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Original article

Glucose-6-phosphate dehydrogenase, glutathione peroxidase, total glutatione and reduced nicotinamide adenine dinucleotide phosphate in milk cells of subclinical mastitic cows

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Abstract

This study aimed to determine the levels of milk cell total protein (TP), reduced nicotinamide adenine dinucleotide phosphate (NADPH), total glutathione (tGSH), activities of glucose-6-phosphate dehydrogenase (G6PD) and glutathione peroxidase (GPx) in subclinical mastitic cows. Milk from each udder was collected and grouped by the California Mastitis Test. Then, a somatic cell count (SCC) was performed, and the groups were re-scored as control (5–87 × 10^3 cells), 1st group (154–381 × 10^3 cells), 2nd group (418–851 × 10^3 cells), 3rd group (914–1958 × 10^3 cells), and 4th group (2275–8528 × 10^3 cells). Milk cell TP, NADPH, tGSH levels, G6PD, and GPx activities were assessed. Microbiological diagnosis and aerobic mesophyle general organism (AMG, cfu/g) were also conducted. In mastitic milk, TP, NADPH, and tGSH levels, and G6PD and GPx activities were significantly reduced per cell (in samples of 10^6 cells). In addition, milk SCC was positively correlated with AMG ($r=0.561$, $p<0.001$), NADPH ($r=0.380$, $p<0.01$), TP ($r=0.347$, $p<0.01$) and G6PD ($r=0.540$, $p<0.001$). There was also positive correlation between NADPH ($r=0.428$, $p<0.01$), TP ($r=0.638$, $p<0.001$) and AMG. NADPH was positively correlated with TP ($r=0.239$, $p<0.05$), GPx ($r=0.265$, $p<0.05$) and G6PD ($r=0.248$, $p=0.056$). Total protein was positively correlated with tGSH ($r=0.354$, $p<0.01$) and G6PD ($r=0.643$, $p<0.001$). There was a negative correlation between tGSH and GPx activity ($r=-0.306$, $p<0.05$). The microbiological analysis showed the following ratio of pathogens: *Coagulase-Negative Staphylococci* 66.6%, *Streptococcus spp* 9.5%, *Bacillus spp* 9.5%, yeast 4.8%, and mixed infections 9.5%.

As a conclusion, when evaluating the enzyme and oxidative stress parameters in milk, it is more suitable to assign values based on cell count rather than ml of milk. The linear correlation between the SCC and AMG, milk cell NADPH, TP and G6PD suggests that these parameters could be used as markers of mastitis.

Key words: subclinical mastitis, milk cell, glucose-6-phosphate dehydrogenase, glutathione peroxidase, NADPH

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Introduction

Mastitis symptoms depend on the state of the cow's defense system, the characteristics of the responsible agents, and the type of intramammary infection (clinical or subclinical). Somatic cell count (SCC) is the main tool for diagnosing subclinical mastitis. Milk somatic cells are comprised of leukocytes (lymphocytes, polymorphonuclear leukocytes (PMN), and macrophages) and epithelial cells (Lee et al. 1980). When the mammary tissue is infected, SCC in milk increase (Başţan 2010). Number of somatic cells in milk and their composition depend on the type of infection and the type of causative agent (Leitner et al. 2000). For instance, the largest intramammary SCC increase in cows was found with *Staphylococcus (Staph.) aureus* infection, and the smallest increase was found with *Corynebacterium bovis* infection (Schepers et al. 1997). A study (Leitner et al. 2000) performed on the effects of different etiological agents on milk SCCs and composition reported that the majority of the cells in the somatic cell composition of healthy milk are epithelial cells. The same study also found that, in milk from acutely infected mammary gland with *Escherichia (E.) coli* and *Staph. aureus*, the SCC increased, and the majority of the cells were PMNs. In addition, in case of acute infection with *Staph. aureus*, mononuclear cell, lymphocyte, and macrophage counts also increased to a certain level. In the case of chronic infection with *Staph. aureus* and *Coagulase-Negative Staphylococci* (CNS), the majority of cells in the somatic cell composition were T-lymphocytes and macrophages. Epithelial cell counts decreased relative to neutrophil, PMN, and macrophage counts upon acute or chronic infection (Leitner et al. 2000).

Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme of the pentose phosphate metabolic pathway (Mehta et al. 2000). The pentose phosphate pathway has two primary functions: synthesizing the ribose-5-phosphate and the reduced nicotinamide adenine dinucleotide phosphate (NADPH) required for reductive reactions (Krebs and Eggleston 1978). NADPH protects the cell from the oxidative damage caused by free radicals (Ames et al. 1993, McCord 1993), and plays an important role in the regeneration of reduced glutathione (rGSH). Reduced GSH and GSH-dependent enzymes protect the cell from reactive oxygen species (Ayala et al. 1991).

Studies in humans have shown that insufficient ROS elimination in epithelial cells of individuals with G6PD deficiency, causes their cells to be more sensitive to *Staph. aureus* infection and apoptosis than healthy cells (Hsieh et al. 2013). The phagocytic activity of PMNs decreased in an individual with low G6PD levels, and the antioxidant capacity of these cells is reduced (Cooper et al. 1972). G6PD is isolated from milk of sows, rats and rabbits (Grigor and Hartmann 1985), lactating mammary glands of rats (Shreve and Levy 1980) and bovine milk cells (Ritter et al. 1977) and is important for NADPH production, which is required to establish oxidative/antioxidative equilibrium (Ritter et al. 1977, Shreve et al. 1980, Grigor and Hartmann 1985).

Pathogens that play a role in the development of subclinical mastitis are *Staph. aureus, Staph. chromogenes, Streptococcus (S.) agalactia, S. bovis*, *S. uberis, E. coli, Pseudomonas aeruginosa* and other CNS (Albenzio et al. 2002). In recent years, CNS has attracted interest among the agents that cause subclinical mastitis (Yağcı et al. 2008).

When the biochemical parameter levels in milk will be investigated, many factors such as PMN, necrotic epithelial cells, and type of infectious agent will complicate the utilization of biochemical parameters (in ml milk) in mastitis diagnosis and prognosis. In previous studies, (Dündar et al. 2000, Weiss et al. 2004, Erişir et al. 2011, Szczubial et al. 2012) biochemical parameters found in 1 ml of milk with subclinical mastitis were evaluated. However, due to differences between the changes in milk somatic cell composition (Leitner et al. 2000), we suggest to evaluate 'parameter per somatic cell'.

No literature was found on changes in G6PD levels in the milk cells of cows with subclinical mastitis. Thus, this study was designed to (1) identify the effect of subclinical mastitis on milk cell G6PD and GPx activity and the levels of NADPH and total glutathione (tGSH) and (2) ascertain per cell parameter levels to illustrate the efficacy of using biochemical parameters to diagnose subclinical mastitis. This study also endeavored to identify the correlation between mastitis agents, SCCs, AMG, and biochemical parameters.

Materials and Methods

Materials

Three to five-year-old Holstein-Friesian cows (n=20) in the same period of lactation were included in the animal material of this study. Milk samples collected from the different mammary lobes (milk from one mammary lobe was accepted as one sample) of the cows were tested by California Mastitis Test (CMT) and grouped based on their CMT reactions (-negative, -/+ suspected, +1, +2, +3, n=15 for each, totally 75 milk samples from 20 cows). After the CMT, the teat was cleaned with 70% alcohol, and after the first 4–5 squirts of milk were discharged, 45–50 ml of milk was collected from each quarter. Milk samples were stored

in a transfer box at +4˚C and transported to the laboratories within 2 h of sampling. The experimental protocols were approved by the Local Animal Ethics Commettee of Mustafa Kemal University (No: 2014-04/1).

Method

Somatic Cell Count

In the study, SCCs were performed on milk samples collected according to CMT scoring, which is a subjective (Holtgrew-Bohling 2016) method, and after re-scoring, using a somatic cell counter, the samples were grouped as follows: control (1–87 × 10^3 cells), mastitis 1st group (418–812 × 10^3 cells), 2nd group (418–851 × 10^3 cells), 3rd group (914–1928 × 10^3 cells), and 4th group (2614–8050 × 10^3 cells) n=15, each). Sodium azide (0.05 ml, 24%) (Sigma-Aldrich) was added to the milk samples in order to preserve the cells. SCC was performed with a Bentley BactoCount IBCm (Bentley Instruments Inc., Chaska, MN, ABD), which is semi-automatic and works according to the principle of flow cytometry.

