https://doi.org/10.17221/67/2019-VETMED

The occurrence of mastitis and its effect on the milk malondialdehyde concentrations and blood enzymatic antioxidants in dairy cows

Frantisek Zigo[1], Juraj Elecko[1], Milan Vasil[1], Silvia Ondrasovicova[2], Zuzana Farkasova[1], Jana Malova[3], Ladislav Takac[4], Martina Zigova[6], Jolanta Bujok[7], Ewa Pecka-Kielb[7], Petra Timkovicova-Lackova[5]*

[1]Department of Animal Breeding, [2]Department of Anatomy, Histology and Physiology, [3]Department of Food Hygiene and Technology, [4]Institute of Judicial and Public Veterinary Medicine and Economics, [5]Institute of Nutrition, Dietetics and Feed Production, University of Veterinary Medicine and Pharmacy, Košice, Slovakia

[6]Department of Pharmacology, Faculty of Medicine, University of Pavol Jozef Šafárik in Košice, Košice, Slovakia

[7]Department of Biostructure and Animal Physiology, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

**Corresponding author: frantisek.zigo@uvlf.sk*

Citation: Zigo F, Elecko J, Vasil M, Ondrasovicova S, Farkasova Z, Malova J, Takac L, Zigova M, Bujok J, Pecka-Kielb E, Timkovicova-Lackova P (2019): The occurrence of mastitis and its effect on the milk malondialdehyde concentrations and blood enzymatic antioxidants in dairy cows. Veterinarni Medicina 64, 423–432.

Abstract: Early identification of mastitis is a serious challenge for dairy farmers and veterinarians in ensuring the health of an animal and the hygienic quality of the produced milk. The purpose of this study was to detect the occurrence and aetiology of mastitis in a dairy herd of 153 milked cows localised in a farm in west Slovakia. During the complex investigation, 606 quarter milk samples were examined (6 quarters were discarded) and classified based on the clinical status, the presence of abnormal udder secretions, the result of the California mastitis test (CMT), the somatic cell count (SCC) and the bacteriological identification of the pathogens causing the intramammary infection (IMI). The study was augmented by the detection of malondialdehyde (MDA) in the milk and the measurements of the blood enzymatic activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) as potential biomarkers for the udder health screening. A positive CMT score was recorded in 19.5% (118) of the examined quarters and 12.5% (76) of the quarters were infected with bacterial pathogens causing latent mastitis (LM; 1.3%), subclinical mastitis (SM; 8.3%), and clinical mastitis (CM; 2.9%). The most commonly isolated bacteria from the infected quarters were coagulase-negative staphylococci (55.2%), *Staphylococcus aureus* (11.8%) and streptococci (10.5%). The concentration of MDA and SCC were significantly higher from both the SM and CM cases than in the milk samples from the healthy cows, while the blood activities of SOD and GPx were lower in the cows with CM compared to the healthy cows. The higher MDA concentrations in the SM and CM milk observed in this study showed the presence of an oxidative stress in the infected milk, accompanied by a decrease in the antioxidative enzymatic activity in the blood of the cows. Therefore, the measurement of the milk MDA concentration and the activity of the blood SOD and GPx may prove insightful for the better screening of the udder health in the early diagnosis of mastitis.

Keywords: milking; biomarkers; lipid peroxidation; udder health; staphylococci

Supported by grants APVV (No. SK-PL-18-0088), VEGA (No. 1-0529-19, Slovak) and PPN-BIL-2018-1-00134-U-00001 (Poland).

https://doi.org/10.17221/67/2019-VETMED

The economic value of dairy cows is mainly determined by the milk yield and longevity. The most important factor affecting the quantity and quality of the produced milk is the occurrence of production diseases, especially mastitis (Zajac et al. 2012).

Mastitis or an intramammary infection (IMI) is characterised by the physicochemical changes of the milk, accompanied by an increase in the somatic cell count (SCC) and bacterial pathogens, in addition to changes in the mammary tissue depending on the type of the disease (Malinowski et al. 2006; Zigo et al. 2019).

All around the world, mastitis is known as a multifactorial disease, closely related to the production system and the environment. The incidence of mastitis increases when the immunological and antioxidant defence mechanisms of the mammary gland are impaired. Dairy cows are exposed to numerous genetic, physiological, and environmental factors associated with both the host and pathogens, which can compromise the host's immunity, hence, increase the incidence of mastitis (Turk et al. 2012; Andrei et al. 2016).

According to Vasil et al. (2009), more than 140 different microorganism species are considered to cause mastitis. Bacteria are the most common causative factor, recognised in more than 95% of mastitis cases. Globally, the most common mastitis-causing bacteria in dairy ruminants are *S. aureus* and coagulase-negative staphylococci (CNS), as well as streptococci and *E. coli*, which may have a similar or higher prevalence than that of staphylococci (Taponen et al. 2007).

Based on the intensity and severity of the clinical signs, mastitis is usually divided into a subclinical and a clinical disease. In clinical mastitis (CM), the signs range from mild to severe and can be systemic, local, or milk related, while in subclinical mastitis (SM) no signs are observed. The most prominent signs of CM are swelling, heat, hardness, redness or pain of the udder. The milk of a cow with CM has a watery appearance with flakes, clots or pus often present. During SM, the udder and milk may appear normal with the infection still present. The increase in the SCC is associated with the reduced milk production in the range of 60–140 litres per cow annually in the animals with SM (Sztachanska et al. 2016).

