

The Impact of Mastitis on Selected Milk Parameters and Lipid Peroxidation Product of Dairy Cows

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Abstract: The aim of the study was to detect mastitis and their effect on levels of oxidative product, using milk malondialdehyde (MDA) and selected milk parameters in dairy cows. During the complex investigation of 123 lactating cows, 612 quarter milk samples were examined and classified by clinical examination, abnormal udder secretions, assessment of the California mastitis test (CMT), with collecting of milk samples for detection of milk malondialdehyde level (MDA), somatic cell count (SCC), milk protein, fat and bacteriological identification of pathogens causing mastitis. Positive CMT score was recorded of 19.2% (118) examined quarters and 12.5% (76) quarters were confirmed bacterial pathogens causing latent (LM; 1.3%), subclinical (SM; 8.3%) and clinical (CM; 3.0%) mastitis. The most common bacterial isolates from the mastitis cases were coagulase-negative staphylococci (CNS; 55.2%), coagulase-positive staphylococci (CPS; 11.8%) and streptococci (10.5%). The concentrations of MDA, as well as SCC, were significantly higher from SM and CM cases than in milk samples from healthy cows. Increased milk protein and decreased milk fat was only observed in dairy cows with CM. The higher MDA concentrations in SM and CM milk observed in this study showed that the oxidative stress of infected milk is higher than normal milk. In conclusion, the measurement of milk MDA level as a significant factor in alterations of the oxidant and antioxidant balance resulting in potent oxidative stress could be a potential biomarker for monitoring health status of the mammary gland.

Keywords: Mammary gland, Mastitis, Diagnostic, Malonaldehyde, Somatic cell count

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I. Introduction

Mastitis is one of the biggest problems of dairy producers causes great losses in the livestock economy. After calving and during the lactation are cows exposed to numerous genetic, physiological, and environmental factors that can compromise host immunity and increase the incidence of mastitis. The milk collected from cows with different type of inflammation, including mastitis, is characterized by an increased number of somatic cell count (SCC; Tab. 1), especially polymorphonuclear cells, changes in protein composition and salt and lactose concentrations, which usually affects its processing properties^{1,2}.

Table no 1: SCC and their relationship to milk production and health status of mammary gland

SCC in milk x 10 ³	Health status of mammary gland	Infected quarters of udders (%)	Decrease in milk production
> 125	very good	X	0
125 to 250	good	6	0
250 to 375	satisfactory	16	6
375 to 500	endangered	32	18
500 to 750	disturbed	48	29
< 750	problematic	up 55	35

Source: Zigo et al.²

It was proved that the antibacterial activity of polymorphonuclear cells generates reactive oxygen species (ROS). The accumulation of the ROS correlating with a chemical and physical changes in the milk and can lead to oxidative stress. It was shown that the occurrence of oxidative stress in cattle may contribute to some disorders (mastitis, retained fetal membranes, udder oedema) or metabolic diseases³.

Increase of oxidative stress in dairy cows as a consequence of high milk production can result in excess accumulation of ROS, which can induce lipid peroxidation and reduction of antioxidative activity due to catalysis of various hydrogen and lipid peroxides⁴.

One of the most reliable of lipid peroxidation end product and widely used indexes of oxidative stress is malondialdehyde (MDA). MDA is produced during the peroxidation of polyunsaturated fatty acids by the action of reactive oxygen species (ROS; Fig. 1), as a result of the depletion of antioxidant systems⁵.

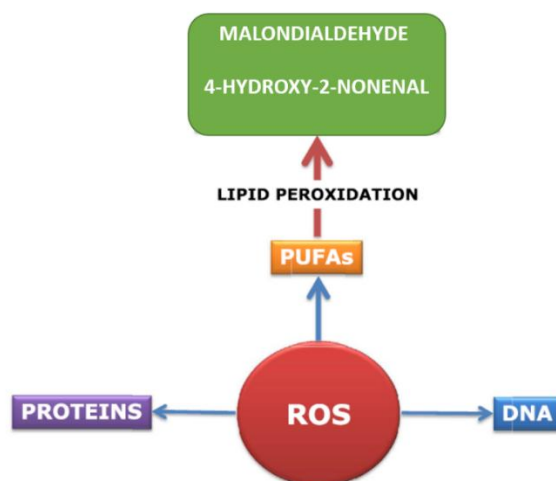


Figure no 1: Lipid peroxidation

Note: Schematic illustrating the attack of ROS on proteins, DNA and polyunsaturated fatty acids (PUFAs), the latter being representatives of the class of lipid biomolecules, ROS peroxidize PUFAs to finally generate MDA and 4-hydroxy-2-nonenal.