Biochemical Analysis

Milk brought to the biochemistry laboratory was centrifuged at 600 × g for 10 min at 4˚C. After centrifugation, the supernatant (milk serum) was eliminated by removing the upper layer of fat with a cotton pad; the remaining cell pellet was washed 2 times with cold phosphate-buffered saline (PBS) and centrifuged at 600 × g for 10 min at +4˚C. Finally, the supernatant was removed, and the remaining pellet was completed to 2 ml with PBS and sonicated (Bandelin Sonopuls HD 2070, Germany) (Akalın et al. 2016) for 5 repetitions of 10 sec each, with a 30-sec cooling period (on ice) between each repetition. After sonication, the homogenates were centrifuged at 13,000 × g for 15 min at +4°C. The supernatant was collected and stored at -86°C until further analysis. Then the total protein (TP), NADPH, and tGSH levels and the G6PD and GPx activities of these cell supernatants were determined.

Determination of G6PD activity

G6PD activity was determined by using the method developed by Beutler (1971) and calculated by the spectrophotometric measurement of the absorbance difference in optical density caused by the conversion of $NADP^+$ to NADPH at 340 nm (UV 2100 UV–VIS Recording Spectrophotometer Shimadzu, Japan). Results are presented as IU/ml, IU/mg protein, and IU/10^9 cells.

Determination of GPx activity

GPx catalyzes the conversion of H_2O to H_2O_2 using GSH. Determination of GSH-Px activity in milk cells was done according to the method described by Beutler (1975). According to this method, GPx catalyzes the conversion of GSH to oxidized glutathione (GSSG) in the presence of H_2O_2. GSSG, formed by GSH-Px in an environment where H_2O_2 is present, is converted back to GSH with the help of glutathione reductase and NADPH. GPx activity was calculated by the spectrophotometric measurement of the absorbance difference in optical density caused by the conversion of NADPH to $NADP^+$ at 340 nm. Results are presented as IU/ml, IU/mg protein, and IU/10^6 cells.

Determination of Total Glutathione Levels

Total glutathione (GSSG + rGSH) levels in cell supernatants and milk serum were calculated using a commercial kit (Sigma CS0260). It is a kinetic method based on the principle of the reduction of 5.5'-dithiobis (2-nitrobenzoic) acid to trinitrobenzoat (TNB) by glutathione. When oxidized glutathione is regenerated by glutathione reductase and NADPH, TNB absorbance at 412 nm can be measured by spectrophotometry. Results are presented in nmol/ml, nmol/mg protein, and nmol/10^6 cells.

Determination of NADPH Levels

NADPH levels in cell supernatants were determined spectrophotometrically using a commercial kit (Sigma MAK038). The reaction principle is based on the spectrophotometric (ELISA reader) analysis of the reduction of formazan dye by NADPH at 565 nm, which is synthesized enzymatically in the pentose phosphate pathway. Results are presented in nmol/ml, nmol/mg protein, and nmol/10^6 cells.

Determination of Total Protein Levels

Total protein levels in milk cell supernatants were determined by the Bradford (1976) method (Coomassie Brilliant Blue G, Sigma 27815-100 G). Protein concentration was determined spectrophotometrically by determining the absorbance at 595 nm. Bovine serum albumin (Merck 112018) was used for a standard. The results are given as mg/ml and mg/10^6 cells.

Microbiological Examination

Milk samples brought to the laboratory were plated on Blood agar, MacConkey agar and Sabouraud Dextrose agar. For bacterial isolation Blood agar and MacConkey agar plates were incubated in aerobic me-

Table 1. Somatic cell count, aerobic mesophyle general organism (AMG) count in milk and total protein (TP) levels in milk cells (Mean±SE) (n=15)

Gruplar	Somatic Cell count in 1 ml milk x 10^3	AMG (Log10)	TP mg/ ml of cell supernatant	TP mg/10^6cells
Control	5-87	-	0,374±0.056 a	0.671±0.170 a
1st Group	154-381	2.59±1.53	0.202±0.034 b	0.038±0.007 b
2nd Group	418-851	2.51±1.26	0.156±0.014 b	0.015±0.002 b
3rd Group	914-1958	2.76±1.50	0.180±0.016 b	0.006±0.001 b
4th Group	2275-8528	3.47±1.45	0.374±0.057 a	0.005±0.001 b
P	-	-	<0.001	<0.001

a–b Means within a column with different superscripts differ.

Table 2. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and total glutathione (tGSH) levels in milk cells (Mean±SE) (n=15).