Ashfaq and Muhammad (2008), reported that the subclinical cases of mastitis are more common than the CM ones. It is estimated that in a herd of dairy cows that there are approximately 15–40 undetected cases of SM for each case of CM.

The lack of symptoms has proved to be the bane of the early detection of mastitis, constituting an important factor for dairy farmers and veterinarians in determining the health of an animal and the hygienic quality of the milk produced. Economic aspects interfere with the routine assessment of the California mastitis test (CMT) because the score evaluation according to the colour reaction is often insufficient. In addition to the assessment of the CMT, a bacteriological examination of the raw milk samples complemented by alternative parameters may aid the better screening of the udder health. Oxidative stress indicators such as the malondialdehyde (MDA) concentration and the activity of antioxidant enzymes may belong to these complementary indicators (Suriyasathaporn et al. 2006; Suriyasathaporn et al. 2010; Turk et al. 2017).

MDA is one of the relatively stable peroxidation products and the most widely used indicator for the assessment of the oxidative stress (Castillo et al. 2006). The oxidative stress is related to some disorders in cattle, for example, mastitis, a retained placenta, an udder oedema or muscle and metabolic diseases (Sharma et al. 2011). It was proven that bacterial infections and other inflammation processes affecting the mammary tissue causing an increased SCC in the milk, especially in the polymorphonuclear leukocytes (PMN) and macrophage numbers, are associated with the generation of the reactive oxygen species (ROS) (Castillo et al. 2006). The accumulation of ROS correlated with a reduction of the antioxidative activity due to the catalysis of various hydrogen and lipid peroxides can lead to the oxidative stress (Suriyasathaporn et al. 2009).

Oxidative stress detection using biomarkers is a relatively new field of research in ruminant health. Therefore, the aim of this study was to determine the incidence and aetiology of the mastitis using the standard methods enhanced by the detection of the milk MDA and the blood activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) as potential biomarkers for the udder health screening.

MATERIAL AND METHODS

Animals and milking. This study was conducted on 180 spotted Slovak dairy cows with an aver-

https://doi.org/10.17221/67/2019-VETMED

age annual milk production of 6971 kg in a farm in west Slovakia. The cows were kept in a free housing system with deep litter based on the repeated spreading of straw every two days. The basis of the cows feeding was a total mixed ration (TMR), which consists of grass silage, maize silage, hay, and concentrate according to international standards (NRC 2001) to meet the nutritional requirements of a 600 kg cow yielding 15–25 kg of milk/day. The cows were milked twice a day in a fishbone milking parlour (DeLaval, UK) 2 × 10 stalls with a standard exit and a single return lane. The first milking started at 4.30 a.m. and the second at 4:30 p.m. The milking routine began with the udder washing with water from a hose to remove any impurities. Subsequently, the udder was thoroughly wiped with disposable paper wipes. The forestripping from each quarter was hand-drawn into a dark-bottomed cup for the visual assessment of the milk's consistency. The milking and pulsation vacuum was set at 42 kPa. The pulsation ratio was 60 : 40 at a rate of 52 c/min and termination was automatically signalled when the milk flow dropped to 0.2 l/min. After the milking process, the teats were disinfected by a teat dipping. The milk was stored in refrigerated milk tanks at +5 ℃ and removed daily around 11.30 a.m.

Herd examination and milk sample collection. During the practical part of the study, 27 cows from the herd were separated in calving boxes or were milked into separate vessels for the first 12 days after calving. A thorough examination of the udder health in the remaining 153 lactating cows included a veterinary history, a clinical examination, and a sensory analysis of the milk from the forestripping of each udder quarter followed by an assessment of the CMT with the subsequent collection of the milk samples. The veterinary history included important information such as changes in the behaviour or the physical state, the date of the last calving, the date of insemination, the hygiene of the holdings, the milking performance, the feed, the milking procedure and recent changes in the daily procedures. After considering the history, the udder from each milked cow was examined by visual inspection and palpation for any clinical signs of oedema, spontaneous triggering of milk, and signs of inflammation or atrophy.

The CMT (Indirect Diagnostic Test, Krause, Denmark) from 606 quarters (6 quarters were atrophied without milk secretion) from the 153 milked cows was performed (Table 1). According to the procedure by Jackson and Cockcroft (2002), the milk from every quarter was mixed with the reagent and the result was read as the trace, a score of 1, 2, 3, 4 or negative depending on the gel formation in the milk sample. A negative score is typical with a normal consistency after stirring. A trace score is seen as slightly thickening, but disappears after prolonged mixing. A score of 1 is seen as the easy thickening of the liquid, which appears after 20 s of mixing, but no tendency toward gelling. A score of 2 is seen as the mixture thickens after 20 s of mixing and a gel formation is suggested.

