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EDITORIAL

Agricultural production worldwide faces numerous challenges, including climate change, technology, social, and economic barriers. These obstacles must be overcome to meet the food demands of the growing population. The key to overcoming these challenges is knowledge. Scientific research can offer new insights into how we can adapt to the changing conditions that affect food production for both humans and animals.

The strategy of the European Union for Southeast Europe (SEE 2020) emphasizes that a knowledge-based economy should be the foundation for improving agriculture in the region and that in this sense, significant investments in research and innovation are necessary. The research and innovations should primarily be tailored to family farms, which are the main subjects of agricultural production in the Republic of Srpska, Bosnia and Herzegovina, and the surrounding area. In other words, the results obtained from scientific research must be accessible to agricultural producers, and this can be most effectively achieved through agricultural advisory services operating in the field. The previous statement applies to large agricultural combines, which are the backbone of agricultural production in some regions.

The results of significant scientific research in the field of food production are presented in these Proceedings.

The Proceedings contains 39 papers presented at XIII International Symposium on Agricultural Sciences "AgroReS 2024" in Trebinje, Bosnia and Herzegovina, from 27 to 30 May, 2024.

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Mastitis makes changes in the blood antioxidant enzyme activity during the transition period of dairy cows: Part II

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Abstract

During the transition period in dairy cows, significant metabolic changes occur, potentially leading to oxidative stress. Early postpartum is also the riskiest period due to the increased susceptibility of dairy cows to mastitis. This study aimed to assess the enzymatic antioxidant status by analyzing the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in blood obtained from cows with mastitis and comparing it with samples from healthy cows. The two years prospective study was carried out to assess the changes occurring in the activity of antioxidant enzymes SOD and GPX in blood serum in three physiological periods: the dry period 21 days before calving, the period from the beginning of lactation until the 21st day in lactation and period from the 22nd to the 42nd day in lactation. All cows were allocated in groups regarding the season years of calving. Categorisation of the observed population was done following screening for mastitis using udder clinical examination, the California Mastitis Test, and bacteriological culturing. The groups included healthy cows, those with abnormal milk secretion (AMS), and cows with intramammary infection (IMI). Enzyme activity was assessed in blood serum using spectrophotometric methods. The physiological stages in the transition period when blood samples were taken, showed a statistically significant influence ($p<0.001$) on SOD and GPX activity in blood serum. There were statistically significant positive correlations between the activity of GPX and SOD in blood serum. Health disorders of the mammary gland showed a statistically significant influence on GPX activity in blood serum ($p<0.05$), but there wasn't a statistically significant influence on SOD activity in blood serum. The findings suggest that maintaining a balanced diet with sufficient antioxidants and managing environmental stressors can mitigate oxidative stress during the transition period, also reducing the risk of mastitis in early lactation.

Key words: dairy cows, oxidative stress, udder disorders

INTRODUCTION

The weeks around parturition are critically important for the well-being, production, and financial success of dairy farms. During this short time, dairy cows undergo significant metabolic shifts. These shifts lead to a surge in energy metabolism and oxygen use, pushing the cows' metabolism to its limits. This intense metabolic activity causes an increase in the production of reactive oxygen species (ROS), which, under normal circumstances, are counteracted by the cow's enzymatic and non-enzymatic antioxidant systems, thereby reducing or preventing oxidative harm (Bionaz et al. 2007; Halliwell & Gutteridge, 2015). Among the enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants primarily include the sulfhydryl

(SH) groups in albumin, alpha-tocopherol, and carotenoids, playing a crucial role in mitigating oxidative stress (Havemose et al., 2006).

The disparity between the production rate of ROS and their neutralisation results in oxidative stress. Studies have demonstrated that oxidative stress can manifest during the transition period, potentially contributing to periparturient disorders or metabolic diseases (Bernabucci et al., 2002; 2005; Lykkesfeldt & Svendsen, 2007). Consequently, health issues tend to concentrate disproportionately within this relatively brief period, making it a crucial phase for dairy producers. The success of the transition period significantly impacts the cow's profitability in the subsequent lactation. Assessing blood redox homeostasis has increasingly enhanced understanding of the mechanisms involved in reproductive and metabolic disorders (Kankofer, 2002; Sordillo & Aitken, 2009). It has become a valuable complementary tool for evaluating the health and metabolic status of dairy cows.

