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RESEARCH ARTICLE

# Changes in Some Biochemical Parameters and Somatic Cell Counts in the Milk of Buffalo and Cattle Suffering from Mastitis

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**ABSTRACT**

The study was conducted on a total of 592 buffaloes and 453 cattle in their different stages of lactation to investigate the biochemical changes occurring in milk due to mastitis. California Mastitis Test (CMT) was used to diagnose the mammary gland infection. The results revealed significant (P<0.0001) increase in pH, electrical conductivity, malondialdehyde and total dissolved solids, while decrease in fat, protein, lactose and solids not fat in milk samples of both mastitic buffaloes and cattle. The total somatic cell and neutrophil counts were significantly higher, while the macrophage and lymphocytes were lower in the milk of mastitic animals. The enzymes including lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase along with sodium were significantly higher in mastitic than healthy buffaloes. It was similar in cattle as well, with the exception of aspartate aminotransferase which was non-significant in cattle. The values of potassium, phosphorous, calcium, magnesium, zinc and iron were significantly higher in the milk of mastitic animals. The copper levels were significantly (P<0.0001) lower in mastitic than in healthy buffaloes, while it showed non-significant difference in cattle. The investigation of enzymes, lipid peroxidation product and milk electrical conductivity in present study appeared suitable diagnostic tools for identification of mastitis.

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## INTRODUCTION

Dairy farming in Pakistan is emerging as an industry with great potential to supply meat and milk not only to the people of Pakistan but also to the other countries of the world. The buffaloes and cattle are the main dairy animals in the country and are contributing to the bulk supply of milk (95%) in the market (Javaid *et al*., 2009). The Nili-Ravi breed of buffalo is producing about 75% of all the milk produced in the country (Sharif *et al*., 2009). Dairy animals encounter various problems including climatic changes, management issues, marketing issues and diseases. Mastitis is one of the important disease entities causing hindrance in the development of dairy sector. Mastitis in early lactation results in long term production losses in dairy industry (DeVliegher *et al*., 2005; Sharif and Muhammad, 2009; Ahmadzadeh *et al*., 2009; Bachaya *et al*., 2011), increases the risk of getting clinical mammary infection in subsequent lactation and an increased risk of premature culling from the herd (Parker *et al*., 2007).

Milk production by the mammary tissue is a complex process which is under the influence of local and systemic hormones along with involvement of some other factors which affect milk yield (Bhutto *et al*., 2010). Mastitis poses innumerable problems to milk production process and also affects its quality. Mastitis is associated with changes in physical, chemical, bacteriological and organoleptic properties of milk, besides causing health hazards to the public. The involvement of polymorpho-nuclear leukocytes and macrophages during inflammatory process triggered by pathogens is crucial against intra-mammary infections. The activities of the resident and newly recruited leukocytes during the early stages of mastitis play vital role in the establishment of intra-mammary infection. Neutrophils are the predominant cell type found in the mammary tissue/secretions during early inflammation and constitute more than 90% of the total mammary leukocytes (Abera *et al*., 2010). Milk somatic

cells consist of several types, including neutrophils, macrophages, lymphocytes and a small percentage of epithelial cells (Zaman *et al*., 2009). When somatic cell count increases in milk, a significant decrease in milk proteins and calcium occur during clinical or subclinical mastitis. The inflammatory cells and damaged epithelial cells release various products including hydrolytic enzymes such as lactate dehydrogenase and *β*-galactosidase (Oliszewski *et al*., 2002). During microbial killing process, free radicals produced from leukocytes result in damage to mammary epithelial cells with resultant decrease in milk production (Barbano *et al*., 2006). Lipid peroxidation by free radicals is a key factor in various mammary tissue pathologies including inflammation. Malondialdehyde is one of the peroxidation products present in the milk of dairy animals which can be used to identify the relationship between somatic cell count and udder inflammation (Suriyastha- porna *et al.*, 2006; Ibrahim *et al*., 2011). Certain minerals including Zn, Cu, and Se play crucial role in ensuring efficient body growth, reduced milk somatic cell count and increased milk production (Cortinhas *et al*., 2010; Hameed *et al*., 2010). These minerals are also present in the secreted milk and their level is affected due to mastitis. In this study, the biochemical changes in milk of both cattle and buffalo are investigated which can be used as diagnostic tool and prognostic markers in mastitic cases.