Table 1. The evaluation of the California mastitis test (CMT) and the Somatic cell count (SCC) in the monitored herd

	All exanimated quarters[1] from 153 cows	Healthy quarters	Quarters with a positive CMT score[2]	Infected quarters[3]	Evaluation of the CMT score and the SCC from examined quarters			
					CMT score	*n*	%	SCC × 10^3
					0 (negative)	488	80.5	94.53 ± 9.85
					T (trace)	26	4.3	282.00 ± 15.39
					1	38	6.3	505.10 ± 27.25
					2	29	4.8	861.56 ± 33.40
n	606	488	118	76	3	18	3.0	1695.50 ± 31.34
%	100	80.5	19.5	12.5	4	7	1.2	5724 ± 57.96

n = the number of quarters

All the examined quarters[1] = the quarters with milk secretion (6 quarters were atrophied); Quarters with a positive CMT score[2] = the quarters with a positive evaluation of California Mastitis Test with the score: trace, 1, 2, 3 and 4; Infected quarters[3] = positive quarters after the bacteriological investigation (see Table 2)

https://doi.org/10.17221/67/2019-VETMED

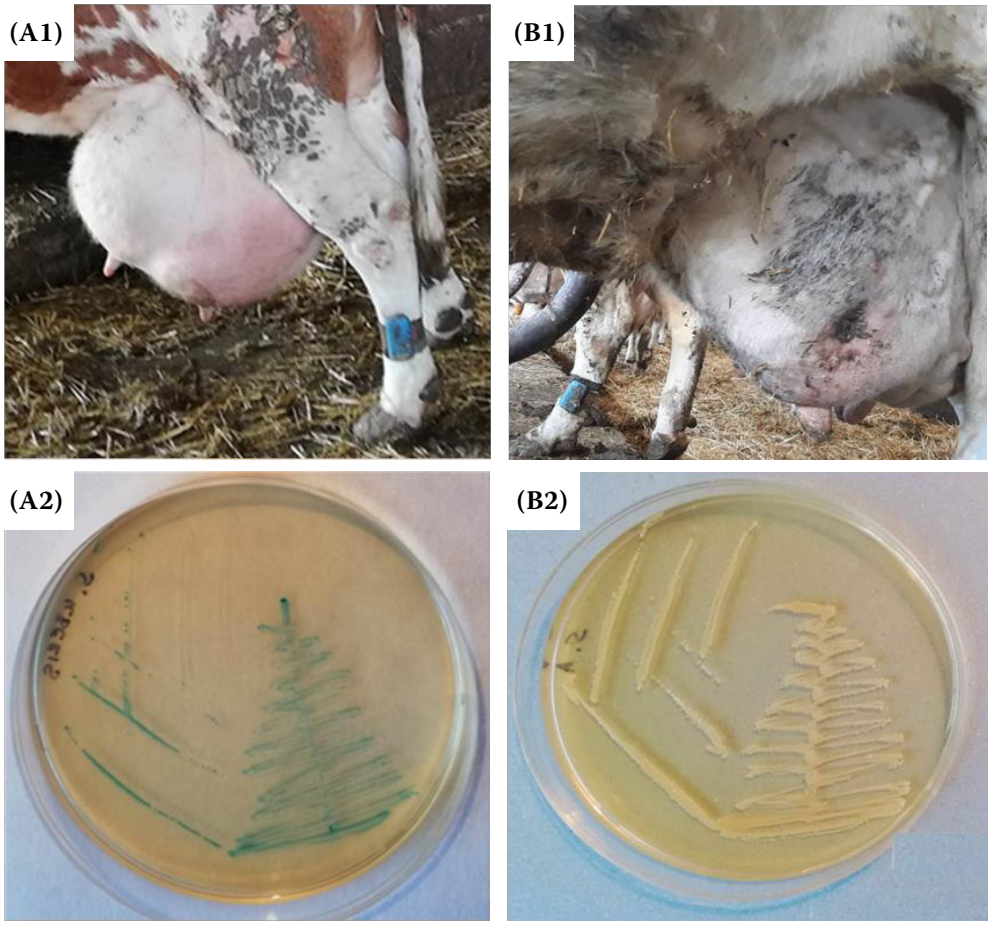


Figure 1. The clinical mastitis cases with a positive bacteriological cultivation

IMI = intramammary infection

The clinical IMI caused of *Str. uberis* (**A1**–**A2**), the clinical IMI caused of *S. aureus* (**B1**–**B2**)

A score of 3 is seen as an immediate concentration with a gel formation. A score of 4 is characteristic with a strong gel formation and the surface of the mixture becomes convex.

Thereafter, two samples (10 ml) of the quarter milk were collected aseptically from all of the 153 milked cows for bacteriological cultivation and SCC measurement in accordance with the guidelines of the National Mastitis Council (2001). The cooled samples were immediately transported to the laboratory of the University of Veterinary Medicine and Pharmacy in Kosice.

