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Evaluation of Oxidative Stress in Dairy Cows through Antioxidant Enzymes Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD)

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Abstract. The research was focused on measurements of oxidative stress in dairy cows in different physiological states (dry period, 0-7 days early lactation, lactation 25-35 days). As indicators of oxidative stress were followed the activities of antioxidant enzyme glutathione peroxidase (GPX) and superoxide dismutase (SOD) on a herd of 15 heads dairy cows of Romanian Spotted breed. It was found that the activity of antioxidant enzymes shown different values in al three physiological states, but with no significantly differences. The results found shown that the stress of calving was installed.

Keywords: oxidative stress, antioxidant enzymes**,** physiological states, dairy cows

INTRODUCTION

Recent research in assessing oxidative stress in farm animals have significantly contributed to understand the fundamental processes involved in metabolic disorders (Lykkesfeldt and Svendsen, 2007).

In dairy cows, advanced pregnancy and early lactation are physiological states that are critical to the health and later productive performance, in witch animals are more sensitive to a variety of metabolic and infectious diseases than the rest of lactation (Sordillo *et al.,* 2007).

Host's defense mechanisms can be directly influenced by many physiological and environmental factors during pregnancy and after parturition. It is also considered a physiological stress the secretory activity of the parenchyma and the onset of milk secretion is accompanied by high-energy demand and increased oxygen demand (Gitto *et al.,* 2002). This leads to an increase in oxygen demand, which increases the production of oxygen-derived reactants, called reactive oxygen species (ROS). Excessive production of free radicals together with damage at the cellular level is controlled by cellular antioxidant defense systems. Antioxidants can be defined as defense substances that delay, prevent or eliminate oxidative damage to a target molecule (Halliwell and Gutteridege, 2007). Antioxidant enzyme systems can be (eg, SOD, GSHPx and catalase) and non-enzymatic systems (eg, vitamin E and selenium). Excessive production of free radicals and ROS and/or a decrease in antioxidant capacity of the body, leads to a degradation of biological macromolecules and disrupting the normal and physiological metabolism (Trevisan *et al.,* 2001). When ROS are produced faster than can be safely neutralized by antioxidant mechanisms oxidative stress results. Therefore, if there is imbalance between increased production of ROS and reduced antioxidant capacity close to the time of parturition may increases oxidative stress and contribute to disorders in dairy postpartum cows (Waller, 2000; Gitto *et al,* 2002).

MATERIALS AND METHODS

The evaluation of oxidative stress was made trough antioxidant enzymes GPx (glutathione peroxidase) and SOD (superoxide dismutase). Biological material was represented by a group of 15 heads of Romanian Spotted dairy cows in different physiological states. Blood samples were collected after the morning milking by jugular puncture in vacuuntainers containing 5 ml Li-heparin and immediately placed in cold. These were centrifuged for 10 minutes after which plasma was harvested. GPX and SOD enzyme activities were determined in whole blood red cell lysates using commercial kits and Ransell Ransod from Randox laboratories, and results were expressed in U/g Hb.

The changes in plasma concentrations of antioxidant biomarkers were analyzed by ANOVA program, and the results were rendered as tables.

RESULTS AND DISCUSSIONS

GPX and SOD levels were monitored during three physiological states, blood was sampled in the dry period (advanced gestation), early lactation (0-7 days) and lactation (25-35 days). The values obtained showed variations between the three physiological states.

Tab. 1

Average value of blood GPX according physiological state

Specification	UM	n	Variables		
			$X \pm s_x$	s	V%
Advanced gestation	U/g Hb	15	81.3 ± 3.91	13.16	16.18
Early lactation (0-7 days)	U/g Hb	15	73.29 ± 2.60	10.09	13.76
Lactation (25-35 days)	U/g Hb	15	79.21 ± 3.93	15.23	19.22

As shown in Table 1, the blood GPx level was higher in cows in advanced gestation compared with the two stages of lactation. Average level of GPx in advanced gestation was 81.3±3.91 U/g Hb, while values related to the stages of lactation were 79.21±3.93 U/g Hb, respectively 73.29±2.60 U/g Hb. The low blood GPX post-partum is considered an indicator of oxidative stress that occurs when GPX reduces plasma lipid peroxidation. Immediately after birth, the reactive oxygen species increased, while blood GPx value started to decrease. These variations have induced an imbalance between production of reactive oxygen species and removing them safely and could indicate a loss of homeostatic control mechanisms to control oxidative during the postpartum period. Also, an increase of the value of GPx in 3-4 weeks after birth may indicate that cows suffered from oxidative stress during parturition.

