Bulletin UASVM, Veterinary Medicine 67(1)/2010
ISSN 1843-5270; Electronic ISSN 1843-5378

Correlations Between Antioxidant Enzymes Activity and Lipids Peroxidation Level in Blood and Milk from Cows with Subclinical Mastitis

Sanda ANDREI, Sorana MATEI, Daniela ZINVELIU, Adela PINTEA, Andrea BUNEA, Simona CIUPE, GROZA I.

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
Faculty of Veterinary Medicine, Str. Mănăştur no. 3-5, Cluj-Napoca, Romania,
Email: sanda_m_andrei@yahoo.com

Abstract. The aim of this study was the evaluation of enzymatic antioxidant status (catalase, superoxid dismutase) and lipids peroxidation level in blood and milk collected from cows with subclinical mastitis in comparison with healthy cows. The following tests were carried out on the 84 lactating cows, 10 of them having a positive diagnosis, representing 12% of the total lactating cows. For these 10 cows positive the diagnosis was confirmed by the increased number of somatic cells present in milk. Increasing number of somatic cells in milk affected the activity of antioxidant enzymes studied different. SOD activity does not change significantly in mastitic milk. The average values obtained were between 1.044±0,17 U/ml at milk from healthy cows and 1.066±0,15 U/ml for the mastitis milk. In the case of catalase activity were observed significant variations between normal and mastitis milk. Values obtained in mastitis milk had a higher average 2.09±0.42U/ml, compared to normal where the average was 1.21±0.35U/ml. Increased activity of antioxidant enzymes in milk were correlated with changes in activity of these enzymes in the blood. Thus, SOD activity in blood from healthy cows had mean values of 1688.4±165.48U/g Hb while in cows with mastitis mean values were 1764.5±110.46U/g Hb. For catase activity, the mean values were 1.46±0,32 U/g Hb in normal blood, while in blood from cows with subclinical mastitis were 2.05±0.17U/g Hb. In milk samples was noted that an increased level of somatic cells can be correlated with an increase in the concentration of MDA, so with increased lipid peroxidation processes.

Key words: subclinical mastitis, antioxidant enzymes, peroxidation, blood, milk

INTRODUCTION

Mastitis in dairy cows are the most important diseases of the mammary gland, with economic implications, due to losses in milk production and the risk posed by consumption of infected milk to public health. Subclinical mastitis infections are characterized by a decrease in milk secretion and its composition changes. A few hours after the infection of the udder with pathogenic microorganisms, the number of somatic cells (SCC) in milk increase in response to activation of inflammatory processes. Macrophages play an important role in overseeing infected gland. When the bacterias invade and colonize the mammary gland, macrophages respond by initiating the inflammatory response, one that attracts polymorphonuclear cells in milk to kill the bacterias. More than 90% of SCC in infected glands are composed of neutrophils [Groza, 2006; Andrei et al., 2009].

The antibacterial activity of neutrophils is mediated via reactive oxygen species (ROS) [Rinaldi et al., 2007]. Various infectious diseases of farm animals, such as pneumonia, enteritis and mastitis are associated with oxidative stress. Although essential for the body, an excess of oxidative reactions of bacterial processes may cause damage to the tissues. An

excess of ROS and the absence of optimal amounts of antioxidants results in oxidative stress development [Andrei et al., 2010; Swaisgood, 1995].

At the cellular level, ROS synthesis and accumulation are controlled by antioxidant enzyme systems including superoxid dismutase (SOD), catalase, peroxidases, the thioredoxine system - thioredoxinreductase, hemeoxygenase, etc. These enzymes are found distributed in different intracellular compartments. Thus, catalase is found in mitochondria and peroxisome where acting with glutathione peroxidase (GPx). The action of glutathione peroxidase in the cytoplasm is coupled with SOD. This will ensure protection of sub cellular structures, regulate the activation of oxygen and prevent formation of hydroxyl radicals. Non-enzymatic antioxidants are molecules that can oxidize easily and quickly in the presence of reactive oxygen species, thereby protecting against oxidation of molecules with a structural role or specialized functions in the body. Unlike the enzymatic antioxidant system contains a small number of antioxidant enzymes, non-enzymatic systems containing a large variety of molecules and act especially in extra cellular space, where enzymes are absent or occur in very small quantities. This system contains antioxidant vitamins (A, E, C), provitamine A, glutathione, uric acid, bilirubin; proteins have a role in binding iron and copper ions (transferrin, ferritin, and ceruloplasmin), various trace elements (copper, zinc, selenium) that are essential for antioxidant enzymes activity [Berger, 2006; Andrei et al., 2005].