	NADPH nmol/ml	NADPH nmol/mg protein	NADPH nmol/10^6 cells	tGSH nmol/ml	tGSH nmol/mg protein	tGSH nmol/10^6 cells
Control	1.233±0.175	4.24± 0.73 b	2.623±0.780 a	42.47±4.07 a	122.88±13.08 c	86.77±23.60 a
1st Group	0.895±0.120	6.35±1.50 ab	0.159±0.021 b	29.76±3.46 b	204.27±38.76 abc	5.33±0.67 b
2nd Group	1.156±0.176	9.03±2.30 a	0.116±0.022 b	33.23±3.05 ab	226.95±22.40 a	3.23±0.33 b
3rd Group	0.995±0.141	5.00±0.70 ab	0.035±0.005 b	36.09±2.36 ab	214.64±18.30 ab	1.28±0.15 b
4th Group	1.525±0.451	3.31±1.12 b	0.020±0.005 b	36.65±3.59 ab	139.82±34.33 bc	0.54±0.05 b
P	-	<0.05	<0.001	<0.05	<0.05	<0.001

a–c Means within a column with different superscripts differ.

dium for 2–3 days at 37°C; then the growth colonies were identified using by biochemical tests (Gram staining, catalase, oxidase, coagulase, sugar fermentations, haemolysis, motility). In addition, for yeast and fungi isolation Sabouraud Dextrose agar plates were incubated at 25°C for 7–10 days under aerobic conditions. Species-level identification of *Aspergillus spp.* was performed by microscopic examination after lactophenol cotton blue staining and *Candida spp*. species-level identification performed by microscopic examination after Gram staining (Quinn et al. 1994, Schultz et al. 2004).

Aerobic Mesophyle General Organism Count

Milk samples were brought to the laboratory in sterile containers and under cold chain. Each milk sample weighed 10 g and was homogenized with 90 ml 0.1% of Peptone Water. The required decimal dilutions were prepared, and Plate Count Agar cultivation was performed. The media were left to incubate for 48 h at 30°C (Swanson et al. 1992). The results are presented as cfu/g according to base $\log_{10}$.

Statistical Evaluation

The values obtained were evaluated by ANOVA in SPSS 15.0 program and group differences were determined by Duncan test. G6PD levels do not show a normal distribution, thus, median values were assigned by using Man Whitney U test. Pearson correlation was performed for correlation analysis and $p<0.05$ indicated as important.

Results

The SCC and AMG in 1 ml of collected milk samples and TP levels in 1 ml of somatic cell homogenate supernatants are presented in Table 1. Total protein levels were found to be inconsistant when assessed as ml and were lower in the 1st, 2nd, and 3rd groups than in the control and 4th groups. When the TP levels in one million cells (10^6) were evaluated, a significant difference between the control group and all the mastitis groups was found ($p<0.001$).

The NADPH levels of the somatic cell supernatants are presented in Table 2. There was a significant decre-

Table 3. Glutathione peroxidase (GPx, Mean±SE) and glucose-6-phosphate dehydrogenase (G6PD, Median) activities in milk cells (n=15).

	GPx IU/ml	GPx IU/mg protein	GPx IU/10^6cells	G6PD IU/L	G6PD IU/g protein	G6PD IU/10^9cells
Control	1.34±0.06	4.44±0.36 a	2.87±0.72 a	0.46 c	1.80 c	1.24 a
1st Group	1.43±0.06	11.03±2.05 b	0.27±0.02 b	0.64 b	5.70 b	0.11 b
2nd Group	1.42±0.06	9.80±1.32 b	0.14±0.01 b	1.29 b	10.20 a	0.11 b
3rd Group	1.36±0.08	8.72±1.14 b	0.05±0.00 b	1.29 b	5.50 b	0.04 c
4th Group	1.46±0.05	4.98±0.93 a	0.02±0.00 b	6.75 a	17.60 a	0.11 bc
P	-	<0.01	<0.001	<0.001	<0.05	<0.05

a–c Means within a column with different superscripts differ.

Table 4. Correlations between the parameters (n=75).

	AMG	NADPH (nmol/ml)	TP (mg/ ml)	tGSH (nmol/ml)	GPx (IU/ml)	G6PD (IU/L)
Somatic Cell Count	0.561 *	0.380**	0.347**	0.174	0.136	0.540*
AMG		0.428**	0.638*	0.164	0.155	0.171
NADPH (nmol/ml)			0.239***	0.155	0.265***	0.248****
TP (mg/ml)				0.354**	0.048	0.643*
tGSH (nmol/ml)					-0.306***	0.198
GPx (U/ml)						0.007

* p<0.001, ** p<0.01, *** p<0.05, **** p=0.056

Table 5. Pathogens isolated from mastitic milks (n=15, totally 60 milk samples).