Experimental group selection for the oxidative stress determination. Based on the veterinary history, the CMT assessment and the clinical examination for the measurement of the milk MDA concentration and the blood enzymatic activities of GPx and SOD, the cows were assigned into one of three groups: control (healthy cows), cows with subclinical mastitis (SM) and clinical mastitis (CM). For the SM group, 43 cows without any clinical signs of mastitis or other illnesses, but with a positive CMT of at least one quarter were included. Of which 77 quarters were selected for MDA detection that showed a positive CMT (a score of 1–4). Seven cows with clinical signs were included to the CM group (Figure 1), from which 15 quarters for MDA detection with a high score of CMT ≥ 3 were selected. Seven (28 quarters) of the perfectly healthy cows with a negative CMT score and without any clinical signs were randomly selected to the control group.

The selected quarter milk samples from each group were then collected into 12 ml plastic tubes for the MDA detection. The blood for the SOD and GPx measurement from the selected cows were withdrawn by puncture of the *vena jugularis* and collected into heparinised tubes. The samples were placed on ice, and immediately transported to the laboratory where they were processed according to the instructions of the enzyme assay kits of the manufacturer and stored at –56 °C until the analysis was performed. The MDA was measured in the milk samples on the day of the collection.

SCC and MDA detection in the milk samples and the blood antioxidant enzymes. The SCC

https://doi.org/10.17221/67/2019-VETMED

in the quarter milk samples was measured using a Somacount 150 apparatus (Bentley Instruments Inc., Minnesota, USA). The lipid peroxidation products in the selected milk samples were determined by the spectrophotometric measurement (Shimadzu, UV 160) of thiobarbituric reactive substances at 532 nm, according to the method described by Andrei et al. (2016). The results were expressed in nmol of MDA per ml of milk (nmol/ml).

After the thawing and warming of the blood samples with erythrocyte lysate, the detection of the SOD activity was performed using a Ransod kit (Randox Laboratories, Crumlin, UK) according to the method of Woolliams et al. (1983). The erythrocyte glutathione peroxidase activity was assessed using a Ransel kit (Randox Laboratories, Crumlin, UK) by the kinetic method (Paglia and Valentine 1967). A Chemistry Analyser Olympus AU 400 (Olympus, Mishima, Japan) was used as the spectrophotometer in this study. The activity of the SOD and GPx enzymes was normalised to the haemoglobin concentration measured by Drabkin's method according to the previous study of Balasubramaniam and Malathi (1992), expressed as units per g of haemoglobin (IU/g Hb).

Microbiological examinations. The bacteriological examinations and identification were performed according to the generally accepted principles (Malinowski et al. 2006). The quarter milk samples (10 µl) were inoculated on a Petri dish with a Columbia blood agar base (Oxoid, UK) with 5% of defibrinated ram blood and incubated for 48 h at 37 °C, the dish was examined after 24 and 48 h of incubation. The suspected colonies were inoculated and cultured on selective media such as *Staphylococcus* medium N°110, Baird-Parker agar, Brilliance™ UTI Clarity Agar, Edwards Medium, MacConkey Agar (Oxoid, OXOID Ltd., Basingstoke, Hants, UK). The parameters, such as the colony size and appearance, pigment production and coagulase, catalase activity, haemolysis, and Gram staining were considered in the determination of the bacterial species. The colonies of *Staphylococcus* spp. were tested for the coagulase activity (Staphylo PK, Imuna Pharm, SR). The growth-confirmed colonies of *Staphylococcus* spp., *Streptococcus* spp. (Figure 1) and *Enterobacteriacae* spp. were further identified biochemically using the STAPHYtest 24, STREPTOtest 24, resp. ENTEROtest 24 (Erba-Lachema, Brno, CZ) and the software TNW Pro 7.0 (Erba-Lachema, Brno, CZ).

Statistical analysis. All the data were analysed statistically using the GraphPad Prism Software, Version 4.00 (GraphPad Software Inc., CA, USA). The data were presented as the mean (M) ± standard error of the mean (SEM). The differences in the selected groups of the control, SM and CM cows were determined by an analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. A statistical analysis by variable probabilities for the Post Hoc Test was used to compare the milk MDA concentrations from the negative and positive milk samples with a CMT score of 1–4. The criteria for the statistical significance was set at $P < 0.05$.

RESULTS

The udder health status assessed by CMT and SCC is shown in Table 1. The CMT was negative in 488 of the 606 quarter milk samples (80.5%) with a mean SCC of 94.5×10^3 cells/ml in the healthy lactating cows. The positive CMTs with a score from the trace to 4 were noted in 118 of the quarter milk samples (19.5%). In 92 of the quarter milk samples with a CMT score of 1–4, there was an observed increase in the SCC over the regular limit (400×10^3 cells/ml) at a mean 505.1, 861.6, 1695.5 and 5724×10^3 cells/ml, respectively.