Mastitis remains a critical issue for dairy farms, causing significant financial losses throughout the milk production process. The primary economic toll on the dairy sector is seen in diminished milk yield and quality. However, current research does not provide strong evidence that directly associates oxidative stress during the transition period with the incidence of mastitis in dairy cows. The clearest connection between oxidative stress and mastitis lies in the inflammatory response triggered by an infection. The onset of mastitis activates the cow's immune system to combat bacterial infection, a process that includes the release of inflammatory molecules and immune cells. These cells can produce ROS as part of their defence pathway. Nonetheless, an overproduction of ROS may lead to oxidative stress, harming nearby tissues and intensifying inflammation. Furthermore, oxidative stress can compromise the cow's immune defences, increasing susceptibility to infections such as mastitis. Thus, a vicious cycle is established where inflammation from mastitis can lead to oxidative stress, which, in turn, may weaken the cow's capacity to fend off infections efficiently.

Therefore, the aim of this study was the evaluation of enzymatic antioxidant status in blood serum during late gestation and early lactation period, from 3 weeks before to 6 weeks after parturition. Hence, the primary objective of this study was to observe the transition period of dairy cows and establish a dynamic of changes in antioxidants enzyme activity (SOD and GPX) in blood serum from cows with mastitis compared with their healthy counterparts.

MATERIALS AND METHODS

A comprehensive two-year longitudinal study was conducted to assess alterations in the activity of antioxidant enzymes SOD and GPX in the blood serum of dairy cows during the transition period from gestation to lactation. Subsequently, udder health screenings were performed from calving to the 42nd day of lactation. The survey involved a population of 211 black-white dairy cows, categorised by parity levels (P) ranging from 1 to 5 or higher. To minimize the impact of additional environmental factors on antioxidant enzyme activity, all cows were kept in the same rearing conditions, receiving similar feeding, and milking regimes throughout the study.

The blood samples for determination of SOD and GPX activities were collected in three physiological periods (PP): the dry period 21 days before calving (transition period 21 - TP_21), the period from the beginning of lactation until the 21st day in lactation (lactation period 21 - LP_21) and the period from 22nd to 42nd day in lactation (lactation period 42 - LP_42). The methodology was described in detail in the previously published paper by Nakov et al. (2023).

Based on the outcomes derived from udder screening involving clinical examination, the California Mastitis Test (CMT), and bacteriological culturing, all cows in the studied population were classified into three distinct groups: healthy cows, cows manifesting abnormal milk secretion (AMS), and cows with intramammary infection (IMI). Additionally, the cows were sorted into groups based on the season of calving (Y_S) to minimize the potential impact of environmental factors on the activity of antioxidant enzymes.

Measuring of the SOD and GPX activity in blood serum was done with spectrophotometric assays expressed as mU/mg protein. SOD activity was measured using kinetic analysis according to Gao et al. (1998), while GPX measuring was used method modified according to Chen et al. (2000).

The statistical model Multivariate General Linear Model (GLM), a multivariate procedure was used to determine the influence of mastitis on changes in SOD and GPX activity in blood serum. The correlation between SOD and GPX activity in blood serum was calculated with Pearson's coefficient of correlation.

RESULTS

Table 1 illustrates the variations in the activity levels of the antioxidant enzymes SOD and GPX in the blood during the transition period. The data presented indicate fluctuations in the activity of these two enzymes throughout the period from gestation to parturition and the beginning of lactation.