Antioxidant activity of milk is due to the presence of antioxidant enzymes such as catalase, glutathione peroxidase, lactoperoxidase and superoxiddismutase, or vitamins such as vitamin A and provitamins and carotenoids, vitamin E, vitamin C.

Superoxiddismutase (EC1.15.1.1.), discovered in 1972 by McCord and Fridovich, is considered the most important enzyme characteristic for aerobic life, in terms of oxidative biochemical processes, and is present in all living cells. SOD shows the highest activity in the animal's blood. In case of pathological conditions (diabetes, cancer, inflammatory diseases, and cardiovascular diseases) enzyme activity is lower, either due to the appearance of inhibitors or due to a limited synthesis [Dejica, 2000]. Milk contains low levels of SOD, 150 times lower than blood. The enzyme present in cow's milk has the same structure with SOD from bovine erythrocytes. [Fox and Kelly, 2006]. The presence of this enzyme is important in maintaining the antioxidant stability of milk. Studies have shown that exogenous addition of SOD causes a reduction in lipid peroxidation processes, providing greater stability of milk.

Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide. In milk, the enzyme can be both mammary gland and bacterial origin, is heat labile and are disactivated within minutes at 65°C. Its absence in milk shows that it has been properly pasteurized. Present in large quantities, catalase is an indication that the udder has held an inflammatory process, with leukocyte influx issuing catalase. It was demonstrated that catalytic activity can be used as a marker of mastitis [Fox and Kelly, 2006].

One of the most used indicators of oxidative stress is the level of lipid peroxidation. Malonyl dialdehyde (MDA), formed by peroxidation of unsaturated fatty acids, is frequently used as a marker of peroxidation.

The aim of this work was to establish correlations between the biochemical indicators of oxidative stress in blood and milk from cows diagnosed with subclinical mastitis. A fierst step was the diagnosis of mastitis, using the method based on electrical conductivity and also the determination of milk somatic cells number. Next, in blood and milk samples, from cows diagnosed with subclinical mastitis compared with those from healthy cows, were examined the following biochemical parameters: activity of antioxidant enzyme (catalase, superoxid dismutase) and the level of oxidative degradation of lipids (classical assay based on the concentration of malonyl dialdehyde). By attending these objectives, we wanted to obtain new

information on the blood antioxidant activity compared with that of milk, and establish a correlation between this activity and oxidative stress induced by subclinical mastitis.

MATERIAL AND METHODS

The research was conducted during February 2010 - March 2010, in a dairy farm from the Apahida village, Cluj County. Of the total of 120 cows (mixed race from Austrian Bălţat with Red Holstein and Red Holstein metis) were 84 lactating cows.

Mastitis diagnosis was achieved with the aid of Waikato mastitis indicator, a physical method for determining the quality of milk by measuring the conductibility. The *somatic cells' counting was* performed using the MT-04 device.

Blood and milk samples were taken from cows diagnosed with subclinical mastitis, and from healthy cows. Blood samples were collected by jugular venepuncture with the use of heparin as anticoagulant. After collection, all the sample was immediately transferred to the laboratory for performing somatic cell counting and determination of biochemical parameters of oxidative stress.

Determination of *blood catalase activity* was performed using Cayman's assay kit. The method is based on oxidation reaction of methanol to formaldehyde and the right amount of hydrogen peroxide. Formaldehyde is then dosed based on reaction with a specific chromogen. Determination of *blood SOD activity* was performed using the kit RANSOD, from Randox Laboratories. For both enzymes the hemoglobin content in blood was measured and the activity expressed as Units/g Hemoglobin (U/g Hb).

Determination of *catalase and SOD activity in milk* was realized by photometric methods. The first step in this analysis was the dissolution of casein micelles, a process known as clearing milk. This was achieved using a solution containing urea and a reducing agent - dithiotrietol. Determination of catalase activity was done by photometric method proposed by Sinha (1972) and adapted for milk. Determination of SOD activity was performed using RANSOD kit, from Randox Laboratories. For both enzyme activity was calculated and expressed in U/ml milk.

Lipids peroxidation level. For quantitative determination of the MDA a photometric method with thiobarbituric acid was used (Pintea et al., 2008). The results were expressed in nmol MDA/ml plasma and MDA nmol /ml milk.

RESULTS AND DISCUSSION

Following tests carried out on the 84 lactating cows, 10 of them had a positive diagnosis, representing 12% of the total so lactating cows. For the 10 cows, positive diagnosis was confirmed by the increased number of somatic cells present in milk. Thus, values obtained were between 500,000 and 1.5 million cells / ml. In healthy cows, somatic cell count has not exceeded the value of 270,000 cells / ml.