Patogens	Number- (%)
CNS (Coagulase Negative Staphylococcus)	28 (66.6)
Streptococcus spp.	4 (9.5)
Bacillus spp.	4 (9.5)
Yeast	2 (4.8)
Mixed Infection	4 (9.5)
Total Infection with pathogens	42 (100)
Total Infection with non-pathogens	18
No Growth	60

Mixed infections included 1 CNS + 1 Yeast, 1 CNS + 1 *Streptococcus spp*, 1 CNS + 1 *Bacillusspp*and 1 yeast+1 *Bacillus spp*.

ase in the mastitis groups compared to the control group as regards nmol/10^6 cells (p<0.001).

tGSH levels in the somatic cell supernatants are also presented in Table 2. When assessed as nmol/mg protein, tGSH levels were suspicious; 2nd group levels were higher than those in the control and 4th groups. However, when assessed as nmol/10^6 cells, the level of the control group was higher than that in all the mastitis groups (p<0.001).

G6PD and GPx activities in the somatic cell supernatants are presented in Table 3. When assessed as IU/L and IU/g protein, the G6PD levels of the 4th group were higher than those in the other groups, whereas when assessed as U/10^9 cells, the control group's G6PD levels were higher than those in the mastitis groups (p < 0.05). As regards GPx activity, when assessed as IU/mg protein, 1st, 2nd and 3rd group GPx activities were higher (p<0.01) from that in the control and 4th groups, whereas a significant decrease was determined in the mastitic groups compared to the control group as regards IU/10^6 cells (p<0.001).

The correlations between the biochemical parameters and SCC and AMG are presented in Table 4. Milk SCC was positively correlated with AMG (r=0.561,

p<0.001), NADPH (r=0.380, p<0.01), TP (r=0.347, p<0.01) and G6PD (r=0.540, p<0.001). There was also positive correlation between NADPH (r=0.428, p<0.01), TP (r=0,638, p<0.001) and AMG. NADPH was positively correlated with TP (r=0.239, p<0.05), GPx (r=0.265, p<0.05) and G6PD (r=0.248, p=0.056). Total protein was positively correlated with tGSH (r=0.354, p<0.01) and G6PD (r=0.643, p<0.001). There was a negative correlation between tGSH and GPx activity (r=-0.306, p<0.05).

Pathogens isolated from the milk with mastitis are presented in Table 5. The ratio of the pathogens was as follows: CNS 66.6%, *Streptococcus spp* 9.5%, *Bacillus spp* 9.5%, yeast 4.8%, and mixed infections 9.5%. Because insufficient pathogens were found for each group, a correlation calculation between biochemical parameters and pathogens could not be conducted.

Discussion

Mastitis causes physical, chemical, and bacteriological changes in milk and pathological changes in mammary glandular tissue (Sharma 2007). In this study, SCC was performed on milk samples which were collected according to CMT scoring and it was found that CMT scoring was not fully coherent with the SCC, which can be explained by the subjectivity of CMT scoring (Holtgrew-Bohling 2016). Correlations between the changes in the biochemical parameters in the supernatant obtained by concentration and homogenization of milk cells and SCC and AMG were determined, and the significant differences between the values of healthy milk and mastitic milk were identified.

When examining the TP levels in 1 ml of cell homogenate supernatant, we found that healthy milk had higher TP levels than the milk with mastitis (1st, 2nd, and 3rd groups) (p<0.001). In the 4th group, the value was closer to that of the healthy milk; this outcome can be explained by the elevated cell count and subsequent relative increase in TP levels. On the other hand, if the TP levels in 10^6 cells are considered, the TP levels per cell decreased as mastitis intensity increased. The correlation between the TP levels of all samples and SCC (r=0.347, p<0.01, n=75) echoed the results reported by Ritter et al. (1987).

In the present study, as the intensity of mastitis increased, G6PD activity in the supernatant of 1 ml of milk cell homogenate increased, but the difference was only statistically significant in the 4th group (Table 3). Milk somatic cells are comprised of leukocytes (PMN), macrophages, and epithelial cells (Baştan 2010). When an infection develops in mammary tissue, the ratio of leukocyte and epithelial cell counts in milk changes (Miller et al. 1993, Zhao and Lacasse 2008, Baştan 2010). Due to the chemotactic agents induced by the infectious agents, the PMNs in blood leak into milk, and PMNs start to play a major role in somatic cell composition (increasing from 5–25% to 90%) (Leitner et al. 2000, Riollet et al. 2000).

