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ANTIOXIDANT STATUS IN DAIRY COWS DURING TRANSITION PERIOD

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Abstract

During the transition period important metabolic changes occur in dairy cows, which can also experience oxidative stress. The two years prospective study was carried out to assess the changes occurring in the activity of antioxidant enzymes superoxid dismutase (SOD) and glutathion peroxidase (GPX) in three physiological periods: dry period 21 days before calving, period from beginning of lactation until 21st day in lactation, and period from 22nd to 42nd day in lactation. Determination of the enzyme activity was assayed on blood serum by using spectrofotometric methods. The average activity of SOD in period before calving was 24.20±2.319 mU/mg proteins, reaching the value of 43.94±3.864 mU/mg proteins in the second period and in the third period from 22nd to 42nd day in lactation, the activity was 26.77±2.201 mU/mg proteins. The average activity of GPx in period before calving was 251.79±9.363 mU/mg proteins, than increasing in period from beginning of lactation until 21st day in lactation (372.45±17.533 mU/mg proteins) and in the third period from 22nd to 42nd day in lactation, the activity was 319.40±14.307 mU/mg proteins. Physiological stages in transition period when blood samples was taken, showed statistical significant influence ($p<0.001$) on SOD and GPx activity in blood serum. Significant difference in the activity of SOD in blood serum was found in period before calving as compared to period from calving to 21st day in lactation, and also in period from calving to 21st day in lactation as compared to period from 22nd to 42nd day in lactation. Significant differences in the activity of GPx in blood serum was found between all three physiological periods, but biggest significant difference in activity was found in period before calving as compared to periods in lactation. There were statistically significant positive correlations between the activity of GPx and SOD in blood serum. Dairy cows experience oxidative stress in transition period from gestation to lactation.

Key words: dairy cows, oxidative stress, superoxid dismutase, glutathion peroxidase.

Introductıon

The transition period, from 3 wk before to 3 wk after parturition, is critically important to health, production, and profitability of dairy cows (Goff and Horst, 1997). Dairy cows experience a drastic change in metabolism around parturition. Daily dry matter intake decreases up to 30 % (Grummer et al., 2004) and at the same time energy demand rises due to the onset of lactation, leading to in negative energy balance. Therefore, cows mobilize body tissues to satisfy the increased energy requirement for milk production, and preferentially use lipids as energetic substrate (Castillo, 2006; Contreras and Sordillo, 2011; Wathes et al., 2013). The intense metabolic processes are accompanied by modification of energetic metabolism and by an increase of oxygen consumption. This enhances metabolism severely, resulting in a raised production of reactive oxygen species (ROS) which are normally neutralized by enzymatic and non-enzymatic antiioxidant systems of living organisms thus limiting or preventing oxidative damage (Bionaz et al. 2007; Halliwell and Gutteridge, 2007). Antioxidant enzyme systems can be superoxide dismutase (SOD), glutathion peroxidase (GPx) and catalase. Non-enzymatic antioxidants are mainly represented by sulfhydryl (SH) groups of albumin, a-tocopherol and carotenoids (Havemose et al., 2004).

The imbalance between the rate of ROS production and their neutralization lead to the oxidative stress (Trevisan, 2001). It was shown that during transition period, oxidative stress can occur and it can contribute to some periparturient disorders or metabolic diseases (Bernabucci et al., 2002; 2005; Lykkesfeldt and Svendsen, 2007). Thus, the occurrence of health problems is centered disproportionately on this relatively short period, which certainly contributes to making this an "interesting" time for dairy producers. The success of the transition period effectively determines the profitability of the cow during that lactation. The evaluation of blood redox homeostasis has increasingly contributed to the knowledge of the processes involved in reproductive and metabolic disorders (Kankofer, 2002**;** Sordillo and Aitken, 2009), and it has become important as a complementary tool for the evaluation of health and metabolic status of dairy cows.