Although there were differences in blood GPx concentrations in the three physiological stages, they were not statistically significant (Tab. 2).

Even if blood GPX activity was inhibited, the body could be protected against oxidative stress through other alternatives. For example, catalase is another antioxidant enzyme that can catabolise hydrogenated peroxides (Droge, 2002), which demonstrates that other antioxidant molecules have played a role in reducing reactive oxygen species in the first week after parturition. Also maintaining the homeostasis is modulated by various substances, which form antioxidant defense system (Lykkesfeldt and Svendsen, 2007).

Tab. 2

The significance of differences between mean values of GPx according to physiological state

Specification	Variables			
	X1-X2	d	q	Significance
Late gestation vs early lactation (0-7 days)	81.3 – 73.29	8.01 ns	2.38	p>0.05
Late gestation vs lactation (25-35 days)	81.3 – 79.21	2.09 ns	0.62	p>0.05
Early lactation vs Lactation (25-35 days)	73.29 – 79.21	-5.92 ns	1.76	p>0.05

Table 3 presents the values of SOD in the blood in three physiological states.

The data shows that the variations in SOD were similar to those of GPX. Thus, the lowest value was recorded during early lactation, SOD having a value of 1786.52±76.36 U/g Hb. In advanced pregnancy SOD value was de1878.61±88.59 U/g Hb, and the other stage of lactation. 1828.52±107.94 U/g Hb.

Tab. 3

Mean value of SOD in the blood depending on the physiological state

Specification	UM	n	Variables		
			$X \pm s_x$	s	V%
Late gestation	U/g Hb	15	1878.61 ± 88.59	343.03	18.25
Lactation (0-7 days)	U/g Hb	15	1786.52 ± 76.36	295.74	16.55
Lactație (25-35 days)	U/g Hb	15	1828.52 ± 107.94	418.04	22.86

SOD is known to be an important factor in protection against harmful free radical activity (Celi *et al.*, 2010) and is considered the first defense mechanism against pro-oxidants (Halliwell and Chirico, 1993).

Low activity of SOD observed in the first week after parturition was probably a consequence of increased reactive oxygen species. Because SOD activity increases production of hydrogenated peroxides, protection against reactive oxygen species would be achieved only with the increase GPX activity. How GPX and SOD activity decreased after parturition, the antioxidant defense mechanisms protection decreased. The results of our research, on the GPx and SOD are confirmed by other researchers. Thus, Sharma *et al.* (2011) showed that the value of GPX and SOD decreases in early lactation than during advanced pregnancy.

The same results were obtained by Celi *et al*. (2010), Bernabucci *et al*. (2005) and Sordillo *et al*. (2007), which have been established a relationship between physiological changes associated with early pregnancy and lactation periods and a decrease in total antioxidant potential. Marker antioxidant SOD activities were similar during the three physiological states with no significant differences in the three groups (Tab. 4).

It is very important that oxidative stress may be influenced by environmental factors and nutrition. Increased cellular metabolism in the body due to mobilization during early lactation milk production increases oxidative stress. In order to reduce oxidative stress in dairy cows, they must be fed higher energy sources and effects of environmental factors to be reduced by ensuring a proper environment.

Tab. 4

The significance of differences between mean values of SOD according to physiological status

Specification	Variables			
	X1-X2	d	q	Significance.
Late gestation vs early lactation (0-7 days)	1878.61 - 1786.52	91.48 ns	0.99	p>0.05
Late gestation vs lactation (25-35 days)	1878.61 - 1828.52	49.48 ns	0.53	p>0.05
Early lactation vs Lactation (25-35 days)	1786.52 - 1828.52	-42 ns	0.45	p>0.05

CONCLUSION

The results obtained in our study, showed a average level of GPx in advanced gestation was 81.3±3.91 U/g Hb, while values related stages of lactation are 79.21±3.93 U/g Hb, respectively 73.29±2.60 U/g Hb. Thus, the lowest value was recorded during early lactation, SOD having a value of 1786.52±76.36 U/g Hb. In advanced pregnancy SOD value was de1878.61±88.59 U/g Hb and the other stage of lactation 1828.52±107.94 U/g Hb.

Following research and through the results we conclude that dairy cows are under oxidative stress and antioxidant defense capacity is reduced during postpartum period and during lactation than during advanced pregnancy.

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