y Ü Vet Fak Derg*, 5, 143-153, 1994.

22. Nicolas-Gaulard I, Moire N, İnra **CB:** Effect of the parasite enzyme, hypodermin A, on bovine lymphocyte proliferation and interleukin-2 production via the prostaglandin pathway. *Immunology*, 84, 160-165, 1995.

23. Colwell DD: Stage specific mortality and humoral immune responses during pulse and trickle infestations of the common cattle grub, *Hypoderma lineatum* (Diptera: Oestridae). *Vet Parasitol*, 99, 231-239, 2001. DOI: 10.1016/s0304-4017(01)00459-9

24. Lopez C, Colwell DD, Panadero R, Paz A, Perez J, Morrondo P, Diez P, Cascallana JL, Santamaria V, Bravo A: Skin immune responses in cattle after primary and secondary infections with *Hypoderma lineatum* (Diptera: Oestridae) larvae. *Vet Immunol Immunopathol*, 108, 285-294, 2005. DOI: 10.1016/j.vetimm.2005.06.001

25. Vazquez L, Dacal V, Lopez C, Diaz P, Morrondo P, Diez-Banos P, Panadero R: Antigen-specific antibody isotypes, lymphocyte subsets and cytokine profiles in cattle naturally infested by *Hypoderma* sp. (Diptera: Oestridae). *Vet Parasitol*, 184, 230-237, 2012. DOI: 10.1016/j.vetpar.2011.09.013

26. Panadero R, Lopez C, Vazquez L, Diaz P, Perez A, Cabanelas E, Morrondo P, Diez-Banos P: Effect of reinfestations on systemic immune responses in cattle naturally infested by *Hypoderma* sp. (Diptera: Oestridae). *Vet Parasitol*, 193, 238-244, 2013. DOI: 10.1016/j.vetpar.2012.11.017

27. Eguchi H, Ikeda Y, Ookawara T, Koyota S, Fujiwara N, Honke K, Wang PG, Taniguchi N, Suzuki K: Modification of oligosaccharides by reactive oxygen species decreases sialyl lewis x-mediated cell adhesion. *Glycobiology*, 15, 1094-1101, 2005. DOI: 10.1093/glycob/cwj003

28. Guzel M, Kontas Askar T, Kaya G, Atakişi E, Erbil Avcı G: Serum sialic acids, total antioxidant capacity, and adenosine deaminase activity in cattle with theileriosis and anaplasmosis. *Bull Vet Inst Pulawy*, 52, 227-230, 2008.

29. Deveci HA, Kükürt A, Nur G, Alpay M, Merhan O, Bozukluhan K, Yilmaz V, Karapehlivan M: Serum paraoxonase activity and total sialic acid in sheep with foot and mouth disease. *Med Weter*, 74, 199-202, 2018. DOI: 10.21521/mw.6078

30. Keleş İ, Ertekin A, Karaca M, Ekin S, Akkan HA: Studies on serum sialic acid and lipid bound sialic acid in cattle with leptospirosis. *YYÜ Vet Fak Derg*, 11, 121-122, 2000.

31. Erel O: A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem*, 37, 277-285, 2004. DOI: 10.1016/j.clinbiochem.2003.11.015

32. Erel O: A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*, 38, 1103-1111, 2005. DOI: 10.1016/j.clinbiochem.2005.08.008

33. Bozukluhan K, Merhan O, Özcan A, Gökçe Hİ, Gökçe G: Investigation of the levels of serum haptoglobin, oxidative indicators and some biochemical parameters in calves naturally infected with *Toxocara vitulorum*. *Ankara Univ Vet Fak Derg*, 64, 75-79, 2017. DOI: 10.1501/vetfak_0000002778

34. Bozukluhan K, Merhan O, Gökçe Hİ, Öğün M, Atakişi E, Kızıltepe Ş, Gökçe G: Determination of some acute phase proteins, biochemical parameters and oxidative stress in sheep with naturally infected sheeppox virus. *Kafkas Univ Vet Fak Derg*, 24, 437-441, 2018. DOI: 10.9775/kvfd.2017.19167

35. Değer S, Değer Y, Ertekin A, Gül A, Biçek K, Özdal **N:** Determination of the status of lipid peroxidation and antioxidants in cattle infected with Dictyocaulus viviparus. *Turkiye Parazitol Derg*, 32, 234-237, 2008.

36. Ozkurt-Borazan G, Aktas MS, Camkerten I, Gokçen A, Ipek H, Sahin T, Uren Paksoy N: Erythrocyte superoxide dismutase, catalase activity and malondialdehit level in hypodermosis. *J Anim Vet Adv*, 10, 84-86, 2011.

37. Merhan O, Bozukluhan K, Gokce HI: Acute phase proteins and biochemical and oxidative stress parameters in *Hypoderma* spp. infested cattle. *J Hellenic Vet Med Soc*, 68, 535-540, 2017. DOI: 10.12681/jhvms.16049