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Age-dependent changes in essential elements and oxidative stress biomarkers in blood of red deer and vulnerability to nutritional deficiencies

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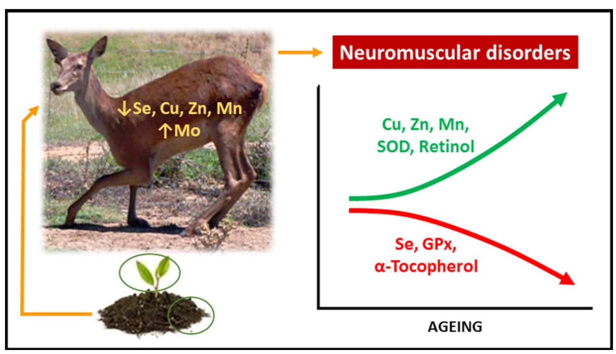
HIGHLIGHTS

- Age-related changes in trace metals and antioxidants are studied in red deer.
- Deer in their first year of life were especially vulnerable to suffer Cu and Se deficiencies.
- Aging reduced blood Zn, Mn and SOD levels in female deer.
- Blood GPx increase in older deer may counteract the decline of other antioxidant mechanisms.
- GPx and SOD may be useful tools for the diagnosis of trace element disorders in ungulates.

GRAPHICAL ABSTRACT



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ABSTRACT

Changes in the concentration of circulating essential elements in animals over life may be indicative of periods of vulnerability to deficiencies and associated diseases. Here we studied age-related variations in essential elements (Se, Cu, Zn and Mn) and some selected oxidative stress biomarkers (GPx, SOD, vitamin A and vitamin E) in blood of an Iberian red deer (*Cervus elaphus hispanicus*) population living in semicaptive conditions. Animals during their first year of life showed to be especially vulnerable to suffer Se- and Cu-related diseases and disorders. Older female deer had lower blood levels of Zn and Mn, which was accompanied by a lower blood SOD activity. On the contrary, GPx blood activity was elevated in older deer, which may help to compensate the reduction of other antioxidants with during aging. Age-related changes in GPx and SOD and their positive relationships with the essential elements suggest that the observed nutritional deficiencies at certain age stages may have a detrimental effect on the antioxidant system, increasing the risk of oxidative stress. Thus, the biomarkers used in the present study may be important tools for the subclinical diagnosis of nutritional disorders and diseases related to the generation of oxidative stress in both domestic and wild ungulates.

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1. Introduction

Exposure of wildlife to metals and metalloids (referred to hereafter as metals or elements) in the environment is a major concern today (Campbell et al., 2010). Some trace elements including selenium (Se), copper (Cu), zinc (Zn), manganese (Mn) and molybdenum (Mo) are essential for biological functions, but outside required ranges, deficiencies and toxicities lead to adverse health consequences. Both natural and anthropogenic processes may determine solubility, mobility and bioavailability of trace elements in the environment, which implies the risk of producing adverse health effects in wildlife species (Medvedev, 1997).

Among their many physiological functions, Se, Cu, Zn, and Mn are functional and structural components of enzymatic systems and are thus key for several biochemical mechanisms (Erikson et al., 2005; Jihen et al., 2009). Importantly, these elements are part of the cellular antioxidant defense system carried out by a complex network of enzymes (e.g. glutathione peroxidase (GPx), superoxide dismutase (SOD)) and endogenous (e.g. glutathione (GSH)) and dietary (e.g. vitamins A and E) antioxidants. Cooperative interaction between endogenous and dietary antioxidants is vital in terms of obtaining maximum protection against the deleterious effects of oxidative stress (Surai, 2003; Kutil et al., 2010; Buettner, 2011; Shen and Jiang, 2012). When it occurs, oxidative stress can result in extensive damage to cellular lipids (lipid peroxidation), proteins, and DNA, being considered an important mechanism in the initiation and/or progression of health problems (Halliwell and Gutteridge, 2007).

There exist a wide variety of antioxidants responsible for preventing the generation and/or effects of the reactive oxygen species (ROS) that cause oxidative damage in cells. The antioxidant enzyme GPx reduces lipid and hydrogen peroxides by using GSH as substrate, where Se plays an important role acting as co-factor in the active center of this enzyme (Surai, 2003, Kutil et al., 2010). The main function of SOD is to detoxify the superoxide anion (Murk et al., 2002). There are two types of SOD depending on the metals in its active core (Buettner, 2011; Shen and Jiang, 2012): (1) Cu/Zn-SOD, which protects the cytoplasm of oxidative damage, and (2) Mn-SOD, which protects mitochondria from free radicals formed by the high metabolic activity in this organelle. Since these enzymes are metal-dependent, deficiencies in these elements may involve decreases in their antioxidant activities, causing greater vulnerability of cells against ROS (Miller et al., 1993). Therefore, the determination of the activity of these metalloenzymes can be used as a biomarker of the nutritional metabolic state of the essential elements they depend on, which can be in turn used as early warning signals of health threats (Surai, 2003).