G6PD has been found in milk, breast tissue epithelial cells, and milk leukocytes (Ritter et al.1977, Shreve and Levy 1980, Grigor and Hartmann 1985). Grigor and Hartmann (1985) reported that, in rats, G6PD activity was substantially low in milk, while the mammary gland epithelial cell G6PD activity was rather high. They also concluded that G6PD found in milk was mainly derived from epithelial cell necrosis. In our study, the higher G6PD activity per cell in healthy milk than in mastitic milk can be attributed to the fact that healthy epithelial cell ratio was found to be higher in healthy milk than in mastitic milk.

The chemotactic effects of the infectious agents cause PMNs in the blood to seep into the milk through diapedesis, and PMNs play a major role in somatic cell composition (they can increase from 5–25% to 90%) (Leitner et al. 2000, Riollet et al. 2000). Milk epithelial cells in SCC also decrease proportionally. Meanwhile, some fluid also leaks into the mammary gland from the veins and alters the content of the milk. The function of PMNs is to phagocytize the pathogen, but they damage mammary tissue during this process. The destructive effects of PMNs on mammary epithelial cells are not yet clearly understood (Zhao and Lacesse 2008). Some researchers (Boulanger et al. 2002, Paape et al. 2002) suggest that PMNs exert their effects by releasing reactive oxygen species (ROS) outside the cell and secreting proteolytic enzymes (degranulation). Free radicals produced by leukocytes during inflammation are one of the body's defenses against microorganisms. Research has shown that, depending on the degree of mastitis and amount of PMN in the milk, the number of free radicals released into the milk increases, leading to damage to the mammary gland epithelial cells (Zhao and Lacasse 2008). Meanwhile, parallel to increased ROS and proteolytic enzymes, mammary epithelial tissue starts to be damaged (Leitner et al. 2000, Zhao and Lacasse 2008). Since PMNs with low G6PD activity will have lower resistance against ROS (Cooper et al. 1972), they can be degraded after phagocytosis of the infectious agents; the ROS they contain will leak into the milk. A PMN cell with insufficient capacity for antioxidant enzymes can be degraded after bacteria phagocytosis, and its contents (ROS and proteolytic enzymes), which are released outside, can cause necrosis in mammary epithelial tissues. Thus, udder epithelial cells undergo necrosis, and their contents mix with milk. Thus, a higher number of epithelial cells in healthy milk than

in mastitic milk (Leitner et al. 2000) and a high level of G6PD in the epithelial cells will cause higher levels of G6PD per cell in healthy milk. On the other hand, in mastitic milk, high leukocyte levels and low epithelial cell colony numbers will reduce the amount of G6PD per cell. A positive correlation between G6PD and SCC was found in all samples, and these results cohere with those of Ritter et al. (1987). In addition, the positive correlation identified between NADPH levels and SCC and TP levels shows compliance with G6PD.

The microbiological results of this study showed that infections with CNS were much more frequent than those with other pathogens. It has been also reported by Yağcı (2008) that CNS in subclinical mastitis are more dominant than the other agents.

When the biochemical parameter levels in milk were investigated, many factors such as PMN, necrotic epithelial cells, fluid leak to milk from extracellular fluid, and type of infectious agent complicated the utilization of biochemical parameters (in ml milk) in mastitis diagnosis and prognosis. In previous studies, (Dündar et al. 2000, Weiss et al. 2004, Erişir et al. 2011, Szczubial et al. 2012) biochemical parameters found in 1 ml of milk with subclinical mastitis were evaluated. However, due to differences between the changes in milk somatic cell composition (Leitner et al. 2000), the authors suggest that evaluating the parameter per somatic cell would be more appropriate. In future studies, separating epithelium, leukocytes, and pathogens in milk with mastitis and identifying G6PD activity in each cell type is recommended.

Conclusion

Considering the parameters analyzed in this study in terms of ml and mg/protein, while different changes among groups were detected, the parameter levels per somatic cell were significantly lower than those with healthy milk. When evaluating the enzyme and oxidative stress parameters in milk, it is more suitable to assign values based on cell count rather than ml of milk. The linear correlation between the SCC and AMG, milk cell NADPH, TP and G6PD suggests that these parameters could be used as markers of mastitis. The study also found that CNS was the dominant pathogens in subclinical mastitis cases.

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