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

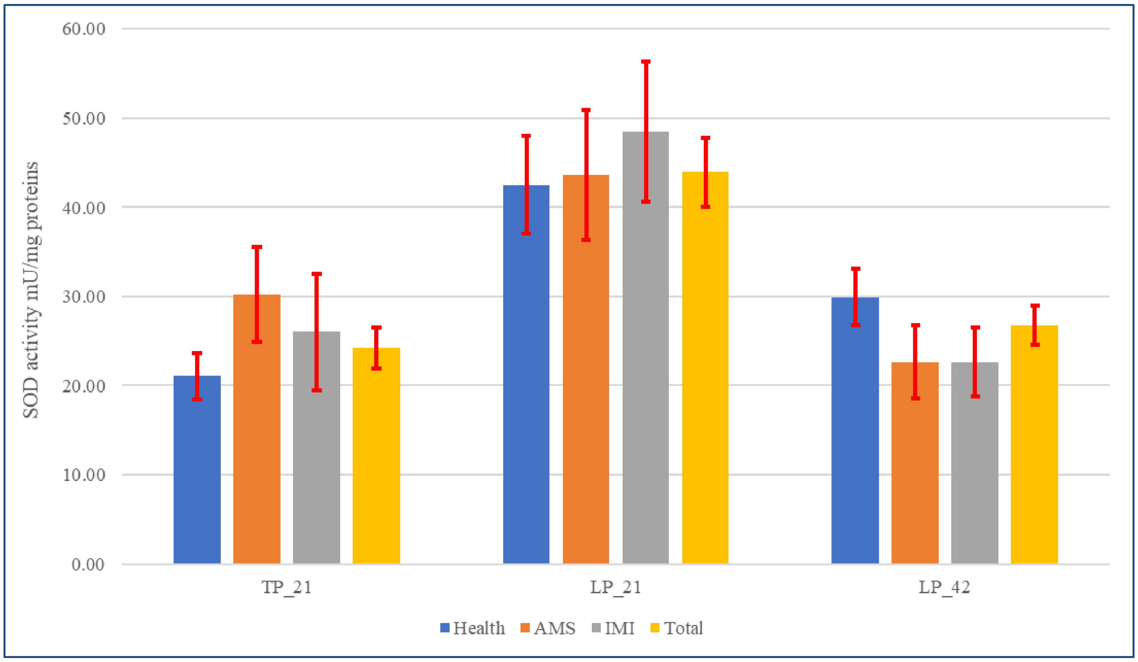
PP	n	SOD	GPx
TP_21	211	24.20±2.319	251.79±9.363
LP_21		43.94±3.864	372.45±17.533
LP_42		26.77±2.201	319.40±14.307

Table 2 presents data for the prevalence of udder quarter disorders in the two physiological periods of early lactation (LP_21 and LP_42) based on the udder screening methods used.

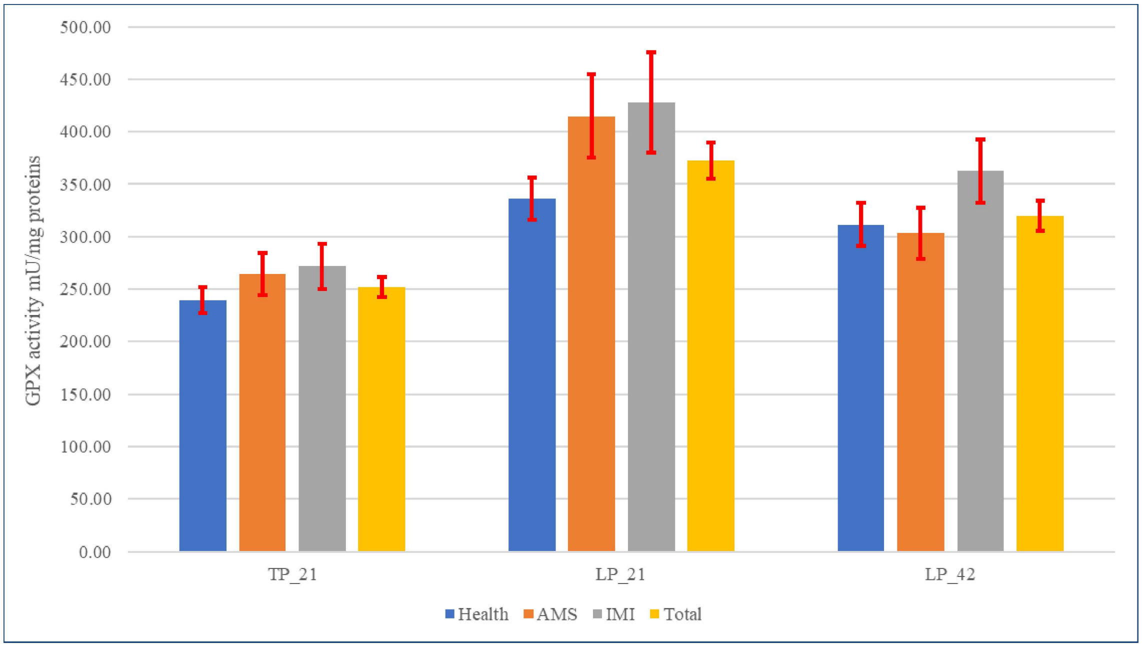
Table 2. The prevalence of AMS and IMI in dairy herd

PP	Total	Healthy cows		AMS		IMI	
	n	n	%	n	%	n	%
LP_21	211	152	72.04	35	16.59	24	11.37
LP_42	211	153	72.51	30	14.22	28	13.27

The dynamic changes in SOD and GPX activity in blood serum at the transition period, related to udder health status in early lactation are shown in Graphs 1 and 2.