## MATERIALS AND METHODS

The present study was conducted on a total of 592 buffaloes and 453 cattle present at two cattle/buffaloes colonies and two public livestock farms near Okara and Sahiwal districts of Pakistan. A total of 121 positive quarters milk samples from cattle and 120 from buffaloes were collected in the present study. The equal number of quarter's milk samples from infected and healthy cattle and buffaloes were investigated for milk changes. The per day milk yield was also recorded. Milk samples of about 50 ml from all the mastitic lactating animals were collected and tested with California Mastitis Test. Total somatic cell count and differential leukocyte counts were also carried out (Lindmark-Mansson *et al*., 2006; Gargouri *et al*., 2008). Milk electrolytes and trace minerals were determined after wet digestion of milk samples. Briefly, 5 ml milk sample and 10 ml concentrated nitric acid were taken into a 250 ml conical flask and heated on Bunsen burner for 20-25 minutes. Thereafter, 5 ml perchloric acid was added with continuous heating till the quantity was condensed to 2-3 ml. Deionized water was then added to make the final volume 50 ml. The digested and diluted samples were used for the estimation of various macro and micro minerals present in the milk. The levels of sodium and potassium (mg/dl) in the milk were determined by using the flame photometer. The total phosphorus (mg/dl) was determined by colorimetric method, on UV spectrophotometer at a wavelength of 720 nm against standard and blank. The copper, calcium, zinc, magnesium, iron and manganese were determined by atomic absorption spectrophotometery (Ahmad *et al*., 2007). The milk lactose, protein, fat and solids not fat were estimated by using Lactoscope. Milk pH was determined by using pH meter. The milk temperature and electrical conductivity were determined with the help of an electrical conductivity meter. Malondialdehyde concentration in milk was determined according to the modified method of Suriyasathaporn *et al*. (2006). The milk samples were centrifuged at 3000 rpm for about 5 minutes to remove the fat. The defatted milk samples were used to study the levels of lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) by using commercially available kits. The enzymes LDH (Cormay, Catalog # 1-239), ALP (Cormy, Catalog # 1-212) and AST (Analyticon, Catalog # 1178) were determined on a spectrophotometer at wavelengths of 340, 405 and 340 nm, respectively (Babaei *et al*., 2007).

**Statistical analysis:** The data collected in this study were analysed by using ANOVA and means were compared by t-test. $P<0.05$ was considered as significance level.

## RESULTS

The means±SD of different variables relating to milk in buffaloes and cattle are presented in Table 1. The temperature of fresh milk samples did not show statistical difference between mastitic and healthy buffaloes and cattle. The pH, electrical conductivity, malondialdehyde and total dissolved solids were significantly higher in milk samples of both mastitic buffaloes and cattle as compared to healthy ones. However, milk yield, milk fat, protein, lactose and solids not fat were significantly ($P<0.0001$) lower in milk samples of mastitic buffaloes and cattle as compared to healthy ones (Table1).

**Table 1:** Analysis of various milk parameters (Mean±SD) of healthy and mastitic buffaloes and cattle

| Parameter/Species | Healthy | Mastitic | P-Values |
|---|---|---|---|
| Buffalo | | | |
| Milk temperature (°C) | 37.1±0.2 | 38.3±0.7 | <0.14 |
| Milk pH | 6.9±0.2 | 7.1±0.2 | <0.0001 |
| Milk yield (L) | 5.9±1.0 | 4.6±1.3 | <0.0001 |
| MEC (mS/cm) | 4.9±0.2 | 6.1±0.7 | <0.0001 |
| Malondialdehyde (nmol/ml) | 17.3±2.9 | 31.3±4.3 | <0.0001 |
| Fat (%) | 6.6±0.3 | 4.6±0.5 | <0.0001 |
| Protein (%) | 4.8±0.2 | 4.0±0.3 | <0.0001 |
| Lactose (%) | 5.1±0.2 | 3.9±0.4 | <0.0001 |
| Solids not fat (%) | 9.9±0.3 | 8.0±0.8 | <0.0001 |
| TDS (mg/L) | 1337.3±68 | 1852.8±138 | <0.0001 |
| Cattle | | | |
| Milk temperature (°C) | 37.4±0.2 | 37.5±3.7 | <0.851 |
| Milk pH | 7.0±0.1 | 7.2±0.3 | <0.0001 |
| Milk yield (L) | 6.3±2.3 | 5.6±1.6 | <0.001 |
| MEC (mS/cm) | 5.1±0.3 | 6.3±0.6 | <0.0001 |
| Malondialdehyde (nmol/ml) | 4.0±0.2 | 8.3±1.6 | <0.0001 |
| Fat (%) | 5.5±0.3 | 4.2±0.5 | <0.0001 |
| Protein (%) | 4.5±0.2 | 3.7±0.3 | <0.0001 |
| Lactose (%) | 4.7±0.2 | 3.9±0.3 | <0.0001 |
| Solids not fat (%) | 11.3±0.2 | 8.3±0.8 | <0.0001 |
| TDS (mg/L) | 1370.8±129 | 1798.6±146 | <0.0001 |

Milk electrical conductivity (MEC); Total dissolved solids (TDS).

