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CHANGES IN VARIOUS METABOLIC PARAMETERS IN BLOOD AND MILK OF DAIRY COWS DURING BOVINE MASTITIS

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ABSTRACT

The aim of this study was to elucidate and compile some of major findings concern to biochemical changes in blood and milk of cow during bovine mastitis. The surf field mastitis test was used to diagnose bovine mastitis and health status of animals was also further confirmed by veterinary doctor. The analysis of blood samples revealed a significant decrease in the hemoglobin and RBC count, whereas significant increase in the leucocytes counts in the blood sample of cows suffering from mastitis. Evaluation of oxidative stress parameters revealed that there was a significant decline in the activity of SOD, CAT and GST and also decline in the level of GSH. However, significant elevation in the level of TBARS was observed in the blood sample of cows with mastitis. Further the study showed a significant increase in the activity of alkaline phosphatase and lactate dehydrogenase in the blood of the cows with bovine mastitis. Whereas significant decrease in the concentration of phosphorus was noted in the blood of cows suffering from mastitis. Additionally, the study presented a significant decrease in the carbohydrate and fat content, whereas significant increase in the protein and total dissolved solids in milk of cows with intramammary infection. It was also noted that in SDS PAGE number of protein bands in the milk sample of infected cows were more than the healthy milk samples. The results of present study is an evidence to suggest the effective defence mechanism of the immune system to prevent and neutralize the free radical induced during intramammary infections and our results conclude that all the noticeable findings in this investigation are due to the impairment in mammary gland due to invading pathogenic bacteria causing bovine mastitis.

Keywords: *Bovine Mastitis, Inflammation, Hemogram, Oxidative Stress, Alkaline Phosphatase, Phosphorus, Lactate Dehydrogenase, Milk*

INTRODUCTION

Mastitis is one of the ancient diseases observed in all milching animals over 5000 years ago. Since then it has been an ever present problem for all those who kept and milked dairy cattle. Mastitis in cows results in significant losses to the dairy industry and is likely the most expensive veterinary disease affecting dairy cattle throughout the world.

The milk is secreted by the epithelial cells lining the alveoli from the blood brought up the blood stream, some constituents of milk are present in the blood and merely transferred to the milk and some constituents not present in the blood has to be synthesized from other constituents of the blood (Sharma *et al.*, 2012). Dairy cows undergo several physiological changes during the onset of lactation that can impact the magnitude and duration of mammary gland inflammatory responses such as oxidative stress, that occurs during imbalance production of oxygen radicals and host's antioxidant defenses (Sordillo and Aitken, 2009).

The incidence of intramammary infections is generally accepted as the most critical periods with respect to mammary health of a dairy cow. During this period the mammary gland undergoes marked biochemical, cellular and immune modulatory changes to accommodate resistance for any infections, to withstand the stress of the inflammation. To counter any bacterial infection PMN (polymorphonuclear neutrophils) constitute the first line of defense. It is the rapid recruitment of sufficient numbers of PMN into the mammary gland and increased phagocytosis at the beginning of infection that prevents establishment of mastitis (Paape *et al.*, 2003).

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Mastitis influences the total milk output and modifies its composition. The relationship of mammary gland inflammation to milk yield and milk composition has received considerable research interest because of tremendous economic implications. Numerous studies have shown clearly that inflammation of the mammary gland influences the total milk output and modifies its composition (Moyes *et al.*, 2014). The magnitude of reduced milk yield and alterations in milk composition is influenced by the severity of the udder infection and type of pathogen causing bovine mastitis. Although, much is known about the influence of mastitis on milk composition, the series of events that occur in the mammary gland after the invasion of mastitis pathogen and how these events influence immune response in cow are not well understood. Therefore, the objectives of the present study is to elucidate the influence of bovine mastitis on haemogram, oxidative stress parameters and biochemical changes in blood of the cow and also to describe changes in milk composition associated with mammary gland inflammation.

MATERIALS AND METHODS

The study was undertaken at Department of Microbiology and Biotechnology Karnatak University, Dharwad. Holstein cows maintained at organized herds near to University were considered for the study. The herds were selected on the willingness of owners to participate in the study and were visited for collection of blood and milk sampling.

Sample Collection

The cows were divided into two groups viz healthy (mastitis negative group) and Infected (mastitis positive group) based on the absences and presence of intramammary infections respectively. Before collection of blood and milk samples, the surf field mastitis test (SFMT) was performed to diagnose the intramammary infection in cattle (Muhammad *et al.*, 1995). Each group contain ten cows and cows showing no results for SFMT were grouped as mastitis negative which were further examined by a veterinary doctor and confirmed as healthy cows free from any other disease. Cows showing positive reaction for SFMT were grouped as mastitis positive (infected animals) for the presence of bovine mastitis.