Graph 1. Average activity of SOD (mU/mg proteins) in blood serum related to the health status of cows' mammary gland



Graph 2. Average activity of GPX (mU/mg proteins) in blood serum related to the health status of cows' mammary gland

In Table 3 are shown the results from the General Linear Model (GLM) used to determine the influence of three PP during the transition period of dairy cows and mastitis prevalence on activity of SOD and GPX in blood serum in terms of oxidative stress.

Table 3. Regression model for the influence of transition period and mastitis on SOD and GPX activity in blood serum of dairy cows

Fixed variable	df	SOD[a]	GPX[b]
Model[a,b]	5	136657.945***	12888995.85***
PP	2	24385.026***	605290.729***
Mastitis	2	366.940^{NS}	136337.019*
Error	628	1773.466	41883.377
Total	633		
$^{a}R^2 = 0,375$; $^{b}R^2 = 0,708$			

*** significant at level p<0.001
* significant at level p<0.05

The results indicated that the transition period in dairy cows had a statistically significant ($p<0.001$) influence on the SOD and GPX activity in blood serum. Therefore, the risk of manifesting oxidative stress in the transition period is high due to an imbalance in the activity of antioxidant enzymes. GLM revealed that udder disorders have a statistically significant influence on GPX activity in blood serum ($p<0.05$) but didn't have a statistically significant influence on SOD activity in blood serum. There was a low ($r = 0.141$), but statistically significant ($p<0.05$) positive correlation between SOD and GPX activity in blood serum.

DISCUSSION

Historically, strategies to enhance the transition into lactation for dairy cows were primarily aimed at infection prevention and optimizing energy consumption during this critical period, treating these concerns as separate entities. Recently, new frameworks have been proposed to understand the onset of various disorders during the transition period in dairy cows. These models propose that a mix of challenges, such as social stress, a deficit in energy balance, exposure to heat stress and endotoxins, along with oxidative stress may trigger inflammation, reduce feed intake, and compromise both metabolic and immune functions in this pivotal time. According to these insights, managing transition cows necessitates a comprehensive approach, recognizing the intricate interplay between the cow's environment, nutrition, and immune system.

The transition from pregnancy to lactation represents a critical phase for dairy cows, marked by substantial metabolic and physiological changes believed to contribute to compromised inflammatory and immune responses in the host (Sordillo & Aitken, 2009). The physiological stress accompanying mammary gland growth, rapid secretory parenchyma differentiation, and the initiation of milk synthesis and secretion results in an escalated oxygen demand. This heightened oxygen utilization gives rise to the production of ROS, intensifying the animal's oxidant status (Nakov et al., 2016).

During the periparturient period, cows experience a decrease in immune function, and the relationship with oxidative stress has been extensively explored (Leblanc 2008; Spears & Weiss 2008). Various factors contribute to making high-producing dairy cows particularly vulnerable to diseases like mastitis, among other common diseases during this period (Chapinal et al., 2012; Nakov et al., 2014). Notably, twenty-five per cent of mastitis cases occur within the first month following calving (Trajchev et al., 2017).

Celi et al. (2010) underscore the pivotal role of SOD as the primary defence mechanism against harmful ROS. The activity of GPX is crucial in safeguarding animal tissues from oxidative damage by aiding in the reduction of hydrogen and lipid peroxides, establishing it as an indicator of oxidative stress (Tuzun et al., 2002). The heightened SOD activity observed from the postpartum period to the 21st day of lactation likely resulted from increased ROS synthesis. However, as SOD activity elevates the production of hydrogenated peroxides, effective protection against ROS necessitates a corresponding increase in GPX activity. The augmented activity of antioxidant enzymes in early lactation suggests that cows experienced oxidative stress during parturition.