The numbers and percentages of the isolates grouped according to the severity of the mastitis are shown in Table 2. Of the 76 infected quarter milk samples, 36 samples were found positive for CNS, 15 for coagulase-positive staphylococci (CPS; *S. aureus* and *S. intermedius*), 8 for *Streptococcus* spp., and 9 for other bacteria. CNS (*S. chromogenes*, *S. haemolyticus*, *S. warneri,* and *S. xylosus*) were the most abundant etiologic factors that represented 47.4% of the infected quarter milk samples and 5.9% from all the examined quarters. SM was noted in 50 milk samples (8.3%). Subclinical mastitis caused by staphylococci was represented by 43.4% of all the mastitis samples. At the quarter levels, 8 (1.3%) and 18 (2.9%) quarters were classified as having latent and clinical mastitis, respectively. The most common pathogens in the CM were *S. aureus*, *Str. uberis* and *S. chromogenes.*

The differences in the lipid peroxidation products (MDA) concentration in the milk of dairy cows with SM and CM are indicated in Graph 1. The increased ($P < 0.01$) milk MDA concentrations

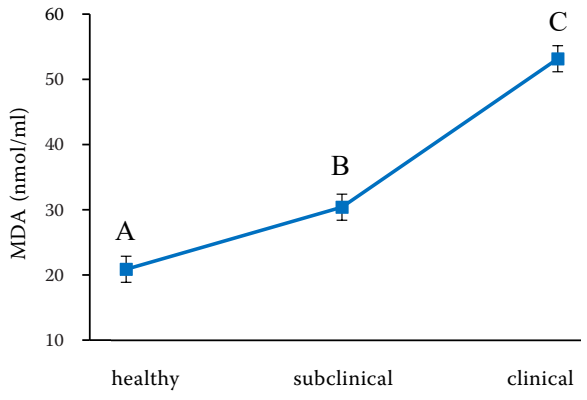
https://doi.org/10.17221/67/2019-VETMED

Table 2. The isolated microorganisms from the infected quarters in the monitored herd

Isolated microorganisms	*n*	%	Latent mastitis[1]		Subclinical mastitis[2]		Clinical mastitis[3]	
			n	%	*n*	%	*n*	%
Staphylococcus spp.								
S. aureus	9	*1.5*	–	–	3	*0.5*	6	*1.0*
S. chromogenes	13	*2.1*	2	*0.3*	8	*1.3*	3	*0.5*
S. haemolyticus	8	*1.3*	–	–	6	*1.0*	2	*0.3*
S. warneri	8	*1.3*	2	*0.3*	6	*1.0*	–	–
S. xylosus	7	*1.2*	1	*0.2*	6	*1.0*	–	–
S. intermedius	6	*1.0*	2	*0.3*	4	*0.7*	–	–
Streptococcus spp.								
Str. uberis	4	*0.67*	–	–	–	–	4	*0.6*
Str. faecalis	2	*0.3*	–	–	2	*0.3*	–	–
Str. suis	2	*0.3*	–	–	2	*0.3*	–	–
Other bacteria								
E. coli	4	*0.7*	1	*0.2*	2	*0.3*	1	*0.2*
Pseudomonas spp.	3	*0.5*	–	–	3	*0.5*	–	–
Enterobacter aerogenes	2	*0.3*	–	–	2	*0.3*	–	–
Mixed infection*	8	*1.3*	–	–	6	*1.0*	2	*0.3*
Total	76	*12.5*	8	*1.3*	50	*8.3*	18	*2.9*

n = the number of isolated bacteria from the 606 examined quarters

Mixed infection* = a mixed infection caused by two or more bacteria; Latent mastitis[1] = are characteristic with normal milk consistency, but an infection is present in the samples of the raw milk without changing the SCC and a negative CMT score; Subclinical mastitis[2] = no signs are observed, the udder and milk appears normal, but an infection is still present with a positive CMT score and an increased SCC; Clinical mastitis[3] = the signs range from mild to severe with a positive CMT score, a high level of SCC, positive bacteriological cultivation, changing the consistency of the milk with the presence of flakes, clots or pus and the reduction or the loss of milk production with clinical signs

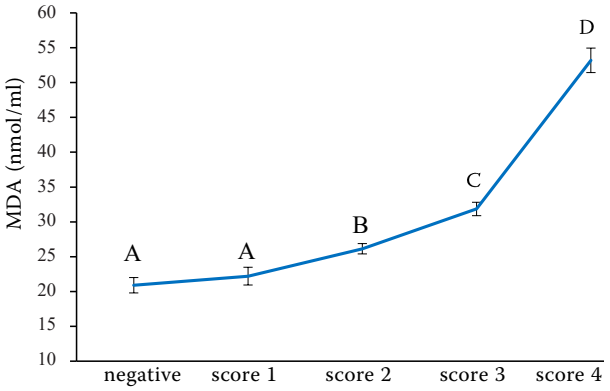


Graph 1. The milk malondialdehyde (MDA) concentrations (nmol/ml) separated by the severity of the mastitis

[A–C]The different superscripts indicate that the means of the MDA differed significantly ($P < 0.01$)

were found in both the CM and SM milk samples. Graph 2 shows the milk MDA concentrations from the CMT-positive quarters with scores of 1–4. The highest MDA concentrations were observed in the milk from the positive quarters with a CMT score of 4. Samples with CMT scores from 2 to 4 had significantly higher ($P < 0.05$) concentrations of MDA when compared to the samples negative for CMT (Graph 2). The differences in the erythrocyte antioxidant enzymes activity among the selected groups of the dairy cows are depicted in Graph 3 and 4, respectively. There was a significant decrease ($P < 0.05$) in the erythrocyte GPx and SOD activities as well as a significant increase ($P < 0.05$) in the MDA concentration in the CM cows. The SOD and GPx activities in the blood of the SM cows did not differ from those of the control animals.