Therefore, the aim of this study was the evaluation of enzymatic antioxidant status in blood during late gestation and early lactation period, from 3 weeks before to 6 weeks after parturition. The activity of main antioxidant enzymes: superoxiddismutase (SOD) and glutathion peroxidase (GPX) in blood serum was determined as indicators for oxidative stress.

Materıals and methods

A two years longitudinal survey was carried out to evaluate the activity of antioxidant enzymes superoxide dismutase (SOD) and glutathione preoxidase (GPx) in blood serum of dairy cows during transition period from gestation to lactation. The study was carried out in a conventional dairy farm localized near city of Strumica in south-eastern part of Macedonia, using 211 pregnant primiparous and multiparous black-white dairy cows. The animals were allocated into groups related with season of calving. Therefore, the dairy cows were grouped according expected date of calving in order to minimize the influence of environment on antioxidant enzyme activity. Cows were kept in the same farm providing with similar house conditions and feeding regime during 2 years of experiment to exclude the influence of additional factors such as feeding. Cows consumed ordinary diet which differed in accordance with the stage of pregnancy and lactation but was the same during consecutive events and included corn and grass silage, hay, commercial concentrate (maize, wheat, barley) as well as vitamin and mineral premix.

Blood samples were collected by jugular puncture in serum tubes from each cow, at the following time points: dry period (advanced gestation) 21 days before calving, period of early lactation from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. After collection, the blood samples were immediately placed in cold. In laboratory, the blood samples were centrifuged at 5000 rpm for 20 minutes at 4^0C, and sera obtained were stored in Eppendorf tubes at -80^0C, no longer than 2 months. Before use the aliquots were thawed. GPX and SOD enzyme activities were determined in blood serum using spectrofotometric assays and enzymes activity were expressed in mU/mg protein. The changes in blood serum concentrations of antioxidant biomarkers were analyzed by Multivariate GLM, and the results were rendered as tables. Following analysis of variance, significant between groups were estimated by the Bonferoni significant difference test.

SOD activities in the blood serum was determined spectrophotometrically at 415 nm using the method of Marklund and Marklund (1974) with some modifications in order to accommodate kinetic analyses in 96-well microplates (Gao et al.,1998). The method was based on the autoxidation of pyrogallol in presence of DTPA in TRIS-HCl buffer, pH= 8.5.

Activity of glutathione peroxidase (GSH-Px) was measured spectrophotometrically at 340 nm. The method was based on the changes in absorbance because of the conversion of NADPH into $NADP^+$ (Paglia and Valentine 1967), and modified according Chen et al. (2000) in order to accommodate kinetic analyses in 96-well microplates.

All chemicals were of the highest purity grade. TRIS, pyrogallol, KH_2PO_4, EDTA and cH_2O_2 were purchased from Merck, Darmstadt, Germany. DTPA, Cu/Zn-SOD, GSH, NADPH and GR were obtained from Sigma Chemical Co., St. Louis, MO, USA. The absorption was determined with a spectrophotometer Bio-Rad 680 XR, microplate reader.

Results

Changes in the blood activity of antioxidant enzymes SOD and GPx during transition period are shown in Table 1.The values obtained showed variations in enzymes activity between the three physiological states.

Table 1. Average activity of SOD and GPX in blood serum according physiological periods before and after calving (mU/mg proteins)

Physiological periods before and after calving	n	SOD	GPx
-21	211	24.20±2.319	251.79±9.363
21		43.94±3.864	372.45±17.533
42		26.77±2.201	319.40±14.307

As shown in Table 1, the blood SOD activity was higher in cows after parturition compared with the period in advanced gestation and period from 22nd to 42nd day in lactation. Average activity of SOD in advanced gestation was 24.20±2.319 mU/mg protein, while values related to the stages of lactation were 43.94±3.864 mU/mg protein, respectively 26.77±2.201 mU/mg protein. The data shows that the variations in GPx activity were similar to those of SOD. Thus, the lowest mean value was recorded during advanced gestation when GPx having a value of 251.79±9.363 mU/mg protein. In early lactation from calving until 21st day in lactation, GPx value was 372.45±17.533 mU/mg protein, and the other stage of lactation from 22nd to 42nd day in lactation the activity was 319.40±14.307 mU/mg protein.