Source: Tsikas⁴.

In cow's milk, MDA levels were measured to evaluate the peroxidation status when milk was kept under different circumstances and SCC is positively associated with MDA level in milk⁶. In fact, malondialdehyde is known to be a mutagen and a suspected carcinogen, it can react with DNA to generate mutations⁴.

On most farms the prevalence of mastitis and the number of infected cows are often unknown. These cases (in particular subclinical mastitis) do not show any clinical signs and are identified only by CMT assessment and SCC determination. One of the additional diagnostic methods of mastitis may be involve determination of malondialdehyde (MDA) in milk as an indicator of oxidative stress⁷.

Oxidative stress in veterinary medicine and particularly in ruminant health is a relatively young field of research. Therefore, the goal of this study was to determine the changes in selected milk parameters and milk MDA levels as a markers on severity of mastitis.

II. Material And Methods

Cows and milking: The practical part of the study was carried out in herd of 180 dairy cows of Slovak spotted cattle in west of the Slovakia. Dairy cows from monitored herd were kept in a free housing system with a separate calving barn and equipped with individual boxes with bedding and were allowed ad libitum access to water. The cows were milked twice a day at 5:30 a.m. and 4:30 p.m. in the 2 x 10 fishing-milking parlour DeLaval 2x5 (Tumba, Sweden). Milking and pulsation vacuum was set at 42 kPa. Pulsation ratio was 60:40 at a rate of 52 c/min.

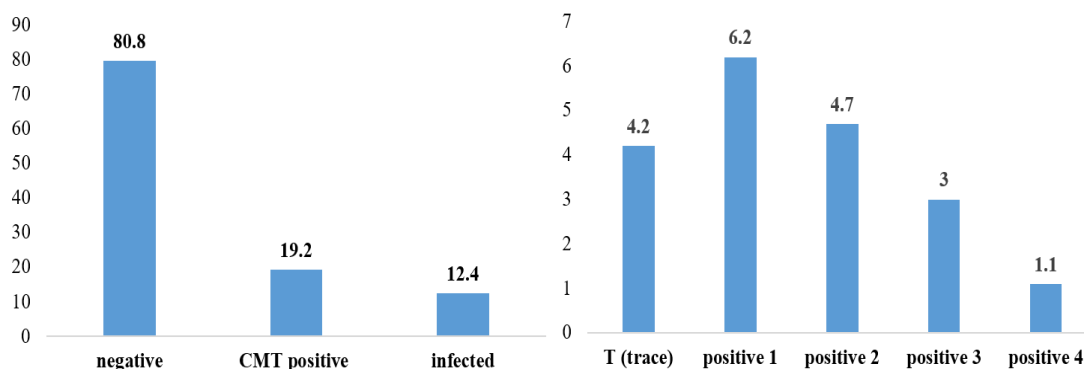
Udder health examination and milk samples collection: During the practical part of the study from 180 pcs were selected 153 lactating cows because 27 cows were separated in calving boxes or were milked into separate vessels for the first 12 days after calving. A complex examination of udder health status of each cow included clinical examination, examination fore-strip of milk, with CMT reaction, subsequent collecting of milk samples for SCC detection and bacteriological examination. CMT (Indirect diagnostic test, Krause, Denmark) was performed from the 153 milked cows on 606 quarters (6 quarters were rejected) according to the visible reactions and results were classified in four scores (Graph 1) according to Jackson and Cockcroft⁸. Quarter milk samples of the secretion (10 ml) for bacteriological cultivation were then collected in sterile test tube after discarding the first three squirts of milk, and then placed in an icebox and transported to the laboratory for examination (Fig. 2).



Figure no 2: CMT assessment and milk sampling with cultivation of bacterial pathogens

Source: Zigo, 2019

Experimental groups selection for MDA determination and milk parameters: Based on evaluation of CMT, clinical examination for measurement of milk MDA concentration and milk parameters were assigned three groups: control (healthy cows), cows with subclinical (SM) and clinical (CM) mastitis. To a SM group were included 43 cows without clinical signs of mastitis or other illnesses but with positive CMT at least one quarter. Out of them were selected 77 quarters for MDA detection that showed a positive CMT (score 1 - 4). To a CM group were included seven cows with clinical signs and out of them were selected 15 quarters for MDA detection with high score of CMT ≥ 3 . From all health cows with a negative score of CMT and without clinical sign was randomly selected seven animals (28 quarters) to the control group. In addition to MDA detection from selected groups were analyzed qualitative milk indicators as SCC, percentage of milk fat and protein.



