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Correlation Between Mastitis Pathogenic Bacteria and Glutathione Peroxidase Activity in Cows Milk

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Abstract: Antioxidant activity of milk is due to the presence of antioxidant anzymes such as catalase, lactoperoxidase, glutathione-peroxidase, xanthin/oxidase, or vitamins and provitamins such as retinoils and carotenoids, tocopherols and ascorbic acid. The aim of this study was to establish a correlation between pathogenic bacteria type involved in cows mastitis and glutathione-peroxidase activity in milk. Determination of the enzyme activity was performed on milk samples, before and after germs inoculation with various dilutions of *Streptoccocus spp.*, *Staphylococcus spp. Pseudomonas spp. Escherichia spp. Candida spp.* To show the glutathione-peroxidase activity during milk bacteria multiplication, an *in vitro* protocol for germs development in liquid medium (Muller Hinton agar with milk) was performed. Glutathione peroxidase activity in milk samples was performed on skimmed milk, using a commercial kit (Ransel, Randox Laboratories) and semiautomatic biochemistry analyzer MasterPlus Screen. We observe the fact that development of the pathogenic bacteria in milk is accompanied by a significant increase of glutathione-peroxidase activity. The level of enzyme activity depends on the type of bacteria. The highest values were in milk samples inoculated with *Escherichia coli*, values that exceeded 10 times the values of normal milk samples. For *Streptococcus viridians, Pseudomonas aeruginosa and Candida spp.* were established modest results, the lowest values being registered from samples containing *Staphylococcus aureus*.

Keywords: mastitis, pathogenic bacteria, glutathione-peroxidase, milk, cows.

INTRODUCTION

Dairy products can be benefical for the oxidative defence of consumers by several enzymatic and nonenzymatic mechanism. Milk antioxidant systems have important roles in preventing lipid peroxidation and maintaining milk quality (Chen J. et al., 2003). Antioxidant activity of milk is due to the presence of antioxidant anzymes such as catalase, lactoperoxidase, glutathione-peroxidase, xanthin/oxidase, or vitamins and provitamins such as retinoils and carotenoids, tocopherols and ascorbic acid (Sanda Andrei et al., 2009; Lykkesfeldt J. and Svendsen O., 2007).

When bacteria invade and colonize the mammary gland, macrophages respond by initiating the inflammatory response, attracting polymorphonuclear (PMN) cells in milk to kill bacteria. More than 90% of somatic cells found in infected glands are neutrophils (PMN). Antibacterial activity of neutrophils is mediated via reactive oxygen species (ROS)

such as hydroperoxides and peroxides (Rinaldi M. et al., 2007). An excess of ROS and the absence of optimal amounts of antioxidants result in oxidative stress development.

Glutathione peroxidase is an antioxidant enzymes in milk, it catalyses the reduction of different peroxides aided by glutathione or other reducing substrates. Two different classes of GPx - selenium-dependent (EC 1.11.1.9) and selenium-independent (EC 2.5.1.18), are known. Both utilize glutathione for reducing hydroperoxides, but selenium-dependent enzymes are also capable of reducing hydrogen peroxide (H_2O_2) (Lindmark-Mansson H. and Akesson B., 2000).

Previous studies have shown that in milk from cows diagnosed with subclinical mastitis, is a statistically significant increase in glutathione peroxidase activity compared to normal milk. This increase is statistically correlated with milk somatic cell count (SCC) and colony forming units (CFU). Increased enzyme activity can be explained, first by hydrolysis of casein-enzyme complex, leading to enzyme release and, on the other hand, the antioxidant defense mechanisms specific for pathogens. The positive correlation between SCC and GPx activity suggest that these enzyme may have potential to detect subclinical mastitis in dairy cows (Sanda Andrei et al., 2011).

The aim of this study was to establish a correlation between pathogenic bacteria type involved in cows mastitis and glutathione-peroxidase activity in milk. Determination of the enzyme activity was performed on milk samples, before and after germs inoculation with various dilutions of *Streptoccocus spp., Staphylococcus spp. Pseudomonas spp. Escherichia spp. Candida spp.*

MATERIALS AND METHODS

Milk samples

To show the glutathione-peroxidase activity during milk bacteria multiplication, an *in vitro* protocol for germs development in liquid medium (Muller Hinton agar with milk) was performed. Some bacterial species isolated from cows with mastitis were introduced in all milk samples according to the folowing table:

Tab. 1.