Figure 1 represent the variations in antioxidant enzymes activity in blood serum of dairy cows during transition period. The chart clearly showed that SOD and GPx activity is increasing in early lactation comparing with period in advanced gestation and there was registered declining activity in late lactation, but not under level of enzymes activity in advanced gestation.

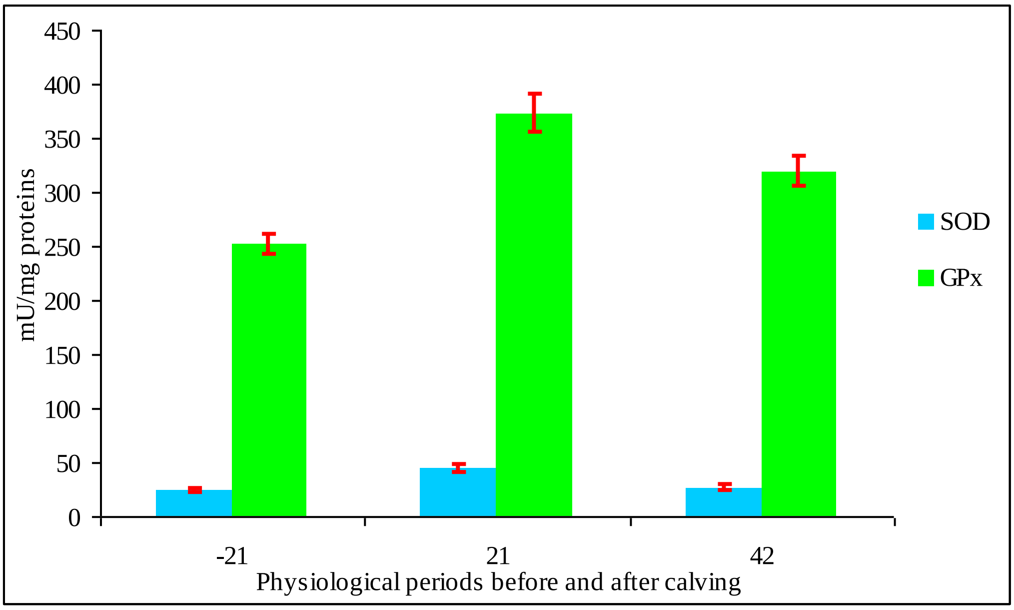

Figure 1. Specific activity of antioxidant enzymes SOD and GPx in different physiological periods (mU/mg proteins)

In Table 6 are shown the results from statistical model used for determination the influence of three physiological states during transition period of dairy cows on activity of SOD and GPx in blood serum in terms of oxidative stress

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Table 2. Regression model for influence of transition period on SOD and GPx activity in blood serum of dairy cows

Fixed variable	df	SOD[a]	GPx[b]
Model[a,b]	2	13.733***	18.294***
Period	2	13.733***	18.294***
Error	630		
Total	632		
[a]R^2 = 0,052; [b]R^2 = 0,039			

*** significant at level p<0.001

The results clearly indicated that transition period in dairy cows statistically significant ($p<0.001$) affect the activity of SOD and GPx in blood serum. Therefore, the risk for manifesting oxidative stress in this period is high due to imbalance in activity of antioxidant enzymes.

There were differences in blood SOD activity in the three physiological stages, but statistically significant were the differences in mean value for SOD activity in early lactation from calving to 21st day in lactation compared with period of advanced gestation and period of late lactation from 22nd to 42nd day in lactation (Table 3). Mean values for SOD activity did not differ significantly between periods of advanced gestation lactation and late lactation from 22nd to 42nd day in lactation.

Table 3. Bonferoni test for difference in mean values of SOD specific activity in blood serum in three different physiological periods before and after calving

Physiological periods before and after calving	21	42
-21	19.737*	2.572
21		17,164*

*significant at level $p<0.05$

Mean values for GPx activity in blood serum differ significantly between all three physiological stages (Table 4). The highest significant difference in mean value for GPx activity was registered between GPx activity in early lactation from calving to 21st day in lactation compared with period of advanced gestation.

