

## Serum concentration of haptoglobin in European mouflon (*Ovis musimon* L.) from a game reserve

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### Abstract

Acute phase proteins are used as markers of inflammation and sub-clinical disease, and are considered potential biomarkers for animal health and welfare. The objective of this study was to analyse haptoglobin as the main positive acute phase protein in the mouflon. A total of 30 clinically healthy mouflon of different age and sex, reared in a game reserve, were used for this study. Haptoglobin was determined in blood serum by colorimetric assay with a commercially accessible kit. The mean value of haptoglobin in the herd was  $0.215 \pm 0.068$  mg/ml. There was no significant difference between animals divided by sex: male  $0.228 \pm 0.073$  mg/ml, female  $0.202 \pm 0.062$  mg/ml. The mean value in young animals was higher than the mean value in the adults  $0.260 \pm 0.081$  mg/ml and  $0.201 \pm 0.059$  mg/ml, respectively ( $P < 0.05$ ). This work was designed to identify the baseline concentration of haptoglobin in a clinically healthy population, thus allowing the comparison of welfare and general health status of the herds or different structured groups within the game reserve. This is the first similar study of haptoglobin as a biomarker in the mouflon.

*Biomarker; herd; welfare; wildlife*

Haptoglobin (Hp) belongs to the acute phase proteins (APP). Acute phase proteins are blood proteins that can be used to assess the innate immune system's systemic response to infection, inflammation or trauma (Murata et al. 2004; Petersen et al. 2004; Ceron et al. 2005). Their main functions include immunomodulation and scavenging activities, they complement activation, opsonization, tissue protection, and regulation of cell proliferation. By definition, these proteins change their serum concentrations by  $> 25\%$  in response to pro-inflammatory cytokines stimulated during the disease process (Eckersall and Bell 2010). Under the influence of interleukin (IL), i.e., IL-1, IL-2, and tumour necrosis factor-alpha (TNF- $\alpha$ ), hepatocytes synthesize and secrete APP (Jain et al. 2011). Pro-inflammatory cytokines also induce local and systemic reactions. Systemic reaction results in activation of the hypothalamus, reduction in growth hormone secretion, and a number of other physiological changes characterised by fever, anorexia, and catabolism of muscle cells. Disease can cause substantial costs for the game farmer and results in serious problems which is an important consideration given the rising concern about animal welfare and food safety. Biochemical indicators can be used as a tool for the surveillance of game herd health status (Žele and Vengušt 2012) or venison quality (Dominik et al. 2013) could be made more precise and valuable with the use of the APP profile.

Research in recent years has shown APP as biomarkers which could be used for general assessment of the health status and welfare of animals, so the aim of this study was to identify baseline concentration of Hp as a part of the APP profile in a clinically healthy population of mouflon.

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## Materials and Methods

The mouflon used in the research came from a game reserve in the eastern part of the Slovak Republic. Samples were collected in winter when the herd was captured for a routine veterinary inspection. The animals showed no signs of disease. Blood was taken from the jugular vein, put into serum gel separator tubes (GEL-ACT, 10 ml, Meus s. r. l., Italy), and immediately centrifuged. Blood serum was stored at  $-20^{\circ}\text{C}$  until analysed. Faecal samples were obtained from the rectum; the sampling was performed at the same time. Samples were divided by sex into males ( $n = 15$ ), females ( $n = 15$ ) and also by age into young mouflon - under one year old ( $n = 7$ ), and adult mouflon - aged over one year ( $n = 23$ ).

The value of haptoglobin in the samples was estimated by colorimetric method with the use of a commercially available kit (Tridelta PHASE Haptoglobin Assay kit, Tridelta Development Ltd, Ireland). Analysis was based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH (Eckersall 2006). The optical density was read on an automatic microplate reader (Opsys MR, Dynex Technologies, USA) at 630 nm. Coprological examination was performed by the flotation method.

Results were statistically processed using standard models for counting the mean value, middle value and standard deviation with the GraphPad software (GraphPad Prism 6, GraphPad Software, Inc.). The differences presented between groups of animals were found using unpaired Student's *t*-test. A *P* value of  $< 0.05$  was considered significant.