10 ml of blood samples from all the animals were collected from the jugular vein in test tubes with and without EDTA. The tubes containing blood without EDTA were kept in a slant position for separation of serum at room temperature for two hours the serum was harvested by centrifugation at 3000 rpm for 15 min and the separated serum was used for the further study (Yildiz and Kaygusuzoglu, 2005).

Hematological Parameters of the Cow

Blood samples from both group of cattle was collected in EDTA (2-3ml) vials. The blood samples were analyzed for Hb, RBC and WBC within 2-4 hrs of collection using the methods described by Jain (1986).

Antioxidants and Stress Enzymes

Preparation of hemolysate was done as per procedure described by Jhambh (2013). The blood samples were collected from both positive and negative group animals in centrifuge tube containing anticoagulant. After centrifugation at 3000 rpm for 10 min, the plasma and buffy coat were removed to harvest the red blood cells (RBC). Part of RBC pellet was diluted with chilled distilled water in 1:10 for the preparation of 10% stock hemolysate which was used for the estimation of SOD, CAT, GST activities and lipid peroxidation (LPO) and rest of the RBC pellet was diluted with chilled normal saline in 1:1 to get 50% RBC suspension for GSH estimation.

Reduced Glutathione

GSH level was measured following the method of Ellman procedure modified by Hissin and Hilf (1973). 5% TCA was added to the tube containing 50% RBC suspension (720 μ l) to precipitate the protein content of the hemolysate.

After centrifugation (10,000 \times g for 5 minutes) the supernatant was taken, 5,5'-dithiolbis-2-nitrobenzoic acid (DTNB) solution (Ellman's reagent) was added to it and the absorbance was measured at 412 nm. A standard graph was drawn using different concentrations of standard GSH solution (1 mg/ml). With the help of the standard graph, GSH contents in the RBC suspension of the blood samples were calculated $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

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Thiobarbituric Acid Reactive Substances

The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) were measured by a modified method of Esterbauer and Cheeseman (1990). For each sample to be assayed, four tubes were set up containing 100, 150, 200 and 250 μL of 10% hemolysate, 100 μL of 8.1% SDS, 750 μL of 20% acetic acid, and 750 μL of 0.8% aqueous solution of TBA. The volume was made up to 4 ml with distilled water, mixed thoroughly and heated at 95°C for 60 minutes. After cooling, 4 ml of n-butanol was added to each tube, the contents mixed thoroughly, and then centrifuged at 3000 rpm for 10 minutes. The absorption of the clear upper (n-butanol) layer was measured using a spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$ and was expressed in $\mu\text{mol TBARS/g Hb}$ ($\epsilon = 1.56 \times 10^5 \text{ mM}^{-1} \text{ cm}^{-1}$).

Glutathione-S-Transferase

GST activity was measured by the method of Habig *et al.*, (1974). The reaction mixture contained suitable amount of the enzyme (50 μl of 10% hemolysate), 1 ml of KH_2PO_4 buffer, 0.2 ml of EDTA, 0.1 ml of 1-chloro-2,4- dinitrobenzene (CDNB) and GSH. The reaction was carried out at 37°C and monitored spectrophotometrically by the increase in absorbance of the conjugate of GSH and CDNB at 340 nm. A blank was run in absence of the enzyme. One unit of GST activity is 1 μmol product formation per minute ($9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$).

Superoxide Dismutase Activity

SOD activity was assayed by the method of Kakkar *et al.*, (1984) with little modifications. Reaction mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 mM, pH 7.0), 0.1 ml of phenazine methosulphate (PMS) (186 μM), 0.3 ml of nitro blue tetrazolium (NBT) (300 μM). 0.2 ml of 10% hemolysate was added to reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 μM) and stopped precisely after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm, results are expressed as units/g Hb.

Catalase

Catalase is a common enzyme found in nearly all living organisms which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. 50 μl of 10% hemolysate was added to a 3.0 ml cuvette that contained 1.95 ml of 50 mM phosphate buffer (pH 7.0). 1.0 ml of 30 mM hydrogen peroxide was added and changes in absorbance were followed for 30 sec at 240 nm at 15-sec intervals. Catalase activity was expressed as Unit /g Hb (Aebi, 1984).

Alkaline Phosphatase Activity (AKP) and Lactate Dehydrogenase (LDH)

The blood investigation for serum AKP and LDH were done in semi automated analyser Vitros 250 using Vitros reagent packs and activity of both enzymes were expressed as U/L.

Phosphorus

Fiske and Subbarow method is employed for the determination of phosphorus in blood sample (Fiske and Subbarow, 1925). Serum is treated with trichloroacetic acid to remove protein and lipid phosphorus. The supernatant fluid is reacted with ammonium molybdate in an acid solution to form phosphomolybdate. A mixture of sodium bisulfite, sodium sulfite and 1-amino-2-naphthol-4-sulfonic acid (Fiske and Subbarow Reducer) reduces the phosphomolybdate to form a phosphomolybdenium blue complex as according to the reaction included. The intensity of the color is proportional to the phosphate concentration and is measured at 660 nm.