It is well-established that the activity of SOD and GPX undergoes dynamic changes during the transition period in dairy cows, potentially indicating a temporary imbalance of ROS, notably between 24-48 hours after parturition. Sharma et al. (2011) demonstrated a decrease in GPX and SOD values in early lactation compared to advanced pregnancy. Even if the activity of blood SOD and GPX is inhibited, the body can still defend against oxidative stress through alternative means. For instance, CAT is another antioxidant enzyme capable of breaking down hydrogenated peroxides, demonstrating that different antioxidant molecules can contribute to reducing ROS in the initial week after parturition (Drodge, 2002). Additionally, maintaining homeostasis involves various substances that collectively form a non-enzymatic antioxidant defence system (Lykkesfeldt & Svendsen, 2007). Our findings regarding the activity of SOD and GPX align with the observations of other researchers. Maurya et al. (2014) also noted elevated SOD and GPX activity in the blood postpartum when compared to the advanced gestation period.

When a dairy cow is affected by mastitis, be it clinical or subclinical, the immune system reacts to the mastitis pathogens, typically bacteria, by releasing various inflammatory molecules and immune cells. Shoji et al. (2003) outline that activated phagocytes generate significant quantities of ROS as part of the mechanisms for the neutralization of foreign organisms. In this context, the heightened activity of SOD and GPX observed postpartum is a consequence of the increased synthesis of ROS triggered by the immune response to mastitis pathogens. Protection against ROS is fully achieved through heightened GPX activity since SOD contributes to the dismutation of superoxide to hydrogen peroxide, which, in turn, serves as a substrate for GPX catalyzing their reduction. Additionally, other antioxidants like vitamin E and vitamin C also play a role in antioxidant mechanisms. Researchers suggest that the presence of mastitis pathogens can influence the activity and expression of antioxidative enzymes in the mammary gland (Matei et al., 2011; Darbaz et al., 2019, Nakov et al., 2023).

Kleczkowski et al. (2008) reported increased SOD activity in cows afflicted by mastitis and the level of activity was strongly associated with mastitis pathogens. Machado et al. (2014) found out that cows with mastitis had lower SOD activity in blood serum and supplement of microelements can increase their activity. Andrei et al. (2010) noted SOD activity in blood from healthy cows at 1688,4±165,48 U/g Hb, compared to 1764.5±110.46 U/g Hb in cows with mastitis.

There is much contradictory literature data on GPX activity in blood during inflammations. Erisir et al. (2006) found decreased GPX activity during the inflammation, Cimen et al. (2000) did not find significant changes in GPX activity, while Mezes et al. (1987) reported increasing in GPX activity during the inflammatory process in the animal organism. In addition, Berry & Meaney (2006) emphasized the GPX role in the protection of mammary gland tissue from the hazardous activity of ROM and alleviating oxidative stress intensity. Andrei et al. (2011) did not find a significant difference in GPX activity in blood between healthy cows and cows with mastitis.

According to Ranjan et al. (2005) and Jhambh et al. (2013), cows with clinical mastitis show lower activity of antioxidative enzymes and higher blood malondialdehyde (MDA) concentrations as an end-product of lipid peroxidation than healthy controls. Parantainen et al. (1987) noticed that the concentration of ROS in blood is elevated in cows that suffer from mastitis while GPX activity is sloping down, and this was in positive correlation with the occurrence of intramammary infections with major udder pathogens.

Kizil et al. (2007) found statistically significant changes in blood antioxidant concentration in healthy cows and cows that suffer from subclinical or clinical forms of mastitis. Based on previously reported research, it can be concluded that selenium as an active component of GPX and vitamin E has a positive effect on udder health and nonspecific defence of the organisms (Dobbelaar et al., 2010; Wang et al., 2010).

CONCLUSIONS

Effectively managing the transition period significantly influences a cow's profitability in the subsequent lactation phase. Dealing with both oxidative stress and mastitis during this challenging period for dairy cows requires a comprehensive approach that underscores the interconnection and interdependence of feeding, rearing conditions, and immunity. Particularly, the SOD/GPX ratio in the blood plays a crucial role in balancing ROS during the transition period. The data indicates that inflammation of the mammary gland markedly increases GPX activity in the blood, while heightened SOD activity in the blood coincides with the onset of lactation. The surge in SOD and GPX activity in the blood likely signifies adaptive changes in cows responding to oxidative stress. Consequently, an imbalance between increased ROS production and a decline in antioxidant capacity around parturition could intensify oxidative stress, contributing to mastitis occurrence in postpartum dairy cows. Providing a well-balanced diet with sufficient antioxidants and managing environmental stressors can help mitigate oxidative stress during the transition period and reduce the risk of mastitis in early lactation.

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