Graph no 1: Quarter milk samples examination with cmt evaluation of 153 lactating cows

Note: Negative – quarters with negative CMT score, CMT positive - quarters with positive evaluation of Californian Mastitis Test with score: trace or 1 – 4, Infected – infected quarters with positive results of cultivation.

Milk parameters and MDA detection: The content of milk fat, total protein was determined in each samples using Infrared Milk Analyzer 150 (Bentley Instruments Inc.). SCC from quarter milk samples were measured using Somacount 150 apparatus (Bentley Instruments Inc., Minnesota, USA)¹. For MDA detection were normal and mastitic milk samples skimmed by centrifugation at 10,000 g for 20 min at 4°C. Defatted milk was used for MDA concentration by spectrophotometric techniques. Malondialdehyde concentration was determined by the thiobarbituric acid (TBA) method, which was modified from method of Andrei et al.⁶

Bacteriological examination of milk samples: Milk samples were examined according to generally accepted principles Malinowski et al.⁹. Quarter milk samples (10 µl) were inoculated on Petri dish with 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. Suspected colonies were inoculated and cultured on selective media such as Staphylococcus medium N°110, Baird-Parker agar, Brilliance™ UTI Clarity Agar, Edwards Medium, Mac Conkey Agar (Oxoid, OXOID Ltd., Basingstoke, Hants, UK). Parameters such as colony size and appearance, pigment production and coagulase, catalase activity, hemolysis, Gram staining have also been taken into account in the determination of bacterial species. Colonies of *Staphylococcus* spp. were tested for coagulase activity (Staphylo PK, Imuna Pharm, SR). Growth-confirmed colonies of *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriaceae* spp. were further identified biochemically using the STAPHYtest 24, STREPTOtest 24, resp. ENTEROtest 24 (Erba-Lachema, CZ) and the software TNW Pro 7.0 (Erba-Lachema, CZ).

Mastitis classification: Based on clinical examination of the mammary gland, assessment of CMT, determination of SCC and bacteriological examination of milk were individual forms of mastitis classified as follows:

Latent mastitis are characteristic only with the presence of bacterial pathogens in samples of milk without changing its consistency and SCC.

Subclinical mastitis are characteristic with positive CMT score, bacteriological cultivation, increased SCC, reduced milk yield without clinical signs.

Clinical mastitis are characteristic with positive CMT score, bacteriological cultivation, high level of SCC, changing the consistency of the milk, reduced or loss of milk production with clinical signs.

Statistical analysis

Data for milk MDA levels and milk parameters in selected groups of dairy cows were expressed as mean \pm standard deviation. Difference between groups were analysed by using analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test and minimum criteria for statistical significance was set at $P \leq 0.05$ for all.

III. Results And Discussion

Graph 1 and table 2 shows the health status udder of 153 lactating dairy cows. From 612 quarter milk samples were recorded negative CMT score in 495 quarter milk samples (80.8%). The positive CMT with score trace or 1 - 4 were recorded from 118 (19.2%) quarter milk samples. From 12.4% infected quarters with positive bacteriological cultivation there was 10.5%, 65.8% and 24.0% classified as latent, subclinical and clinical mastitis, respectively.

Table no 2: Mastitis forms and number of isolated bacterial pathogens from 76 infected quarters

PATHOGENS	LATENT		SUBCLINICAL		CLINICAL	
	n	%	n	%	n	%
CNS ¹	5	6.6	22	28.9	5	6.6
CPS ²	2	2.6	7	9.2	4	5.3
Streptococcus spp. ³	-		4	5.3	4	5.3
Other bacteria ⁴	1	1.3	11	14.5	3	3.8
Mixed inf. ⁵	-		6	7.9	2	2.6
TOTAL	8	10.5	50	65.8	18	24.0

Note: n - number of isolated bacteria from exanimated quarters, CNS¹ – coagulase negative staphylococci (*S. chromogenes*, *S. haemolyticus*, *S. warneri*), CPS² - coagulase positive staphylococci (*S. aureus* and *S. intermedius*), Strept. spp.³ – *Streptococcus* spp. (*S. uberis* and *S. faecalis*), Other bacteria⁴ – *E. coli* and *Enterobacter aerogenes*), Mixed infection⁵ - mixed infection caused two or more bacteria

According to Vatour et al.¹⁰ the annual incidence of CM in dairy herds is generally lower than 5%, but in a small percentage of herds the incidence may exceed 30 – 50% of the animals, causing mortality (gangrenous mastitis) or culling of up to 70% of the herd. The economic losses are more associated with mastitis occurs as a low grade infection, a subclinical state, which affects 10 - 15% of lactating animals, increasing milk leucocyte content, reducing milk production and increasing milk bacterial content.