Milk Samples

Milk samples (10 ml) were collected into tubes after the udder was thoroughly washed with clean water and dried with clean paper towel. 2 ml of milk sample was incubated at 75°C for 15 minutes and centrifuged at 15,000 $\times g$ (Pourkabir and Gharib, 2010). The supernatant was collected and used for estimation of carbohydrate (Sadasivam and Manickam, 1996) and protein content in the milk (Lowry *et al.*, 1951). Fat in milk was determined with little modification of the lab manual for analysis of milk and milk products (Manual for Analysis of Milk and Milk Products, 2005). A total solid in whole milk was determined as per the FSSAI Ministry of Health and Family Welfare India, New Delhi (FSSAI, 2012).

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SDS-Polyacrylamide Gel Electrophoresis

2 ml of milk sample was incubated at 75°C for 15 minutes and centrifuged at 15,000 ×g. The supernatant was collected and diluted (1:1) with sample buffer (3% SDS, 5% 2ME, 10% glycerol, 0.005% BPB, 6% Tris base, pH 7.3). They were then incubated at between 90 and 100°C for 5 mins and then again centrifuged at 15,000 ×g for 5 mins. The resultant supernatant was applied to polyacrylamide gels that contained 12% SDS. Gel casting was done with Biorad gel cast system and was placed in the electrophoresis unit. About 15-20 µl of the sample was loaded into PAGE and was run at 100 V for 3-4 hours. After the completion of electrophoresis the gel was taken out carefully and rinsed with distilled water to remove excess SDS and then stained with 0.25 % Coomassie brilliant blue R-200 staining solution for overnight. Destaining of the gel was done for 20-30 minutes and then gel documentation was done in the Biorad gel documentation system.

Statistical Analysis

Statistical analysis was conducted by using 16.0 for windows. All data's were analyzed by using Student's t-test to know the significance values between the groups. P<0.001 was considered as significance level.

RESULTS AND DISCUSSION

Effect of Bovine Mastitis on Heamogram of the Cow

The heamoglobin of healthy animal was 9.74 g/dl whereas the heamoglobin of infected animal was 7.78 g/dl. The number of RBC count in the healthy animals was $8.82 \times 10^6 / \text{mm}^3$ whereas the number of RBC count in the infected animal was $5.97 \times 10^6 / \text{mm}^3$. The total number of leucocytes in the healthy animals was $9.06 \times 10^3 / \text{mm}^3$ and the total number of leucocytes in the infected animal was $12.61 \times 10^3 / \text{mm}^3$ (Figure 1).

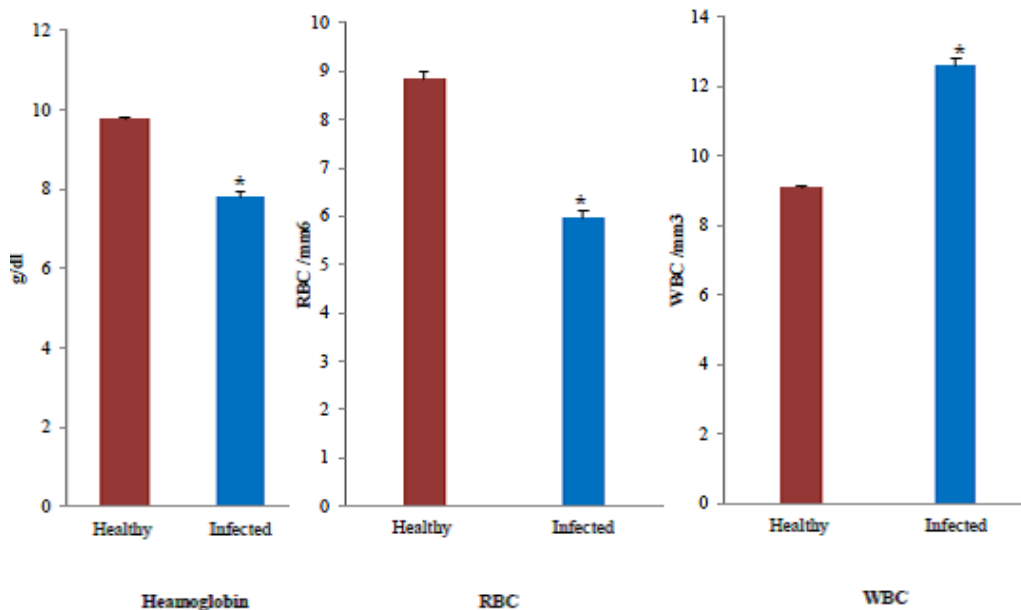


Figure 1: Effect of Bovine Mastitis on Heamogram of the Cow

Mastitis is characterised by increased milk SCC and neutrophils are the first cells to migrate from blood into an inflamed area after initiation of inflammation (Jain, 1993). The main function of neutrophils is phagocytosis and intracellular killing of engulfed bacteria by two distinct mechanisms, the respiratory burst and digestion by lysosomal enzymes (Woessner, 1992). In the present study effect of bovine mastitis on heamogram of the cow showed that the heamoglobin content of the cows with bovine mastitis significantly decreased when compared with that of healthy animals. Similarly, the RBC count in the