The means±SD of total somatic cells and differential leukocytic counts are presented in Table 2. The total somatic cell and neutrophil counts were significantly higher in mastitic than healthy buffaloes and cattle. However, the macrophage and lymphocytes were significantly ($P<0.0001$) lower in milk of mastitic than healthy buffaloes and cattle.

The results of various milk serum enzymes and minerals in healthy and mastitic buffaloes and cattle are presented in Table 3. The enzymes including lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase along with sodium levels were significantly higher in mastitic than healthy buffaloes. Similar findings were observed in cattle with the exception of aspartate aminotransferase which was non-significant. The values of potassium, phosphorous, calcium, magnesium, zinc and iron was significantly higher in milk samples of mastitic than healthy buffaloes and cattle. The copper levels were significantly ($P<0.0001$) lower in mastitic than healthy buffaloes, while it showed non-significant difference in cattle (Table 3).

**Table 2:** Milk total somatic cell (Mean ± SD) and differential score of mastitic and healthy buffaloes and cattle

| Parameter/Species | Healthy | Mastitic | P-Values |
|---|---|---|---|
| Buffalo | | | |
| Total milk somatic cell count (x$10^5$/ml) | 3.64±0.43 | 62.69±30.4 | <0.0001 |
| Neutrophil (%) | 22.48±2.39 | 60.81±5.7 | <0.0001 |
| Macrophages (%) | 25.82±3.12 | 12.87±4.09 | <0.0001 |
| Lymphocyte (%) | 30.80±2.64 | 16.27±2.68 | <0.0001 |
| Cattle | | | |
| Total milk somatic cell count (x$10^5$/ml) | 3.40±1.48 | 41.34±25.49 | <0.0001 |
| Neutrophil (%) | 17.86±6.47 | 52.70±7.85 | <0.0001 |
| Macrophages (%) | 45.76±5.66 | 25.86±4.56 | <0.0001 |
| Lymphocyte (%) | 19.74±3.10 | 13.29±2.47 | <0.0001 |

**Table 3:** Changes in various milk enzymes (U/L) and minerals (mg/dl) (Mean±SD) of mastic and healthy buffaloes and cattle

| Parameter/Species | Healthy | Infected/Mastitic | P Value |
|---|---|---|---|
| Buffalo | | | |
| LDH | 176.94±14.14 | 1528.09±362.49 | <0.0001 |
| AST | 18.19±1.74 | 42.54±15.17 | <0.0001 |
| ALP | 248.00±11.83 | 414.07±37.34 | <0.05 |
| Sodium | 46.61±2.00 | 74.80±9.46 | <0.0001 |
| Potassium | 33.94±1.75 | 24.45±17.65 | <0.005 |
| Phosphorous | 12.67±3.62 | 80.25±9.00 | <0.0001 |
| Calcium | 42.84±3.30 | 30.13±1.24 | <0.0001 |
| Magnesium | 17.85±1.15 | 14.06±0.73 | <0.0001 |
| Copper | 14.27±0.80 | 9.54±0.61 | <0.0001 |
| Zinc | 42.53±1.72 | 37.03±0.91 | <0.0001 |
| Iron | 18.80±0.88 | 15.40±1.35 | <0.0001 |
| Cattle | | | |
| LDH | 149.61±102.07 | 647.91±348.72 | <0.0001 |
| AST | 140.33±26.75 | 152.15± 57.31 | 0.561 |
| ALP | 149.71±11.17 | 157.79±23.77 | <0.0001 |
| Sodium | 59.31±5.76 | 89.46±12.13 | <0.0001 |
| Potassium | 161.48±9.78 | 147.04±16.59 | <0.0001 |
| Phosphorous | 31.84±1.78 | 24.60±10.24 | <0.0001 |
| Calcium | 97.43±3.37 | 80.74±2.71 | <0.0001 |
| Magnesium | 9.51±0.63 | 8.12±0.46 | <0.0001 |
| Copper | 0.12±0.03 | 0.14±0.21 | 0.727 |
| Zinc | 4.10±0.25 | 3.49±0.14 | <0.0001 |
| Iron | 11.33±1.26 | 7.38±0.59 | <0.0001 |