https://doi.org/10.17221/67/2019-VETMED

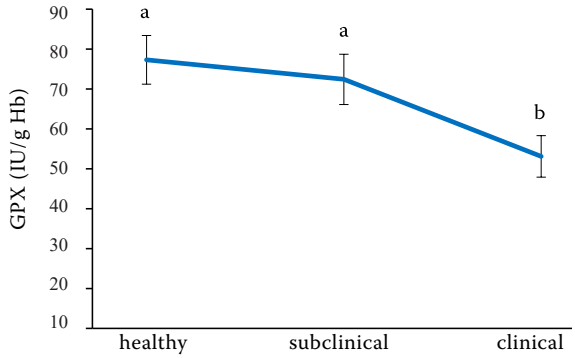


Graph 2. The comparison of the malondialdehyde concentrations (nmol/ml) from the healthy quarters and the quarters with a positive CMT score

CMT = California mastitis test; MDA = malondialdehyde; SCC = somatic cell count

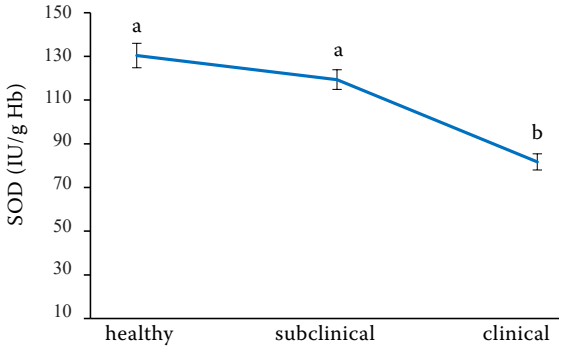
The statistical analysis by the Post Hoc Test were used to compare the milk MDA concentrations from the negative (healthy) quarter milk samples with an SCC of 94.5 × 10^3 per ml and a positive quarter milk samples with a CMT score of 1–4 in the SCC of 505.1, 861.6, 1695.5 and 5724 × 10^3 per ml, respectively. The variable MDA probabilities for the Post Hoc Tests – the error: between MS = 5.97, df = 45.000 were used.

$^{A–D}$The different superscripts indicate that the means of the MDA concentrations differed significantly ($P < 0.01$)



Graph 3. The comparison of the erythrocyte glutathione peroxidase (GPx) activity separated from the healthy and the affected cows

^{a,b}The different superscripts indicate that the activity of the GPx differed significantly ($P < 0.05$)



Graph 4. The comparison of the erythrocyte superoxide dismutase (SOD) activity separated from the healthy and the affected cows

^{a,b}The different superscripts indicate that the activity of the SOD differed significantly ($P < 0.05$)

DISCUSSION

The occurrence or the number of infected cows (quarters) is often unknown in most farms. As opposed to clinical mastitis, SM is characterised by no obvious signs and can only be detected by the microbiological examination of the milk or by the determination of other milk abnormalities. The most common methods are the evaluation of the CMT with the SCC in the milk (Turk et al. 2017).

In Europe, the ECC directive 92/46 from 1992 states that milk with an SCC over 4 × 10^5 cells/ml cannot be used for human consumption. Generally, an SCC greater than 2 × 10^5 cells/ml has been used as indicator for udder inflammation and oxidative damage. In this study, we noted a negative CMT score in 488 of the 606 investigated quarter milk samples (80.5%) with an SCC under 100 × 10^3 cells/ml in the healthy lactating cows. Also, an SCC over the regular limit with a positive CMT scores of 1–4 was present in 92 quarter milk samples (15.2%) ranging from 505.1 to 5724 × 10^3 per ml.

An increased SCC may be associated with the generation of larger amounts of ROS which are considered as the main factors responsible for lipid peroxidation. The products of lipid peroxidation, thus, may be utilised as indicators of the oxidative stress in the tissues and secretions. In our experiment, the increased SCC in the milk was positively associated with the milk MDA concentration. Milk with a higher SCC was shown to have more infiltrated PMNs causing an increase in the rate of the oxidative reactions and apoptosis. Previous studies have shown the relationship between the SCC and MDA concentration in both the quarter milk and the bulk tank milk samples (Suriyasathaporn et al. 2006; Suriyasathaporn et al. 2010).

Moreover, recent research showed that the milk MDA concentration was a marker of a decreased milk yield in cows with CM and high SCC (Sharma

https://doi.org/10.17221/67/2019-VETMED

et al. 2011). In our study, the milk from the cows with CM and SM, respectively, had higher MDA concentrations as compared to milk from the healthy udders. Therefore, it could be considered a potential indicator of subclinical mastitis. The mean concentrations of the MDA were higher in the SM milk when compared with the normal milk, and the MDA could be considered as an indicator of the SM udders. Similar results were reported by Suriyasathaporn et al. (2012) who discovered increased milk MDA levels in cows with SM and CM infected with staphylococci or streptococci.