Results obtained from analysis of antioxidant enzyme activity in milk and blood samples (mean and standard deviation) are presented in Tables 1 and 2.

Superoxid dismutase (SOD) radically accelerates the dismutation of the toxic superoxide to hydrogen peroxide and is considered the first intracellular defense against reactive oxygen species. The cytosol of all eukaryotic cells contains CuZn-SOD. Determination of SOD is important in the evaluation of antioxidant status, under physiological or pathological conditions. As can be seen in table 2, the SOD activity has an

average of 1688 U/g Hb for healthy cows. A small increase of SOD activity can be observed in cows with subclinical mastitis.

According to the literature, SOD activity in cow's milk ranges from 0.92 to 1.27 U/ml, this activity was not influenced by factors such as lactation stage or age of the animal [Lindmark-Maensson and Aekesson, 2000]. As you can see, the results obtained when we analyzed samples have minor variations in SOD activity (Table 1). Average values obtained ranged from 1.044 U/ml in milk from healthy cows to 1.066 U/ml in milk from cows with subclinical mastitis, both values being close to normal limits presented in the literature. In this study, the SOD was not affected by the number of somatic cells. The SOD activity is not correlated with the somatic cells of milk and not influenced by an elevated cellular numbering. Holbrook and Hicks (1978) have examined the SOD in bovine milk and reported that the enzyme was identical, from the point of view of its electrophoretic properties, to those of bovine erythrocyte SOD, with no evidence that SOD was derived from bacterial or somatic cells present in milk.

Tab 1.

The activity of antioxidant enzymes in normal and subclinical mastitis milk

Milk samples	SOD U/ml milk	Catalase U/ml milk
Normal	1.044±0.17	1.21±0.35
Subclinical mastitis	1.066±0.15	2.09±0.42

Tab 2.

The activity of antioxidant enzymes in blood of normal cows and cows diagnosed with subclinical mastitis

Blood samples	SOD U/mg Hb	Catalase U/ mg Hb
Healthy Cows	1688.4±165.48	1.46±0,32
Cows with subclinical mastitis	1764.5±110.46	2.05±0.17

The results obtained in determination of blood catalase indicate that the activity of this enzyme shows no significant changes. Thus, cows with sub clinical mastitis were underlined average value of 2.05 U/mg Hb, while in healthy animals mean values obtained were 1.46 U/mg Hb. In the analysis of catalase in milk samples was observed that enzyme activity increases in mastitis milk compared to normal milk. Catalase activity present in milk varies according to diet, lactation stage and especially for mastitis. It was demonstrated that catalase activity can be used as a marker of mastitis, in these cases the activity of catalase is much higher compared with normal milk [Fox and Kelly, 2006]. Our results confirm those of Hamed et al. (2008) who found a positive correlation between the catalase activity and the milk somatic cell counts. They signaled that catalase plays a central role in milk redox control. Especially during mastitis, catalase activity increases markedly, making it a useful indicator of mastitis. Also, they demonstrated that the catalase activity increased more with neutrophils than with macrophages and lymphocytes, indicating its role in free-radical decomposition in the case of bacterial infection resulting in an important increase of milk somatic cells.

From the experimental point of view, in oxidative stress state, in addition to modification of antioxidant enzymatic activity and decreased concentrations of non enzymatic antioxidants, it is noted an increase in ROS concentration and also of the concentration of the products resulting from oxidative degradation of lipids, protein and nucleic acids.

Effect of mastitis on the fat content is not yet fully understood, articles from the literature provided contradictory data. It is known that mastitis milk fat is easily susceptible to the action of lipases produced by leukocytes that occur in the mammary gland in response to inflammation. The action of these enzymes causes a decrease in glycerides concentration and an increase of fatty acid peroxidation processes. Milk with higher content of somatic cells is more susceptible to the processes of lipolysis and peroxidation [Pterovski and Stefanov, 2006].

One of the most important substrate for peroxidation is represented by polyunsaturated fatty acids (PUFA) of lipid from cell membranes and subcellular components. PUFA peroxidation process lead to the formation of aldehydes and ketones with low number of carbon atoms, among them is malonyl dialdehyde (MDA). MDA concentration is frequently used as markers of peroxidation in different biological media [DelRio et al., 2005].

The results obtained in the determination of malonyl-dialdehyde in the samples analyzed are presented in Table 3.

Tab 3.