High-producing cows are highly susceptible to subclinical intramammary infection due to *Staphylococcus aureus* or *Streptococcus* spp., and losses in milk yield are related to an increase in composite milk SCC. Authors Reksen et al.¹¹ from 25 affected cow's quarters isolated CPS (68.0%), mainly *S. aureus*, followed by streptococci (28%), *Klebsiella* spp. (12%), *Pseudomonas* spp. (8%) and *E. coli* (4%).

Sharma et al.¹² and Tenhagen et al.¹³ reported that staphylococci were the predominant organisms isolated from mastitis milk samples followed by streptococci, *E.coli*, *Pseudomonas* spp. and *Klebsiella* spp.

The present findings are in accordance with the findings of our study. From 76 infected quarter milk samples, 51 (67.1%) were found positive for staphylococci, 8 (11.0%) for streptococci and 7 (9.2%) for *E. coli* with *Pseudomonas* spp. From staphylococci, CNS and CPS gave the highest percentages 47.4 % and 19.7% representation on the aetiology of mastitis from the affected samples, respectively (Tab. 2).

High SCC present in milk is the main indicator of mammary gland infection, caused by specific and non-specific microorganisms, which cause contagious and environmental mastitis. Malinowski et al.⁹ carried out a study to determine the relationship between SCC and mastitis etiological agents. They reported that milk samples with SCC lower than 200,000 cells/ml were mostly (59.6%) culture negative. Coagulase-negative staphylococci *Staph. aureus* was mostly noted in samples with 200,000 to 2,000,000 of SCC/ml. Samples having more than 2 million/ml of SCC were infected mainly with streptococci and gram negative bacilli. Very

high SCC (≥ 5 million/ml) was connected with infections caused by *Prototheca* spp. (64.5%), yeast-like fungi (60.2%) and *Streptococcus* spp. (55.1%).

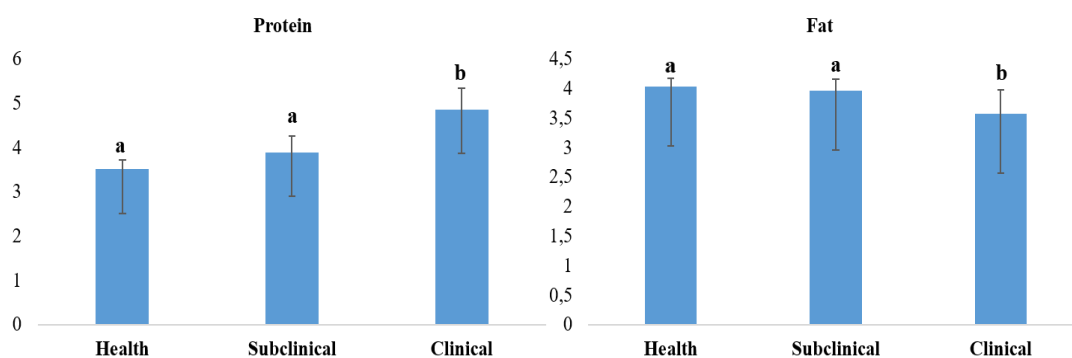
In a study in which dairy cows were followed-up throughout the whole lactation, the geometric mean SCC was over 600,000 cells.ml⁻¹ in quarters with persistent CNS infection, but only 60,000 cells.mL⁻¹ in healthy quarters¹⁴.

In many countries, the problem of environmental mastitis caused of CNS has gradually increased since 2005. CNS have been acknowledged more and more frequently as a cause of SM and CM in dairy cattle^{13,15,16}.

In addition to increased SCC, especially in cows suffering from mastitis caused by *Staphylococcus* spp., there is a change in protein composition and this affects the physical and chemical properties of milk. Factors that determine the biological and technological properties of milk include proteins, fat, carbohydrate, and protein fractions, including caseins¹.

