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O. MERHAN, K. BOZUKLUHAN, H. I. GOKCE

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## ■ Acute phase proteins and biochemical and oxidative stress parameters in *Hypoderma spp.* infested cattle

Merhan O.<sup>1\*</sup>, Bozukluhan K.<sup>2</sup>, Gokce H.I.<sup>3</sup>

<sup>1</sup> Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey.

<sup>2</sup> Kars School of Higher Vocational Education, University of Kafkas, Kars, Turkey.

<sup>3</sup> Department of Internal Medicine, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Burdur, Turkey.

**ABSTRACT.** The aim of the present study was to determine concentrations of acute phase proteins (APP), oxidative stress and some biochemical parameters in naturally infested cattle with *Hypoderma spp.* For this purpose, 10 clinically healthy cattle as controls and 25 Brown Swiss cattle with *Hypoderma spp.* were used. Blood samples were collected to tubes from jugular vein. Parts of blood samples were stored without any process as a whole blood. The serum was separated from the remaining blood samples. The reduced glutathione (GSH) in whole blood and the level of malondialdehyde (MDA), haptoglobin (Hp), ceruloplasmin, serum amyloid A (SAA), AST, GGT, ALP, CK, albumin, urea and total protein levels in serum were colorimetrically determined. The present study indicated that the concentrations of Hp, SAA, ceruloplasmin, AST, GGT, ALP, CK, and MDA were significantly increased, and albumin, total protein, GSH concentrations were significantly decreased in the *Hypoderma spp.* infested group compared to the control group. Additionally, the increase in serum Hp levels was proportional to the number of *Hypoderma spp.* and it was statistically significant. In conclusion, the production of APP increased in a response to acute phase response in animals with subcutaneous warbles. Furthermore, liver functions were also shown to be impaired and oxidative stress developed as a result of metabolic products of the parasite in *Hypoderma spp.* infested cattle.

**Keywords:** acute phase proteins, biochemical parameters, cattle, *Hypoderma spp.*, oxidative stress

*Corresponding Author:*

Oguz Merhan, Department of Biochemistry, Faculty of Veterinary Medicine,  
University of Kafkas, Kars, Turkey.  
E-mail: oguzmerhan@hotmail.com

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## INTRODUCTION

Cattle hypodermosis (warble flies infestation) is a subcutaneous skin myiasis caused by larvae of *Hypoderma* species. The disease in cattle is caused by *Hypoderma* (*H.*) *bovis* and *H. lineatum* throughout the world. The adults of the yellowish black colored hypoderma fly species lay their eggs on the hair of cattle, particularly on the legs and lower body regions. The larvae originating from the eggs penetrate into the skin of the animal at their 1st stage, and then move into the spinal column and after that settle under the skin of animal's back. Larvae in the 2nd stage drill a hole in the skin to facilitate their respiration, and one week later, 3rd-stage larvae is formed and taken into a capsule, as a result of a reaction by the host. Larvae are located under the skin of dorsal and lumbar regions of the animal as swollen structures, and then they mature. The mature larvae drop away from the swollen structures into the dark corners of the soil to form pupae, and ultimately they turn into adult flies (Özcel 2013, Özkutlu and Sevgili, 2005).

The disease is highly common in tropical and subtropical countries. It damages the internal organs and skin, and impairs the immune system of the host. *Hypoderma spp.* flies also interrupt feeding and cause stress in cattle. Thus, the disease causes huge economical losses due to the damage in skin, and reduction in milk and meat production in animals (Boullard et al., 2002; Özcel 2013).