High-producing dairy cows are highly susceptible to IMI caused by *Staphylococcus aureus,* CNS or *Streptococcus* spp., and losses in the milk yield are related to the increase in the composite milk SCC. The most frequent pathogens isolated from the mastitis milk samples included staphylococci followed by streptococci, *E. coli, Pseudomonas* spp. and *Klebsiella* spp. were reported by Shaheen et al. (2016) in dairy cows. The isolated pathogens from the milk samples in our study comply with the previous findings. The infected quarter milk samples were the most frequently found positive for CNS (47.4%), *S. aureus* (11.8%), streptococci (11.0%) and for other bacteria (11.8%). In many countries, the problem of environmental mastitis caused by CNS has gradually increased since 2005. In recent times, CNS has been frequently acknowledged as a cause of SM and CM in dairy cattle (Contreras et al. 2007; Pyorala and Taponen 2009; Lange et al. 2015). The results of the bacteriological evaluation of the milk in our experiment revealed the predominance of CNS such as *S. chromogenes, S. haemolyticus, S. warneri,* and *S. xylosus*, and are in accordance with the previous reports. The pathogenicity of the different CNS species varies widely. Generally, these bacteria, as the most common mastitis-causing pathogens in cows, are associated with a low-grade infection in the subclinical state, which affects 10–15% of the lactating animals and is characterised by an increase in the SCC, a reduction in the milk production and a high bacterial content in the milk (Monday and Bohach 1999).

Although CNS usually cause relatively mild clinical signs and their pathogenicity is lower than that of *S. aureus*. However, in many cases, persistent SM or chronic mastitis caused by *S. chromogenes* and *S. warneri* lead to a decrease in the antioxidant potential and is associated with the accumulation of ROS and the oxidation products (Castillo et al. 2006; Sharma et al. 2011; Zigo et al. 2019).

Synthesis of ROS and their accumulation are controlled by antioxidant defence systems. Several mechanisms are available to prevent oxidative damage including enzymatic scavengers such as GPx and SOD (Andrei et al. 2011; Celi 2011).

According to Bernabucci et al. (2005), the decreased erythrocyte SOD and GPx activities after calving and during lactation in dairy cows indicate a higher oxidative stress and lower antioxidant status. In our study, the SOD and GPx activities were decreased in the cows with CM, which was probably caused by the depletion of the antioxidant enzymes resulting from the increased ROS generation rate by the inflamed gland. Sharma et al. (2010) also observed a significant decrease in the SOD and GPx activities in the infected milk in their study on bovine staphylococcal mastitis. In our study, the SOD and GPx activities in the blood from the cows with SM were similar to those in the healthy controls probably due to the low severity of the inflammation in the affected mammary glands with SM. However, the induction of the oxidative stress was detected in the cows with SM (Turk et al. 2012).

In the study of Sharma et al. (2011), conducted on 20 Holstein × Sahiwal cross bred dairy cows, the activity of SOD, GPx and catalase (CAT) was confirmed to associate closely with the antioxidant capability of the body. Furthermore, the enhanced enzymatic activity of CAT, which was accompanied by the increase of the plasma MDA level, was observed in the cows 30 days after calving as compared to cows 30 days before calving. On the other hand, the decreased GPx and SOD activities were observed in early lactating cows and this appears to be the reason for their increased susceptibility to mastitis and other health problems.

In conclusion, the results obtained in this work showed that bacteria such as CNS, *S. aureus, Str. uberis*, and *E. coli* were the principal causative factors of IMI, leading to increased SCC and lipid peroxides in the milk of the affected cows. In contrast, the antioxidant enzyme activities were only lower in the cows with CM compared to the healthy cows. Conclusively, CM leads to systemic oxidative stress with the depletion of the enzymatic antioxidant mechanisms and the lipid peroxidation products accumulation, while the oxidative stress seems to be more restricted to the mammary gland in SM. In both forms of mastitis, the milk quality is affected by

https://doi.org/10.17221/67/2019-VETMED

the accumulation of the oxidation products, which positively correlates with the SCC. Therefore, the measurement of the milk MDA concentration could be a potential biomarker for the better screening of the udder health in the early diagnosis of mastitis.

REFERENCES

Andrei S, Matei S, Fit N, Cernea C, Ciupe S, Bogdan S, Groza IS (2011): Glutathione peroxidase activity and its relationship with somatic cell count, number of colony forming units and protein content in subclinical mastitis cow's milk. Romanian Biotechnological Letters 16, 6209–6217.

Andrei S, Matei S, Rugina D, Bogdan L, Stefanut C (2016): Interrelationships between the content of oxidative markers, antioxidative status, and somatic cell count in cow's milk. Czech Journal of Animal Science 61, 407–413.

Ashfaq K, Muhammad G (2008): Pathogens associated with bovine and bubaline mastitis in peri-urban areas of Faisalabad, Pakistan. Pakistan Journal of Life and Social Sciences 6, 86–88.

Balasubramaniam P, Malathi A (1992): Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. Journal of Postgraduate Medicine 38, 8–9.

Bernabucci B, Ronchi B, Lacetera N, Nardon A (2005): Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. Journal of Dairy Science 88, 2017–2026.

Castillo C, Hernandez J, Valverde I, Pereira V, Sotillo J, Alonso-Lopez M, Benedito JL (2006): Plasma malondialdehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Research in Veterinary Science 80, 133–139.