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blood sample of infected cows revealed that there was a significant decrease in the number of RBC of the cows with bovine mastitis when compared with that of healthy animals. However, the results of total number of leucocytes in blood sample of both healthy and infected cows revealed that there was a significant increase in the total number of leucocytes of the cows with bovine mastitis. Similarly, studies from Egypt suggests that there was decrease in the hemoglobin percentage and RBC and increase in the WBC count of the buffalo suffering from intramammary infection (Zaki *et al.*, 2008). Correspondingly, previous studies also reported similar changes in the hemogram level of the cows with bovine mastitis compared to healthy cows (Zaki *et al.*, 2010; Pyorala, 2003). In the present study decrease in the hemoglobin content is due to reduction in the number of RBC due to the inflammation of the mammary gland. However, the stress of disease may be responsible for change in the clinic opathological parameters in blood of cows with mastitis.

Effect of Bovine Mastitis on Antioxidants and Stress Enzymes in the Cow

The level of GSH in the blood of the both healthy and bovine mastitis positive group of animal's was 14.73 and 7.21 unit/gHb respectively. Similarly, the GST activity in the blood of the healthy animals was 7.85 unit/gHb whereas the GST activity in the blood of infected animal was 3.59 unit/gHb. The SOD activity in the blood of healthy and diseased animals was 51.12 and 27.79 unit/gHb respectively. The CAT activity in the blood of the healthy animals was 159.99 unit/gHb but in the blood of infected animals it was 64.56 unit/g Hb. The TBARS level in the blood of the healthy animal's was 4.08unit/gHb whereas in infected animals blood it was 10.12 unit/gHb (Figure 2).

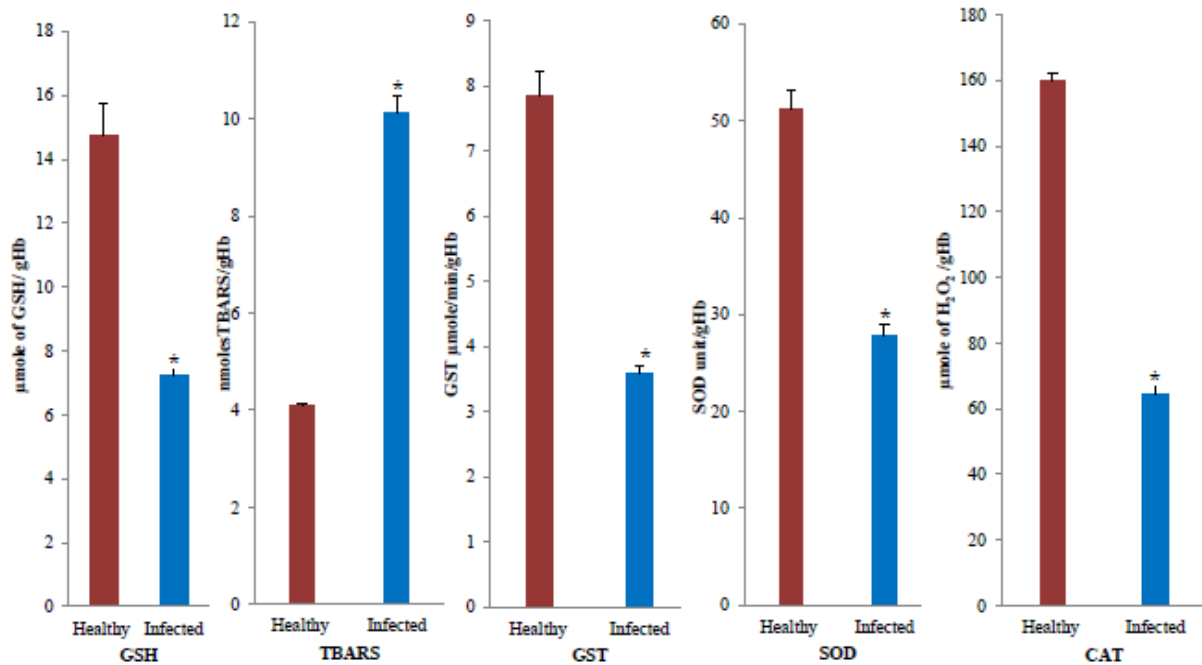


Figure 2: Effect of Bovine Mastitis on Oxidative Stress Parameters

GSH plays a fundamental role in the antioxidant biology of mammals. Severe GSH depletion is associated with pathologic consequences including, but not limited to, susceptibility to the development of lipid peroxidation (Anundi *et al.*, 1979). GSH is the major cellular sulfhydryl compound that serves as an effective reductant and a nucleophile that interacts with numerous electrophilic and oxidizing compounds (Mitchell *et al.*, 1976).