Concentration of MDA (nmoli ml) in samples collected from healthy cows and cows with subclinical mastitis

Samples	MDA nmoli/ml plasma	MDA nmoli/ml milk
Healthy Cows	37.86±2.68	16.84±3.94
Cows with subclinical mastitis	129.59±22.40	48.97±17.14

In milk samples was seen that an increased level of somatic cells can be correlated with an increase in the concentration of MDA. Also, in cows with mastitis, the level of serum lipid peroxidation is increased in comparison with healthy cows. These data are similar to those presented by the literature. Thus the study presented by Suriyasathaporna et al. (2006) was demonstrated that serum lipid peroxidation levels in cows with mastitis compared to healthy increases, while the glutathione-peroxidase activity is lower compared to values recorded in healthy cows.

CONCLUSIONS

- In conclusion, the present study demonstrated that mastitis infections, even in subclinical phase, determine the appearance of oxidative stress state. This condition is manifested both in blood and in milk secreted by infected mammary glands. Occurrence of oxidative stress has been demonstrated both through induced changes in antioxidant enzyme activity level and by increasing levels of lipid peroxidation in blood and milk.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS –UEFISCSU, project number 1044/2009 PNII – IDEI 1482/2009.

REFERENCES

1. Andrei, Sanda, Adela Pintea, B. Sevastre, and A.I. Baba (2005). Study regardind the mechanisms involved in oxidative stress induction in rats i.p. injected with asbestos. Iaşi,Universitatea de Stiinte Agricole şi Medicină Veterinară “Ion Ionescu de la Brad”, Lucrări Ştiinţifice, 48(7): 72-79.

2. Andrei Sanda, Adela Pintea, A. Bunea,I. Groza,L. Bogdan, S. Matei, S. Ciupe, and D. Crainic (2009). Non-enzymatic antioxidants concentration and lipids peroxidation level in milk from cows with subclinical mastitis. *Bulletin USAMV series Veterinary Medicine* 66(1): 196-202.
3. Andrei Sanda, Adela Pintea, I. Groza, L. Bogdan, S. Ciupe, and S. Matei (2009). Milk antioxidant enzymes activity in cows with subclinical mastitis. Iaşi, Universitatea de Ştiinţe Agricole şi Medicină Veterinară "Ion Ionescu de la Brad" Lucrări Ştiinţifice, seria Medicină Veterinară 52:1-6 .
4. Berger, M. (2006). Nutritional manipulation of oxidative stress: review of the evidence, Clinical Nutrition and Metabolism, www.science direct.com, 1-7
5. Dejica, D. (2000). Stresul oxidativ in bolile interne, Editura Casa Cartii de Stiinta, Cluj-Napoca.
6. Del Rio, D., A. J. Stewart , and N. Pellegrini (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism & Cardiovascular Diseases 15: 316-328.
7. Fox P., and A. Kelly (2006). Indigenous enzymes in milk: Overview and historical aspects—Part 1, International Dairy Journal 16: 500–516.
8. Groza I.S. (2006). Ginecologie, andrologie si obstretica veterinara – Compendiu, Editura Academiei Romane, Bucuresti.
9. Hamed, H., Abdelfettah El Feki, and G. Ahmed (2008). Total and differential bulk cow milk somatic cell counts and their relation with antioxidant factors. C. R. Biologies Physiology / Physiologie 331:144–151.
10. Holbrook, J., and C.L. Hicks (1978). Variation of superoxide dismutase in bovine milk. J. Dairy Sci. 61:1072–1077.
11. Lindmark-Maensson, H., and B. Aekesson (2000). Antioxidative factors in milk. British Journal of Nutrition 84 (1):S103-S110.
12. Pintea Adela, Andrei Sanda, C.Bele (2008). Biochimie Medical veterinara – lucrari practice, Editura AcademicPres, Cluj-Napoca.
13. Petrovski, K., and E. Stefanov (2006). Milk composition changes during mastitis, www.milkproduction.com/library/articles.
14. Rinaldi, M., P. Moroni, M. J. Paape, and D. Bannerman (2007). Evaluation of assays for the measurement of bovine neutrophil ROS. Veterinary Immunology and Immunopathology 115:107–125.
15. Suriyasathaporna, W., U. Vinitketkumnuen, T. Chewonarin, S. Boonyayatra,and Y.H. Schukken (2006). Higher somatic cell counts resulted in higher malondialdehyde concentrations in raw cows' milk. International Dairy Journal 16:1088–1091.
16. Swaisgood, H. (1995). p. 472-475. Enzymes Indigenous to Bovine Milk – Hanbook of milk composition, Academic Press. Inc.