Acute phase response (APR) is a nonspecific reaction develops associated with inflammation, tissue damage, infection, neoplastic growth, and immunological disturbances (Murata et al., 2004; Eckersall and Bell, 2010). Acute phase proteins (APP) are synthesized during the APR by the liver and their synthesis and secretions are regulated by inflammation mediators (cytokines; interleukin 1 (IL-1), interleukin 6 (IL-6) and  $\alpha$ -tumor necrosis factor ( $\alpha$ -TNF), glucocorticoids, etc.) (Whicher et al., 1991; Gruys et al., 2005). Blood concentration of APP is variable according to the animal species. Haptoglobin (Hp) and serum amyloid A (SAA) have been known to have diagnostic value for cattle and sheep (Petersen et al., 2004; Tothova et al., 2014). Studies have been showed that APP can be used in distinguishing bacterial and viral infections, differential diagnosis of clinic and subclinic diseases, determining the prognosis of sick

animals (Toussaint et al., 1995; Petersen et al., 2004).

Oxidative stress develops in association with the distribution of balance between oxidants and antioxidants. If this balance shifts in a favor of oxidants, oxidative stress may develop (Halliwell 1994; Fang et al., 2002). Oxidative stress is very important in the pathogenesis of many diseases, such as aging process, cardiovascular diseases, infertility, muscle, and liver diseases. Free radicals formed during normal cell metabolism cause oxidative damage by affecting molecules, such as lipids, carbohydrates, proteins, and nucleic acids (Gutteridge 1993; Celi and Gabai, 2015). Free oxygen radicals causing peroxidation of unsaturated fatty acids in cell wall cause the formation of new radicals by affecting fatty acids while taking released hydrogen atoms and turn into lipid peroxides. Malondialdehyde (MDA), the end product of lipid peroxidation, impairs ion transport and enzyme activities, and damages cell membrane. Therefore, MDA is used to determine the severity of cell damage. On the other hand, antioxidant defense system (reduced glutathione 'GSH') prevents damages caused by free radicals and/or lipid peroxidation (Percival 1998; Değer et al., 2008).

There is only one reported research study (Özkurt-Borazan et al., 2011) on oxidative stress in cattle infested with *Hypoderma spp.* while there is no published report on APPs in cattle infested with *Hypoderma spp.* with our best knowledge. Oxidative stress and APP in cattle infested with *Hypoderma spp.* have not been well-studied and need to be further investigated. Therefore, the objective of this study was to investigate whether *Hypoderma spp.* induce the production of APP and development of oxidative stress in naturally infested cattle. Furthermore, the alterations in some biochemical parameters were also investigated in cattle infested with *Hypoderma spp.*

## MATERIALS AND METHODS

35 cattle of Brown Swiss breed, aged between 2 and 4 years, were used in the study. These animals were obtained from dairy farms in Kars, Turkey and its vicinity. These farms were visited during the March and May and the animals were examined for the presence of subcutaneous warbles in the dorsal and lumbar regions of them. Subcutaneous

warbles (8 to 15 in each cattle) were diagnosed in 25 animals, and these animals were then used in the study. The remaining 10 clinically healthy ones were obtained from a dairy farm in Kars, Turkey, and used as a control group. 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 (number of breaths, pulse, fever, native examination of the feces, etc.) were carried out for each animal and blood samples were collected. The research work was carried out with the approval of the Institutional Ethics committee of Kafkas University, Faculty of Veterinary Medicine (KAU 2016/76).

Peripheral blood samples were collected in dipotassium ethylenediamine tetra acetic acid (EDTA)-coated evacuated tubes and tubes without anticoagulant. Blood samples collected in tubes without anticoagulant were used to separate serum samples. These samples were kept at -200C until used. Whole blood samples with anticoagulant were used to determine GSH concentrations, whereas serum samples were used to measure the concentrations of MDA, Hp, ceruloplasmin, SAA, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatine kinase (CK), albumin, urea and total protein.