The present study showed a significant depletion in the level of GSH in the blood of the cows with bovine mastitis when compared with that of healthy cows. The depletion in the level of GSH of the infected cows

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may be attribute to the increased oxidant mediated cell injury during inflammation of udder, that enhanced the participation of GSTs in the removal and reduction of (hydro) peroxides at the expense of GSH utilization.

Lipid peroxidation is the most extensively studied manifestation of oxygen activation in biology. LPO is broadly defined as “oxidative deterioration of poly unsaturated fatty acids (PUFA)” which are fatty acids that contain more than two carbon carbon double bonds (Halliwell, 1990). LPO is a chain reaction between PUFA and reactive oxygen species (ROS) and it produces lipid peroxides and hydrocarbon polymers that are highly toxic to the cell. Malonyldialdehyde is an end product of peroxidation of polyunsaturated fatty acids and related esters used as a marker of lipid peroxidation.

The current investigation revealed that there was a significant increase in the level of TBARS in the blood of the cows with bovine mastitis. The findings of our study are in corroboration with the reports of Saleh *et al.*, (2007), they used TBARS values as a marker of lipid peroxidation in cattle. Likewise, a study from Pakistan and China suggests that change in the level of MDA in the milk of the infected animal were in similar fashion (Hussain *et al.*, 2012; Yang *et al.*, 2011). The increase in the concentrations of MDA is the indicator of generation of free radicals that induce lipid peroxidation in mammals and other organisms (Comelekoglu *et al.*, 2000).

Therefore, in the current investigation increase in MDA level was probably due to the increased level of free radicals in the blood of infected animals due to udder inflammation.

Reactive oxygen species must be continuously inactivated to keep only a small amount necessary to maintain normal cell function. Antioxidants, in general, are compounds which dispose, scavenge, and suppress the formation of ROS, or oppose their actions. Among the well-known biological antioxidants, SOD and catalase have a significant role. GSTs belong to a family of phase II enzymes that catalyze the conjugation of GSH into a wide variety of electrophilic compounds (Gong *et al.*, 2005).

The findings of our study indicates that there was a significant decrease in the activity of SOD, CAT and GST in the blood of the cows with bovine mastitis. Incontrast Holbrook and Hicks observed no significant differences in the SOD activity throughout the lactation of non mastitic cows (Holbrook and Hicks, 1978).

In the present study, decline in the level of antioxidant enzymes like SOD, CAT and GST observed in cows with bovine mastitis is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage.

Bovine Mastitis on Biochemical Constituents of the Blood

The AKP activity in the blood of healthy and bovine mastitis positive cows was found to be 85.69 u/l and 218.6 u/l respectively.

Similarly, the LDH activity in the healthy animal's blood was 1221 u/l whereas it was found to be 1338 u/l in the blood of cows with bovine mastitis. However, the phosphorus level in the blood of healthy and bovine mastitis infected cows was 7.18 and 5.1 mg/dl respectively (Figure 3).

Biochemical analysis of mastitic animals may help in diagnosis of subchemical abnormalities and become a helpful means for practice under field conditions. AKP is a membrane bound enzyme found at bile pole of hepatocytes and also found in pinocytic vesicle and Golgi complex. It is present on all cell membranes where active transport occurs.

The present investigation indicated a significant increase in the activity of AKP in the blood of the cows with bovine mastitis.

Likewise, similar studies have reported that there was increase in the activity of AKP in the blood of the cows and goats with mycoplasmosis (Amany *et al.*, 2008). Similarly, there was increase in the level of AKP in the milk of the buffalo and cows suffering from the mastitis (Hussain *et al.*, 2012). In the present study the increase in the AKP activity may be due to the adaptive rise to the persistent stress during the inflammation of udder.

Most of the cells contain LDH and when these cells are lethally injured, loss of membrane integrity can be assessed by monitoring activity of LDH. The LDH release is commonly used as a marker for necrotic/osmotic cell death (Valentovic and Ball, 1998).

