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Original Research Article

# Influence of different periods of the year and age on the parameters of antioxidative status and oxidative stress in the blood serum of breeding bulls

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ARTICLE INFO

*Article history:*
Received 9 February 2016
Received in revised form
11 March 2016
Accepted 19 March 2016
Available online 29 March 2016

*Keywords:*
Serum
Antioxidative/oxidative status
Period of the year
Age
Breeding bulls

ABSTRACT

The sources of variations that may cause physiological differences between blood serum biochemistry parameters of bulls have not been investigated in detail. Aim of the present study was to establish influence of different periods of the year and the age of breeding bulls on parameters of antioxidative status and oxidative stress in their serum and to correlate these monitored variables. Research was performed on two groups, each comprising 9 Simmental bulls: a younger group (YB) (aged 2–4 years) and older one (OB) (aged 5–10 years). Blood samples for biochemical analyses were collected from jugular vein in cold (CP) and warm periods (WP) of the year. Reduced glutathione (GSH), uric acid (UA), total protein (TP), albumin (ALB), thiobarbituric acid reactive substance (TBARS), and protein carbonyl content (PCC) serum concentration were determined, as well as activities of selenium-dependent glutathione peroxidase (Se-GSH-Px), total superoxide dismutase (TSOD), manganese superoxide dismutase (MnSOD), copper–zinc superoxide dismutase (CuZnSOD) and catalase (CAT). Serum values of SeGSH-Px, MnSOD, UA and TP in OB were significantly higher compared to those in YB during CP of the year. Significantly higher PCC concentration in serum of YB and OB were established in CP of the year than in WP. TBARS serum concentration in YB was significantly higher in comparison to that in OB during CP of the year. It can be concluded that both OB and YB show a great sensitivity to climate condition alterations during CP in comparison to WP of the year and that YB show even greater sensitivity.



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http://dx.doi.org/10.1016/j.repbio.2016.03.003
1642-431X/

## 1. Introduction

It is well known that numerous factors, such as animal species, breed, nutrition, general health, antioxidative status, housing, intensity of exploitation, environmental temperature and body temperature during spermatogenesis, spermiogenesis and the maturation of spermatozoa in the epididymis, may influence the quality of semen and concentration of spermatozoa in domestic animals [1–4].

During metabolic processes in the organism, many toxic reactive molecules, termed reactive oxygen species (ROS also named as pro-oxidants), are produced. Namely, aerobic metabolism is related to the toxic effects of oxygen due to the oxidation of basic biological molecules, which can change cell function and/or jeopardize cell survival [5,6]. In an organism, *i.e.* in its cells, ROS may be produced due to the presence of endogenous and/or exogenous factors and are physiologically essential in very low concentrations, whereas higher concentrations may have detrimental and even toxic effects. ROS may induce lipid peroxidation, damage to DNA, proteins and carbohydrates, and may oxidate almost every organic molecule [7–10].

Oxidative stress is a condition induced by a disturbed balance between the production and elimination of ROS, but may also be induced as a consequence of the overproduction of oxidants and/or decreased antioxidative protection. Thus, in cases when the balance between pro- and antioxidants is disturbed in favor of prooxidants in the systemic circulation of breeding bulls, oxidative stress may be reflected in the functioning of the testis. The intensity of oxidative stress may be monitored by measuring the oxidation products of biological molecules and/or by determining enzymatic and non-enzymatic antioxidants [11,12]. Lipid peroxidation is an oxidative process of the degradation of lipids frequently induced by ROS [13,14]. Thiobarbituric acid reactive substrates (TBARS) are produced as degradation products of lipid peroxidation, and by determining their concentration it is possible to obtain a reliable assessment of the quantities of lipid peroxides present in an organism [15]. The process of protein oxidation usually results in the production of new functional groups, such as hydroxyl and carbonyl groups [16]. Carbonylation of proteins is an irreversible process which can be used as biomarker of oxidative stress [17].

In order to prevent detrimental effects, which mostly take place in the cells, the organism has developed several mechanisms by which it can neutralize oxidative stress, such as the prevention of damage, reparative processes regarding oxidative damage, physical protection from damage mechanisms, and antioxidative protective mechanisms [18]. Antioxidative protection may be endogenous and exogenous, enzymatic and non-enzymatic, and may synergistically act to neutralize the negative effects induced by ROS. The most effective enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), while non-enzymatic antioxidants comprise glutathione (GSH), vitamins C and E, carotenoids, natural flavonoids, albumins (ALB), uric acid (UA), as well as other compounds and minerals, such as selenium [19,20]. Antioxidant enzymes act to scavenge free radicals by converting them to less harmful molecules [21]. SOD isoenzymes catalyze the dismutation of superoxide to hydrogen peroxide ($H_2O_2$) and $O_2$, and are crucial for defense against ROS in eukaryotic cells [22]. Although both GSH-Px and CAT decompose $H_2O_2$, their contributions vary depending on the amount and site of $H_2O_2$ production [23].

In relation to non-enzymatic antioxidants, GSH can directly scavenge free radicals or act as a substrate for GSH-Px and glutatione S-transferase during the detoxification of $H_2O_2$ and lipid hydroperoxides [24–26]. Another non-enzymatic antioxidant is UA, one of the most important physiological antioxidants in the total antioxidative capacity of blood plasma. It directly reduces peroxyl and hydroxyl radicals and singlet oxygen, and has the capability to bind the ions of transition metals, which may initiate lipid peroxidation [27]. In addition, proteins are high molecular weight antioxidants which have the ability to prevent the production of free radicals by binding free metal ions in order to perform their transport or storage [5]. ALB is considered as the major circulating non-enzymatic antioxidant which protects cells scavenging ROS [28,29].

According to our knowledge, studies on the influence of different periods of the year and age on antioxidative status and oxidative stress in the blood serum of breeding bulls are not available as yet. We assume that new insights regarding the influence of environmental stress (coldness and warmness) and the age of bulls on their physiological adaptive responses could be of relevance because of the impact which can be detected through the parameters of antioxidative status and oxidative stress. Thus, the aim of the present study was to establish the influence of the different periods of the year and the age of breeding bulls on the parameters of antioxidative status and oxidative stress in their blood serum and to determine correlations among the monitored variables.

## 2. Materials and methods

### 2.1. Housing and feeding of animals

The study was performed on 18 breeding bulls of the Simmental breed aged from 2 to 10 years old. The animals were assigned into two age groups comprising 9 bulls each. The first group consisted of younger bulls aged from 2 to 4 years old ($3.1 \pm 0.6$; mean ± SD), while in the second group there were older bulls aged from 5 to 10 years old ($7.4 \pm 1.9$; mean ± SD). The bulls were kept in stalls with an outlet and windows that were closed only during the cold period of the year. The animals were fed with a feed mixture produced according to a standard recipe (Department for Nutrition of Domestic Animals at the Faculty of Agriculture of the University of Zagreb in Croatia). The feeding of the animals was performed twice per day, and the feed comprised approximately 10 kg of hay, 8 kg of concentrate, 6 kg of haylage, straw *ad libitum* and a mineral supplement (MS) (Table 1). Water was provided to the bulls *ad libitum*.

The average air temperature and humidity values in the continental part of Croatia where the bulls were kept are shown in Table 2. The average daily temperature and relative humidity were used to calculate the daily temperature-humidity index (THI) using the following formula: THI = (0.8 × temperature) + [(% relative humidity/100) × (temperature − 14.4)] + 46.4, according to Amundson et al. [30] and Mader [31]. The average THI data for the cold and warm periods of the year are given in Table 2.