Celi P (2011): Biomarkers of oxidative stress in ruminant medicine. Immunopharmacology and Immunotoxicology 33, 233–240.

Contreras A, Sierra D, Sanchez A, Sanchez A, Coralles JC, Marco JC, Paape MJ, Gonzalo C (2007): Mastitis in small ruminants. Small Ruminant Research 68, 145–153.

Jackson P, Cockeroft P (2002): Clinical Examination of Farm Animals. Blackwell Science Ltd, Wiley-Blackwell, Oxford, UK. 54–166 p.

Lange C, Brito MA, Reis D, Marco AM, Guimaraes A, Azevedo AL, Salles EB, Alvim MC, Silva FS, Meurer IR (2015): Species-level identification of staphylococci isolated from bovine mastitis in Brazil using partial 16S rRNA sequencing. Veterinary Microbiology 176, 3–4.

Malinowski E, Lassa H, Klossowska A, Smulski S, Markiewiecz H, Kaczmarowski M (2006): Etiological agents of dairy cows' mastitis in western part of Poland. Polish Journal Veterinary Sciences 9, 191–194.

Monday SR, Bohach GA (1999): Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. Journal of Clinical Microbiology 37, 3411–3414.

National Mastitis Council (2001): National Mastitis Council Recommended Mastitis Control Program. Available at: www.nmconline.org/publications/f (Accessed October 5, 2019).

NRC – National Research Council (2001): Nutrient Requirements of Dairy Cattle. 7th edn. National Academic Press, Washington, DC, USA.

Paglia DE, Valentine WN (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine 70, 158–169.

Pyorala S, Taponen S (2009): Coagulase-negative staphylococci – Emerging mastitis pathogens. Veterinary Microbiology 134, 3–8.

Shaheen M, Tantary HA, Nabi SU (2016): A treatise on bovine mastitis: disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic management, prevention and control strategy. Journal Advanced Dairy Research 4, 1–10.

Sharma N, Mukherjee R, Ingale SL, Jadhav RK (2010): Therapeutic and anti-oxidant activity of vitamin E and selenium in bovine Staphylococcal mastitis. Indian Journal Veterinary Research 19, 25–31.

Sharma N, Singh N, Singh O, Pandey V, Verma P (2011): Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Australasian Journal of Animal Sciences 24, 479–484.

Suriyasathaporn W, Vinitketkumnuen U, Chewonarin T, Boonyayatra S, Kreausukon K, Schukken YH (2006): Higher somatic cell counts resulted in higher malondialdehyde concentrations in raw cows' milk. International Dairy Journal 16, 1088–1091.

Suriyasathaporn W, Vinitketkumnuen U, Chewonarin T, Chupia V, Pinyopummintr T (2009): The indicative influence of oxidative stress on low milk yields in dairy cattle. Thai Journal of Veterinary Medicine 39, 237–243.

Suriyasathaporn W, Vinitketkumnuen U, Chewonarin T (2010): Relationships among malondialdehyde, milk compositions, and somatic cell count in milk from bulk tank. Songklanakarin Journal of Science and Technology 32, 23–26.

Suriyasathaporn W, Chewonarin T, Vinitketkumnuen U (2012): Differences in severity of mastitis and the pathogens causing various oxidative product levels. Advances in Bioscience and Biotechnology 3, 454–458.

https://doi.org/10.17221/67/2019-VETMED

Sztachanska M, Baranski W, Janowski T, Pogorzelska J, Zdunezyk S (2016): Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. Polish Journal of Veterinary Sciences 19, 119–124.

Taponen S, Koort J, Bjorkroth J, Saloniemi H, Pyorala, S (2007): Bovine intramammary infections caused by coagulase-negative staphylococci may persist throughout lactation according to amplified fragment length polymorphism-based analysis. Journal of Dairy Science 90, 3301–3307.

Turk R, Piras C, Kovacic M, Samardzija M, Ahmed H, Da Canio M, Urbani A (2012): Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis. Journal of Proteomics 75, 4412–4428.

Turk R, Koledic M, Macesic N, Benic M, Dobranic V, Duricic D, Urbani A, Mestric ZF, Soggiu A, Bonizzi L, Roncada P (2017): The role of oxidative stress and inflammatory response in the pathogenesis of mastitis in dairy cows. Mljekarstvo 67, 91–101.

Vasil M, Elecko J, Farkasova Z, Bires J (2009): The reduction on the occurrence of mastitis in dairy herd using the innovation of housing conditions, sanitary of milk storage and applying the therapy of mastitis during the lactation. Folia Veterinaria 53, 186–189.

Woolliams JA, Wiener G, Anderson PH, McMurray CH (1983): Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Research in Veterinary Science 34, 69–77.

Zajac P, Tomaska M, Murarova A, Capla J, Curlej J (2012): Quality and safety of raw cow's milk in Slovakia in 2011. Potravinárstvo 6, 64–73.

Zigo F, Elecko J, Vasil M, Farkasova Z, Zigova M, Takac L, Takacova J (2019): Etiology of mastitis in herds of dairy cows and ewes situated in marginal parts of Slovakia. EC Veterinary Science 4, 72–80.

Received: May 29, 2019
Accepted after corrections: September 18, 2019