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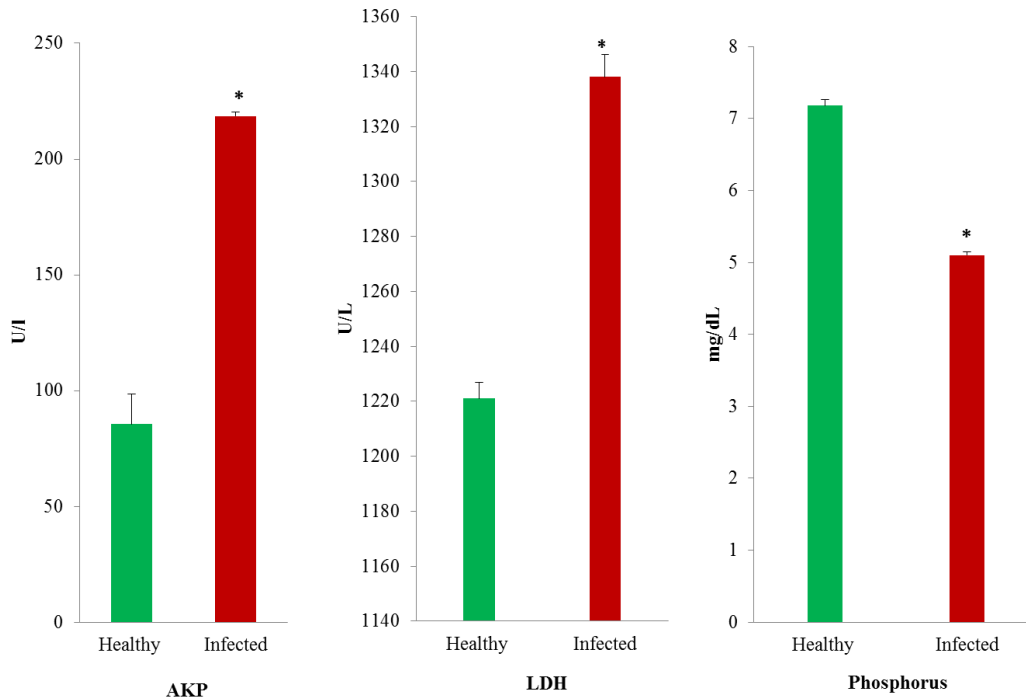


Figure 3: Effect of Bovine Mastitis on Biochemical Parameters of Blood

The present investigation indicated that a significant increase in the activity of LDH in the blood of the cows with bovine mastitis. Similarly, Mona and Co-workers reported that there was increase in the level of LDH in the blood of buffalo and cows suffering from the sub clinical mastitis (Zaki *et al.*, 2008; 2010). Studies from Iran suggest that during sub clinical mastitis there was increase in the level of LDH in blood of the infected animal (Kalantari, 2013). The rise in LDH activity in tissue suggested high turnover of pyruvate to lactate and vice-versa to yield required energy to overcome induced metabolic stress (Kackar *et al.*, 1997; Mishra *et al.*, 1998). Therefore, in the present study elevated in the activity of LDH in the blood of the infected cows was probably due to the turnover of pyruvate to lactate to yield required energy to overcome the metabolic stress due to the intramammary infection.

Phosphorus has a variety of important biological functions that makes it essential for good health of the animal. Oxidative phosphorylation, oxygen delivery, glycolysis, and maintenance of cellular structural integrity are among the processes that require Phosphorus. Phosphorus is necessary for generation of adenosine triphosphate, without which many physiologic processes could not occur. In the current investigation the results I showed a significant decrease in the level of phosphorus in blood of the cows with bovine mastitis. Similar studies conducted by Mona on buffalo and cows revealed that there was decrease in the concentration of phosphorus in the blood of buffalo and cows suffering from sub clinical mastitis (Zaki *et al.*, 2008; 2010). Effective reduction of Phosphorus in blood of the cow suffering from bovine mastitis was probably due to the influx of blood constituents into the mammary gland during infection.

Bovine Mastitis on Biochemical Composition of Milk

The carbohydrate content in the milk sample collected from of healthy and bovine mastitis positive cows was 47.8 and 40.5 mg/ml respectively. Similarly, fat content in the milk sample of healthy and infected animals was 37.5 and 30.8 mg/ml respectively. Whereas, the protein content in the milk sample showed that the protein in the milk of healthy animals was 36.4 mg/ml and in infected animal milk sample it was noted to be 43.4 mg/ml. Alike total dissolved solids (TDS) in the milk sample of healthy animals contain 1.28 mg/ml TDS. Whereas, the milk of infected animal contain 1.70 mg/ml TDS (Figure 4).