## Results

The serum concentration of acute phase proteins was determined in 30 mouflon. The health status of all mouflon used in this study was based on clinical observation and deemed to be good without any sign of disease. Coprological examination did not show presence of parasite infestation. The mean value of Hp for the herd was  $0.215 \pm 0.068$  mg/ml. The mean values of samples divided by sex were  $0.228 \pm 0.073$  mg/ml in males, and  $0.202 \pm 0.062$  mg/ml in females. The samples divided by age into young and adult differed significantly,  $0.260 \pm 0.081$  mg/ml and  $0.201 \pm 0.059$  mg/ml, respectively ( $P < 0.05$ ), as shown in Fig. 1.

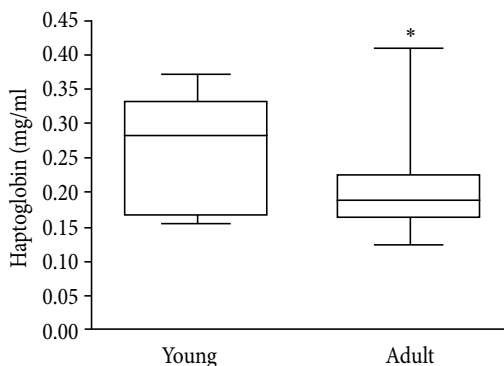


Fig. 1. The mean value of haptoglobin concentration with respect to age. The plot shows median (line within box), 25<sup>th</sup> and 75<sup>th</sup> percentiles (box), 5<sup>th</sup> and 95<sup>th</sup> percentiles (whiskers). Asterisk (\*) marks a significant decrease of haptoglobin expression in adults compared to the young ( $*P < 0.05$ ).

## Discussion

There have not been as many studies on APP in wild ruminants as in small ruminants or cattle. There has been research into Alpine ibex (Rahman et al. 2010), reindeer (Orro et al. 2004), impala and musk ox (Bertelsen et al. 2009) indicating that a similar response

occurs. In goats and sheep, Hp is considered a major APP with an increase that can be up to 80 fold in inflammatory conditions, with its value almost negligible in animals (Gonzales et al. 2008; Lephherd et al. 2009). In wild ruminants, the primary structures of the main APP and their changes during cases of sarcoptic mange have been studied in Alpine ibex as mentioned above. In this species, serum amyloid A (SAA) and alpha 1-acid glycoprotein (AGP) acted as major APP, whereas Hp and ceruloplasmin (Cp) acted as moderate APP. It has been suggested that APP could be used for the identification of subclinically infected animals and thus reduce the possibility that these animals are introduced into healthy populations (Rahman et al. 2010). This study creates a possible reference range of Hp concentration in mouflon. The value of  $0.215 \pm 0.068$  mg/ml for the herd as a whole is slightly higher than the widely accepted Hp concentration in healthy ruminants which is 0.2 mg/ml (Eckersall and Bell 2010). The reason for this may be species singularity or stress which can elevate plasma IL-6 and APP concentrations (Nukina et al. 2001), and certainly acts on the animals during capture. Also it should be noted that the Hp concentration was significantly higher in the young compared to the adults. The reason for the higher values seen in the younger animals in the herd may be due to physiologically higher values in lambs or weaker immune systems in the young, and therefore a possibility of the presence of certain percentage of animals with ongoing subclinical health problems. As this was the first study in a mouflon herd, no suitable studies were found for relevant comparison. Based on the results obtained from the research of Hp in small ruminants (Kostro et al. 2009; Meling et al. 2012), we can deliberate that Hp could be the APP that best reflects pathological states; however, in order to obtain more complete and valuable information it is advisable to perform APP profiles including another APP, such as SAA or AGP.

In order to assess herd health, it is first necessary to establish a reference baseline concentration of the selected indicators. Acute phase proteins show high sensitivity which justifies their role as biomarkers in the field of veterinary medicine. If less expensive analytical methods become available, the calculation of mean acute phase response scores could be a useful tool for the evaluation of herd health in zoos and game reserves.

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