Ceruloplasmin concentrations were determined according to the spectrophotometric method developed by Colombo and Rictorich (1964), while serum Hp concentrations were determined by methods of Skinner et al. (1991) (UV-1201, Shimadzu, Japan). GSH and MDA concentrations were measured using spectrophotometric methods as described by Beutler et al. (1963), and Yoshioka et al. (1979), respectively (UV-1201, Shimadzu, Japan). SAA (Tridelta development limited, Ireland), AST, GGT, ALP, CK, total protein, albumin, and urea concentrations were determined using colorimetric commercial test kits (DDS, Turkey) according to the manufacturer's instructions (Epoch, Biotek, USA). In order to determine the associated with the number of parasites and the levels of Hp, infestation was assessed as "mild/moderate" in animals with less than 10 subcutaneous warbles; and was assessed as "severe" when there were more than 10 subcutaneous

warbles.

SPSS (2011) 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 subcutaneous warbles with those observed in control group. The levels of significance accepted were  $P < 0.05$  and  $P < 0.01$  as stated below. A simple statistical analysis was performed to determine the associate with between the number of parasites and the levels of Hp.

## 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. It was determined that the majority of the larvae in infested animals with hypoderma were in the 2nd stage, and fewer of them were in the 3rd stage.

In this study, there were significant increases in the concentrations of Hp, SAA, ceruloplasmin, AST, GGT, ALP, CK and MDA ( $P < 0.01$ ) in the *Hypoderma spp.* infested group compared to the control group. There were no significant differences in the serum urea levels in the infested group compared to the control group. However, albumin ( $P < 0.01$ ), total protein ( $P < 0.05$ ) and GSH ( $P < 0.01$ ) values were significantly lower in infested group than in the control group (Table 1). The increase in serum Hp levels was in proportion to the number of parasites and it was statistically significant ( $P < 0.01$ ).

## DISCUSSION

Larval secretions of *Hypoderma spp.* are comprised of three main components (hypodermins A, B and C) with potent anti-inflammatory and immunosuppressive effects in primoinfested cattle. Those immunosuppressive effects of Hypoderma antigens are well documented in vitro (Chabaudie and Boulard, 1992; Moire 1998; Panadero et al., 2009), to the extent that they are being assayed as candidates to the inhibition of hyperacute rejection in human xenotransplantation (Malassagne et al., 2003; Chen et al., 2014; Hu et al.,

**Table 1.** Acute phase proteins, biochemical and oxidative stress parameters in control and *Hypoderma spp.* infested groups. Data are presented as mean±standard error (X±SEM).

Parameters	Control	Infested
Hp (g/L)	0.062±0.003	0.334±0.010**
SAA (µg/mL)	16.47±0.91	115.39±4.02**
Ceruloplasmin (mg/dL)	12.78±0.70	26.04±1.04**
Albumin (g/L)	3.32±0.92	2.49±0.86**
AST (U/L)	68.21±1.53	87.54±2.40**
GGT (U/L)	34.36±3.31	76.65±3.66**
ALP (U/L)	74.14±4.35	93.57±4.02**
CK (U/L)	157.49±17.06	441.76±30.44**
Urea (mmol/L)	7.24±0.21	7.94±0.27 <sup>NS</sup>
Total protein (g/L)	69.51±2.47	62.38±2.17*
MDA (nmol/mL)	1.86±0.08	3.43±0.12**
GSH (mg/dL)	73.28±1.69	48.08±1.43**

Significant differences in values between infested and control groups are indicated by asteriks:

\* $P < 0.05$ ; \*\* $P < 0.01$ .; NS: Non significant

2015). The larvae induce hypodermosis affect weight gain, welfare, bovine immune defense mechanisms and the leather industry (Chabaudie et al., 1991; Taşçı et al., 1994). In addition, the migrating fly larvae trigger serious allergic reactions and inflammation in cattle. APP, nonspecific markers of inflammation, are produced by the liver. The migrations of larvae induce APR and trigger the synthesis of APP in associated with the development of humoral and cellular immune responses in infested cattle (Nicolas-Gaulard et al., 1995; Colwell 2001; Lopez et al., 2005; Vazquez et al., 2012; Panadero et al., 2013). The blood concentrations of some of APP increase, so called positive APP, while some of them decrease which is called negative APP during APR (Petersen et al., 2004; Gruys et al., 2005). In the present study, a significant increase was observed in the blood concentrations of Hp, SAA and ceruloplasmin whereas a significant decrease was detected in albumin concentrations compared to those of healthy animals.