Lactate dehydrogenase (LDH); Aspartate aminotransferase (AST); Alkaline phosphatase (ALP)

## DISCUSSION

Mastitis results in biochemical changes in milk of both cattle and buffalo with very minor difference between the two species as evident from the results of the present study. The electrical conductivity and pH of milk from mastitic animals were significantly higher due to the presence of clinical and sub-clinical infections which can be used as an adjunct test for diagnosis of mastitis in animals along with other available tests for mastitis including surf field mastitis test. The increase in electrical conductivity of mastitic milk could be due to higher concentration of salts released due to increased permeability of cell membrane because of inflammatory process and thus might be responsible for increase in pH of milk samples. To the best of our knowledge there is no report on electrical conductivity results in mastitic buffaloes. However, Roy *et al*. (2009) reported the result of electrical conductivity of milk samples from mastitic cattle. They also reported an increase in electrical conductivity of milk samples from mastitic animals of both clinically and sub-clinically infected animals. The significant decrease in milk fat, protein, lactose and solids not fat in mastitic buffaloes and cattle observed may be linked with impaired synthetic activity of mammary tissues or it may also occur due to the damaging effects of pathogens to the mammary parenchyma. These results are similar to previous studies showing decrease in fat, protein and lactose in milk (Lindmark-Mansson *et al*., 2006; Ahmed *et al*., 2007). There are few studies where increase in milk lactose from mastitic animals has been reported (Dhillon *et al*., 2000). In present study, an increase in the level of sodium in milk samples from mastitic animals was observed, while other minerals including potassium, calcium, zinc, iron, magnesium and phosphorus decreased. Similar results have previously been reported for cattle (Ahmad *et al*., 2007; Betavani *et al*., 2007). This shows no specie variation in changes of mineral in milk samples as the change in levels of these minerals was also observed in buffaloes. The change in milk pH is thus related with the increase in sodium levels in the milk and probably the electrical conductivity also is influenced by the change in sodium levels in milk. The copper concentration showed variation between species as the copper level was lower in the milk of mastitic buffaloes than the healthier ones but it did not show any change in the milk of infected and non infected cattle. This might be due to species differences in some biochemical mechanisms contributing to change in levels of copper in buffalo but not in cattle. However, further studies are needed to confirm such differences and explore the underlying mechanisms.

The total somatic cell and neutrophil counts in present study were significantly higher in infected cattle and buffaloes indicating mammary gland infection. As inflammatory response of udder is directly related to neutrophil influx. Neutrophil population in milk is a useful indicator in the evaluation of mammary gland infection as whenever the inflammatory process starts, the neutrophil number increases. Similar findings have been previously reported (Moroni *et al*., 2006; Piepers *et al*., 2009; Zaman *et al*., 2009). However, it was observed that lymphocyte, monocyte and macrophage population significantly decreased in the milk of infected animals. Similar findings as in our study regarding differential cell count have been reported earlier (Schukken *et al*., 2003; Gargouri *et al*., 2008).

The increase in milk enzymes including lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase in mastitic animals may be linked with tissue damage occurring in mammary tissue and is very much an expected change (Batavani *et al*., 2003). There is no specie variation observed as all these enzymes behaved in

similar fashion in milk of buffalo and cattle. To the best of our knowledge, there is no previous report indicating the changes of these enzymes in mastitic buffaloes. Such changes however, are reported in ewes and cattle (Batavani *et al*., 2003; Babaei *et al*., 2007; Ibrahim *et al*., 2011). The findings of present study showed variation in levels of AST as a significant increase in AST levels in buffalo milk was observed, while non-significant difference was observed in cattle milk. Babaei *et al*. (2007) also observed a non-significant difference in the AST levels of milk samples from mastitic and healthy cattle. The increased levels of various enzymes in milk occur mainly due to increased permeability of microcirculatory vessels in inflamed areas along with leakage from degenerated/necrotic parenchymal cells and leukocytes.

**Conclusions:** In present study, higher activities were observed for different enzymes and lipid peroxidation product which can be used to diagnose mastitis. The milk fat, protein, lactose and solids not fat (%) were decreased showing negative impact on udder tissues. Along with the gold-standard screening test (CMT), the milk electrical conductivity test can also be used for diagnosis of subclinical mastitis.

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