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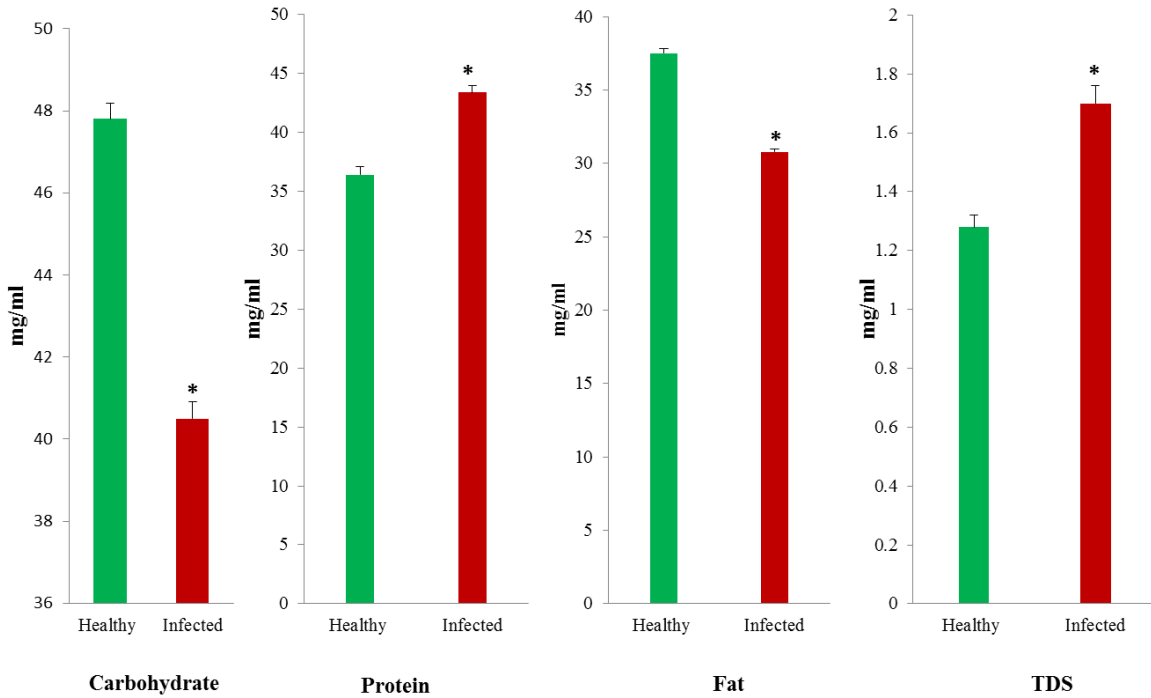


Figure 4: Effect of Bovine Mastitis on Biochemical Composition of Milk

The influence of mastitis on milk composition depends on the severity of infection and the pathogen involved in it. During the inflammation of udder, three mechanisms are involved in milk composition changes like a decrease in synthesis of milk, an increase in the permeability of the milk barrier and an increase in the proteolytic activities in milk (Roux *et al.*, 2003).

Milk sugar or lactose is a disaccharide found exclusively in milk and formed by the union of two simple sugars, glucose and galactose. The concentration of lactose in the milk is relatively constant, the molecules from which lactose is made are found in much lower concentrations in milk. Each time the blood flows through the udder, almost 20% of the glucose is taken up, However, some of the glucose is taken up by the cells of the udder may be used as a source of energy for the formation of milk (Blackwood and Stirling, 1932). The present investigation of milk sample showed that a significant decrease in the carbohydrate content in the milk of the cows with bovine mastitis when compared with that of healthy animals. Similarly, various reports showed a decrease in the concentration of the lactose in milk of buffaloes and cows suffering from bovine mastitis compared to healthy animals (Hussain *et al.*, 2012; Lindmark-Mansson *et al.*, 2006; Ahmad *et al.*, 2007). The decrease in the concentration of lactose in milk is caused by the leakage of lactose into the extracellular fluid and blood after the destruction of the normal lactose barrier by the pathogenic bacteria (Mielke, 1975).

In the present study, the results of estimation of protein content in milk sample showed a significant increase in the milk protein of the cows with bovine mastitis when compared with that of healthy animals milk. Mastitis also associated with increases in the concentrations on many different enzymes in milk (Pyorala, 2003). Moussaoui *et al.*, (2003; 2004) have also showed similar changes in milk composition, including the level and type of protein constituents in the bovine mastitis infected milk. Compared with normal milk, mastitis milk has higher levels of several enzymes, especially, proteases that could potentially damage mammary tissue (Long *et al.*, 2001; Raulo *et al.*, 2002). It is generally accepted that during mastitis, there is an increase in milk proteins particularly those originating from blood however, those milk proteins synthesized in the mammary gland like casein decreases (Lee *et al.*, 1992). As such, mastitis results in decrease of useable milk proteins like caseins and increase in the undesirable whey

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protein fractions like serum albumin, globulin (Sharma *et al.*, 2004). In the present study, increase in protein concentration of bovine mastitis infected milk may be due to the more sloughing off of epithelial cells and migration of leucocytes from blood into milk.

Milk fat plays a central role in the dairy products and farm efficiency. Fat is a major contributor to the energy density of whole milk and is essential to many of the physical properties, manufacturing qualities and organoleptic characteristics of dairy products. Concentration of fat in milk is very important from the economic point view as the grading of the milk is done based on its fat content. The present study showed a significant decrease in the fat content in the milk of the cows with bovine mastitis. Similarly, there was reduction in the fat content in the milk of buffalo and cow suffering from bovine mastitis (Hussain *et al.*, 2012; Lindmark-Mansson *et al.*, 2006; Ahmad *et al.*, 2007). Whereas, some scientist recorded an increase in the fat content of mastitic milk (Pyorala, 2003). Decrease in fat content during intramammary infection is due to the reduced synthetic and secretory capacity of the mammary gland, and also condition within the infected mammary gland enhance the activity of milk lipoprotein lipase (Bansal and Randhawa, 2003).