Milk fractions, such as proteins, in milk from dairy cows from which *Streptococcus* spp. were isolated have a longer coagulation time and a higher proportion of whey proteins. Also, the proportion of fats and proteins is altered due to contamination with bacterial pathogens and immune cells entering the site of inflammation¹⁷.

In our study, the average fat content in the milk of investigated cows ranged from 3.6 to 4.1%. In dairy cows with a clinical form of mastitis, in addition to reduced milk yield, a reduced milk fat content was reported, probably related to high serum protein transfer and increased SCC, as reflected in an increase in milk protein in cows with clinical mastitis compared to healthy cows (Graph 2).

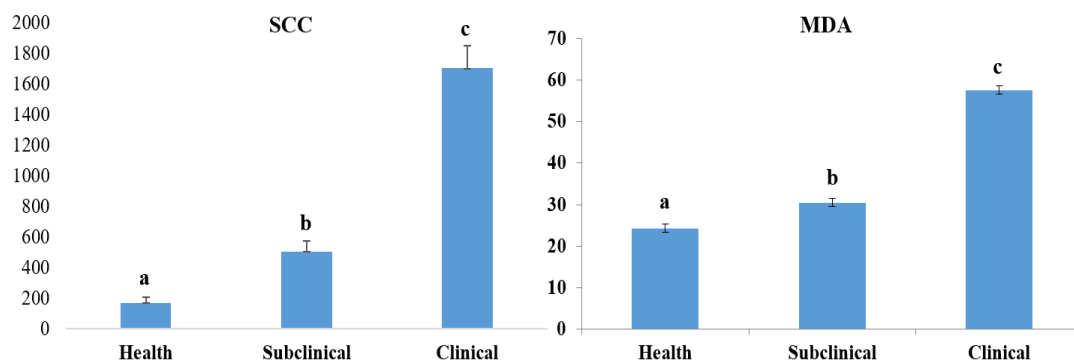


Graph no 2: Comparison of milk protein and fat separated by severity of mastitis

Note: ^{a-c}Different superscripts indicate that means of milk protein and fat differed significantly ($P < 0.05$).

In addition to the increased level of SCC in milk was positively associated with blood and milk MDA levels as the main cause of lipid peroxidation which is used as an indicator of oxidative stress. Milk with higher SCC has shown to have more infiltrated polymorphonuclear cells, and this caused an increase of oxidative reactions and apoptosis. Lipid peroxidation is a well-established mechanism of oxidative damage caused by reactive oxygen species, and measurement of the MDA provides a convenient index of oxidative stress¹⁸. The higher MDA levels with increases SCC demonstrated in previous study, that the auto-oxidative activity of infected milk is higher than normal milk.

Similar results were observed in our study when the mean level of MDA was significantly higher ($P < 0.05$) from mastitis milk samples compared with normal milk. In addition, a recent study showed that MDA was a mediator of a decreased milk yield in cows with CM and high SCC³. Our results indicate that not only milk from cows with CM but also with SM had higher MDA concentrations as compared to normal milk (Graph 3). Therefore, milk MDA concentration would be considered as an indicator of SM udders. Similar results have been reported by Suriyasathaporn et al.¹⁹. Authors investigated the increased milk MDA levels in cows with SM and CM milk samples infected of staphylococci or streptococci. There was a significant difference ($P < 0.05$) between the SM and CM milk samples compared with the milk samples from healthy cows. Milk from mastitic udders is of low quality and less suitable for consumption. In addition, the increased malondialdehyde due to high SCC further reduces milk quality.



Graph no 3: Comparison of scc and milk mda concentrations (nmol.mL⁻¹) separated by severity of mastitis
 Note: ^{a-c}Different superscripts indicate that means of SCC and MDA differed significantly (P < 0.05).

IV. Conclusion

The results of the current study showed that bacteria such as CNS, *S. aureus* and streptococci were the principal factor causing IMI leading to increased SCC, milk protein and lipid peroxides in affected cows. It should be kept in mind that polyetiological origin of mastitis in dairy cows means that the effectiveness of generally applicable mastitis control program in the reduction of environmental mastitis bacteria, in combination with the main contagious pathogens of the mammary gland tends to be limited. It is therefore necessary to implement new knowledge for diagnosing mastitis in dairy herds. The measurement of milk MDA level as a significant factor in alterations of the oxidant and antioxidant balance resulting in potent oxidative stress, could be a potential biomarker for diagnosing mastitis and monitoring health status of udder.

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