The pathogenic bacteria inoculated in milk samples

Entry	Samples	Dilution
1.	Normal milk	-
2.	Normal milk + Müller Hinton agar	-
3.	Milk + *Streptococcus viridans*	1
4.	Milk + *Streptococcus viridans*	2
5.	Milk + *Staphylococcus aureus*	1
6.	Milk + *Staphylococcus aureus*	2
7.	Milk + *Pseudomonas aeruginosa*	1
8.	Milk + *Pseudomonas aeruginosa*	2
9.	Milk + *Escherichia coli*	1
10.	Milk + *Escherichia coli*	2
11.	Milk + *Candida spp.*	1
12.	Milk + *Candida spp.*	2

Normal milk samples and and milk samples inoculated with bacteria in various dilution were placed in a thermostat at 37 ˚C for 24 hours. We used two types of dilutions: on

the first dilution the quantity of the bacteria in milk was 100 ml with a density of 0.5 McFarland and for the second dilution de amount of pathogenic bacteria was 500 ml with a density of 0.5 McFarland.

Glutathione peroxidase activity in milk samples was performed on skimmed milk, using a commercial kit (Ransel, Randox Laboratories) and semiautomatic biochemistry analyzer MasterPlus Screen. The final results were reported in units per ml of milk ($U.mL^{-1}$ milk) (Sanda Andrei et al., 2011).

RESULTS AND DISCUSSIONS

The *in vitro* experiments results regarding the activity of milk glutathione-peroxidase from milk samples inoculated with pathogenic bacteria involved in mastitis are the following:

Tab. 2.

Glutathione-peroxidase activity in milk samples inoculated with pathogenic bacteria involved in cows mastitis

Samples no.	**Isolated bacterial species**							
	Normal milk	**Müller Hinton agar**	***Staphylococcus aureus***	***Streptococcus viridans***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida spp.***	**Glutathione-peroxidase activity ($U.mL^{-1}$)**
1.	+							35.9
2.	+	+						29.9
3.	+			+				130.2
4.	+			+				157.4
5.	+		+					121.4
6.	+		+					134.5
7.	+					+		133.4
8.	+					+		172.8
9.	+				+			151.5
10.	+				+			245.6
11.	+						+	169.8
12.	+						+	175.1

Glutathione peroxidase is an antioxidant enzymes in milk, it catalyses the reduction of different peroxides aided by glutathione or other reducing substrates. Two different classes of GPx - selenium-dependent (EC 1.11.1.9) and selenium-independent (EC 2.5.1.18), are known. Both utilize glutathione for reducing hydroperoxides, but selenium-dependent enzymes are also capable of reducing hydrogen peroxide (H_2O_2) (Torres A. et al., 2003; Swaisgood H.E., 1995). In our study, the average value for GPx activity in normal milk was 33 $U.mL^{-1}$. Our results are in line with those of Lindmark-Mansson and Akesson (2000) who reported that GPx activity in cow's milk has values ranging between 12 and 32 $U.mL^{-1}$ whereas its activity is correlated significantly with selenium concentration.

The development of pathogenic bacteria in milk is accompanied by a significant increase of glutathione-peroxidase activity (table 2). The level of enzyme activity depends on the type of bacteria. Thus, the highest values were in milk samples inoculated with *Escherichia coli*, values that exceeded 10 times the values of normal milk samples. For *Streptococcus viridians, Pseudomonas aeruginosa and Candida spp.* were established modest results, the lowest values being registered from samples containing *Staphylococcus aureus.*

During phagocytosis, phagocytic cells generate superoxide and other reactive oxygen species, which are involved in antibacterial activity. Many pathogens possess antioxidant defenses such as superoxide dismutase and catalase that may explain their survival. In bacteria involved in mastitis infections, such as *Streptococcus agalactiae*, various studies have shown that the glutathione can be synthesized. In bacteria these molecule plays an important roles in the protection of cell against oxidative stress. The resistance of bacteria to hydrogen peroxides was found to be dependent on the accumulation of glutathione, which can activate glutathione- glutathione peroxidase - glutathione reductase system in cells (Kino K. et al., 2007; Sanda Andrei et al., 2011)

CONCLUSIONS

The activity of glutathione-peroxidase in normal milk showed averages values of 33 U/ml, data in concordance with scientific literature Enzymatic activity increases in milk, regardless the type of pathogenic bacteria and applied dilution, but is different depending on species. The highest activity was determined for *Escherichia Coli* species and the lower values were for *Staphylococcus aureus* species.

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