Other kind of antioxidants includes the dietary vitamins, especially the fat-soluble vitamins A (retinol) and E (α-tocopherol). Vitamin A active free form, is essential for many metabolic functions, participating in metabolic regulation and modulating gene expression and cell and tissue differentiation, being essential for vision and embryonic development, and for the proper functioning of the hematopoietic, reproductive, immune and nervous systems, among others (Kefer et al., 2009). Furthermore, promotes the activity of other components of the antioxidant system such as GSH and GPx (Simms and Ross, 2000). Vitamin E is the main liposoluble antioxidant found in nature and it is known for its protective role in biological membranes against oxidative stress (Chow, 2009). Vitamin E is known to reduce the Se requirement by maintaining body Se in an active form and by preventing the destruction of membrane lipids, to maintain the functional integrity of cellular membranes by preventing lipid peroxidation of unsaturated fatty acids, thereby inhibiting the production of hydroperoxides, and reducing the amount of GPx needed to eliminate peroxides formed in cells (Hassan et al., 2012).

Wild ungulates hunting, management, and farming (semicaptive conditions) are important socioeconomic activities in rural areas across Europe, being the Iberian red deer (*Cervus elaphus hispanicus*) one of the most important and widely distributed large game species in Spain today (Milner et al., 2006). These animals depend on food and mineral resources available in the environment (plants, soils, water) to keep the required physiological ranges of essential elements and to avoid associated health risks. Homeostasis of essential elements can be affected by nutritional and physiological factors (e.g. dietary composition, metabolism), but also by constitutional factors such as age (Goyer and Clarkson, 2001). Thus, variations in the physiological concentrations of essential elements and nutrients over life may be indicative of vulnerability to deficiencies and functional and/or structural abnormalities related to oxidative stress and associated diseases (WHO, 1996; Flueck et al., 2012), which may have important implications for the management of both wild and semicaptive populations (Christensen et al., 2016; Oliver-Guimerá et al., 2017). In ungulates, there exist two diseases related to deficiencies of certain essential elements. One of these diseases is associated with Se and/or vitamin E deficiencies and it is known as white muscle disease (WMD) (Pourliotis et al., 2009); the second one is known as enzootic ataxia (EA), which is a pathology that causes slow and progressive paralysis in the hind limbs in association with Cu deficiency (Wilson et al., 1979).

In this work we aim to evaluate the levels of elements with a key role in the antioxidant system (Se, Cu, Zn, and Mn), the activity of related antioxidant enzymes (GPx and SOD), and levels of antioxidant vitamins (A and E) in blood of red deer in relation to age in a herd in which signs and lesions of WMD and EA have been observed. This study may contribute to a better understanding of physiological variations of essential elements and antioxidants homeostasis with age, helping to highlight the value of considering the effect of this factor in management practices and for the evaluation of health risks in both wild and captive populations.

2. Materials and methods

2.1. Study area

The study area is located in the southwest of the Iberian Peninsula (province of Cádiz, Andalucia, Spain; Fig. 1), and belongs to a 12,000-ha agricultural and hunting estate close to the Natural Park of Los Alcornocales. Animals sampled were from a farm of Iberian red deer subjected to intense management to improve venison production and quality of antlers through genetic selection, covering an area of 350 ha. The farm has 100 plots separated from each other by fences (semicaptive conditions) with dimensions of 1–7 ha by plots, within which deer are distributed by 7–8 deer/ha in accordance to their gender and age. At the time of sampling, total deer in this farm was about 410 females (plus calves) and 72 males. This area has a Mediterranean climate characterized by an average temperature of 18 °C and an average rainfall of 440 mm (Rivas-Martínez, 1987). The habitat is Mediterranean woodland characterized by the presence of cork oak (*Quercus suber*), evergreen oak (*Quercus ilex*) and wild olive tree (*Olea europaea* var. *sylvestris*) with scrubland areas and scattered pastures. An aquifer supplies the water for game species and irrigation. The large game species present in the area include Iberian red deer, roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*).

2.2. Sampling

A total of 132 deer, 44 juveniles (<2-year-old) and 88 adults (≥2-year-old), were sampled between June and July 2013. The age of the sampled animals was recorded by the farmer, who follows individually every deer in the farm from birth to death as part of the management process. Age categories respond to the management strategy of the studied semicaptive deer population, which considers juvenile as sexually immature animals while that >1-year-old form part of the reproductive herds of the farm. Adults were females, but the gender of juveniles was only provided for 24 animals (females). Animals were taken to the handling facilities, where they were immobilized through