It is well-known that blood concentrations of Hp and SAA are very low in healthy animals and their concentrations increase during the inflammation (Gruys et al., 1994; Petersen et al., 2004). Blood Hp and SAA concentrations have been shown to increase

in bacterial (Skinner et al., 1991; Horadagoda et al., 1994), viral (Höfner et al., 1994; Ganheim et al., 2003) and parasitic (Wells et al., 2013) diseases in animals. Increases in Hp and SAA concentrations determined in this study were most probably due to stress, tissue damage or granulomatose reaction on the skin caused by the migrated larvae. In addition, Hp concentrations of 0.2-0.4 g/L were defined as mild and 1-2 g/L as severe infection (Skinner et al., 1991). As Hp levels were 0.334 g/L (approximately 0.3 g/L) in this study, the infection was determined to be mild. Furthermore, in this study, was found to associate with the Hp level and the number of parasites.

Ceruloplasmin in association with copper is used to evaluate animal health and it increases phagocytic and antimicrobial capacity of some blood cells (Cerone et al., 2000; Tothova et al., 2014). It is thought that, increased ceruloplasmin levels obtained in the present study might be due to effects of the larvae on the immune system.

It has been reported that the blood concentrations of albumin, a negative APP, is affected by impaired liver function, reduced intestinal absorption, and starvation (Gruys et al., 2005; Russell and Roussel 2007;



Kaneko et al., 2008). In the present study, blood concentrations of albumin were found to decrease in animals infested with *Hypoderma spp.* The decrease in albumin concentrations was most probably occurred due to APR and/or damaged liver as indicated by the increased liver enzymes obtained in this study. APR is a very fast response, developing with increased concentrations of APP within a few hours, which remain elevated as long as the inflammatory stimulus persists. In this study, we investigated the last period of the infection (subcutaneous stage); and the dynamics of the parameters along the life cycle of the parasite should be further analyzed in the future.

Liver enzymes such as AST and GGT are used to detect liver parenchymal damages, while ALP is used to determine cholestasis. Furthermore, CK is used to detect muscle damages (Kaneko et al., 2008). In the present study, blood concentrations of AST, GGT, and ALP significantly increased in *Hypoderma spp.* infested cattle. Increases in AST and GGT indicate presence of cellular damage in liver while increase in ALP shows damage to the bile ducts and/or cholestasis in these animals. Furthermore, increase in CK obtained in the present study also shows the presence of muscle damages has been reported previously in *Hypoderma spp.* infested animals (Boullard 2002; Özcel 2013). Decrease in blood total protein concentrations obtained in this study might be related to decreased albumin concentrations and increased loss of protein due to serine proteinase (hypodermin) produced by the larvae (Dacal et al., 2009). In our study, we found that liver biomarkers showed cellular

damage in the liver and also in the bile ducts and/or cholestasis, but how hypodermins could be capable to provoke such damages will be investigated in our laboratories in a molecular level.

Parasitic infestations cause cellular and tissue damages due to increase in free radicals (Değer et al., 2008). Ozkurt-Borazan et al. (2011) reported that concentrations of MDA increased whereas superoxide dismutase and catalase concentrations decreased in *Hypoderma spp.* infested cattle. In the present study, MDA concentrations increased while GSH concentrations decreased in *Hypoderma spp.* infested cattle which are in line with the findings by Ozkurt-Borazan et al. (2011). These changes are most probably related to the presence of cellular and tissue damages caused by increased free radicals reported in parasitic infestations (Değer et al., 2008).

## CONCLUDING REMARKS

Production of APP increased in a response to APR in animals with subcutaneous warbles. Moreover, the levels of Hp associated with the number of parasites. Furthermore, liver functions also shown to be impaired and oxidative stress developed in a result of metabolic products of the parasite in *Hypoderma spp.* infested cattle.

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest. ■

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