Table 4. Bonferoni test for difference in mean values of GPx specific activity in blood serum in three different physiological periods before and after calving

Physiological periods before and after calving	21	42
-21	120.660*	67.603*
21		53.057*

*significant at level $p<0.05$

Estimation of interdependence between SOD and GPx activity in blood serum of dairy cows was performed with Pearson's coefficient of correlation, showed in Table 5

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Table 5. Pearson's coefficient of correlation for SOD and GPx specific activity in blood serum of dairy cows

Pearson's	SOD
GPx	0,141*

*significant at level $p<0.05$

There was low, but statistical significant positive correlation between SOD and GPx activity in blood serum.

Discussion

In the past, efforts to improve the transition to lactation in dairy cows have focused largely on preventing infections and maximizing energy intake in transition cows, and these have generally been treated as independent issues. However, new models are emerging to explain the development of numerous transition disorders. A combination of insults, including social stress, negative energy balance, heat stress, endotoxin exposure, and oxidative stress may promote inflammation, suppress feed intake, and impair both metabolic and immune function during the transition period. These models suggest that transition cow management must be viewed in a holistic way, with the cow's environment, nutrition, and immune function interacting in many complex ways.

Oxidative stress in veterinary medicine and particularly in ruminant health is a relatively young field of research. As oxidative stress is indicative of an imbalance between oxidants and antioxidants, methods for quantifying oxidative stress mostly include direct or indirect measures of oxidants and antioxidants (Celi, 2011).The role of antioxidants in health and disease was studied extensively in both human and animal medicine (Valko et al., 2007). SOD is known to be an important factor in protection against harmful free radical activity and is considered the first defense mechanism against pro-oxidants (Celi et al., 2010). Plasma GSH-Px activity contributes to the oxidative defense of animal tissues by catalysing the reduction of hydrogen and lipid peroxides (Halliwell and Chirico, 1993) and is also considered an indicator of oxidative stress (Tuzun et al., 2002).

Immediately after birth, the reactive oxygen species increased, while blood SOD and GPx value started to decrease. An increased activity of SOD observed in period after parturition to 21st day in lactation was probably a consequence of increased synthesis of 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. The increased activity of antioxidant enzymes in early lactation may indicate that cows suffered from oxidative stress during parturition. The low blood SOD and GPX post-partum is considered an indicator of oxidative stress that occurs when GPX reduces plasma lipid peroxidation. 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.

It is clearly established that SOD and GPx activity show dynamic changes within transition period in dairy cows and may indicate a temporary imbalance of ROS which is mainly expressed between 24-48 h after parturition. Thus, Sharma et al. (2011) showed that the value of GPX and SOD decreases in early lactation than during advanced pregnancy. However, even if blood SOD and 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 (Drodge, 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 non-enzymatic antioxidant defense system (Lykkesfeldt and Svendsen, 2007).

The results of our research related with the GPx and SOD activity are confirmed by other researchers. Maurya et al. (2014) reported increased SOD and GPx activity in blood after parturition compared with period of advanced gestation. Festila et al., (2012) reported higher blood SOD and GPx level in cows in advanced gestation compared with the two stages of lactation. According them, the low blood GPX post-partum was 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. How GPX and SOD activity decreased after parturition, the antioxidant defense mechanisms protection decreased. 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. Also, it is very important that oxidative stress may be influenced by environmental factors and nutrition. 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.

Conclusıons

The results obtained in this study showed dynamic changes in enzymatic antioxidative capacity of blood serum during transition period of dairy cows. The activity of antioxidant enzymes SOD and GPX is higher in period of early lactation compare with period of advanced gestation but later following lactation the activity of antioxidant enzymes is declining as results of scavenging the ROS. An increase of SOD and GPx activity in blood are likely to represent adaptive changes of cows in response to oxidative stress. 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.

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