Total solid content of milk is of quite importance as these affect product quality. 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 the present investigation the results showed a significant increase of TDS in milk of the cows with bovine mastitis. Similarly, researchers from Pakistan reported an increase in the total dissolved solids in the milk of the animals infected with bovine mastitis (Hussain *et al.*, 2012).

In the present study, elevated in the TDS in the mastitic milk is due to the influx of blood constituents into the milk during infection.

SDS-Polyacrylamide Gel Electrophoresis of Milk Sample

The protein from milk sample was subjected to SDS-PAGE to determine the effect of bovine mastitis on milk protein profiling using 12 % separating gel and 5% stacking gel. The protein in the milk of healthy cows and the protein in the milk of the cow with bovine mastitis along with standard medium range protein molecular weight markers were run on SDS-PAGE.

Four bands were observed in milk sample of cow with bovine mastitis (Lane 2, 3, 4, 5 and 6) whereas only two bands were observed in the milk sample of the healthy cow (Lane 7). On comparison with standard protein markers (Lane 1) the apparent molecular weights of mastitis infected milk protein were 31, 40, 66 and 70 KDa whereas the apparent molecular weight of healthy milk protein were 35 and 70 KDa respectively (Figure 5).

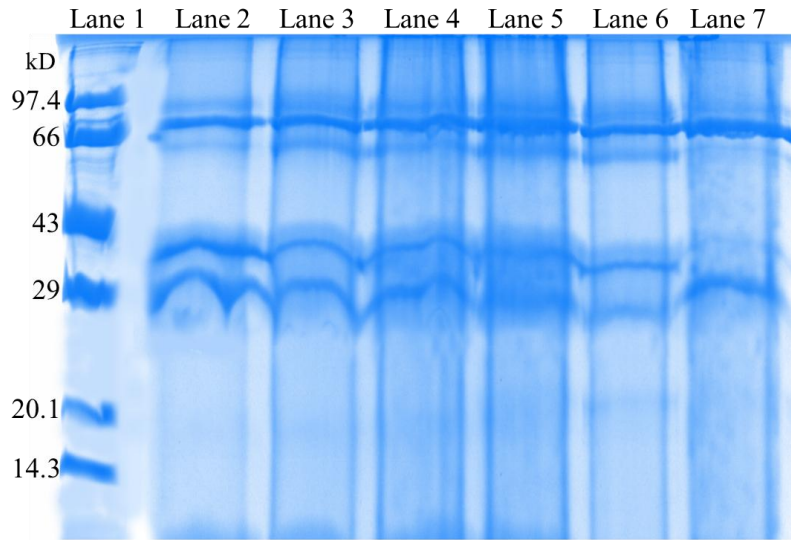
In the present study of milk protein profiling on SDS-PAGE showed that on comparison with standard protein markers the apparent molecular weights of mastitis infected milk protein were 31, 40, 66 and 70 KDa whereas, the apparent molecular weight of healthy milk protein were 35 and 70 KDa respectively. Whereas, the studies of Pourkabir and Gharib (2010), have showed that the apparent molecular weight of healthy milk was 22 KDa and that of milk of the cow with mastitis caused by different bacteria were 330, 220, 67 and 60 KDa.

In the present study increase in protein content of bovine mastitis infected milk may be due to the more sloughing off of epithelial cells and migration of leucocytes from blood into milk, the more number of band on the SDS PAGE in mastitic milk sample was may be due to the presence of serum proteins which might have passed into milk because of vascular permeability changes due to inflammation of mammary gland.

The study was conducted to elucidate the defensive ability of the cow by evaluating the serological changes, oxidative stress parameters and some biochemical changes in blood involved during mastitis and its impact on composition of milk. The study also revealed that significant increase in the level of AKP, LDH and decrease in the level of phosphorus in the blood of IMI cows. Further there exists a negative correlation between Hb, RBC, SOD, CAT, GST GSH, phosphorus in blood and fat, carbohydrate and bovine mastitis whereas positive correlation exists between leucocytes TBARS, AKP and LDH of the

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blood and protein and TDS of the milk and bovine mastitis. Additionally, the results of SDS-PAGE, showed that the increase in the number of protein bands in the infected milk was due to influx of undesirable proteins during intramammary infection. All the noticeable findings in this study are due to the impairment in mammary gland due bovine mastitis and is represented in Figure 6.



Lane 1- Marker, Lane 2, 3, 4, 5 and 6- Bovine mastitis milk, Lane 7- Healthy milk

Figure 5: SDS-Polyacrylamide Gel Electrophoresis of Milk Sample

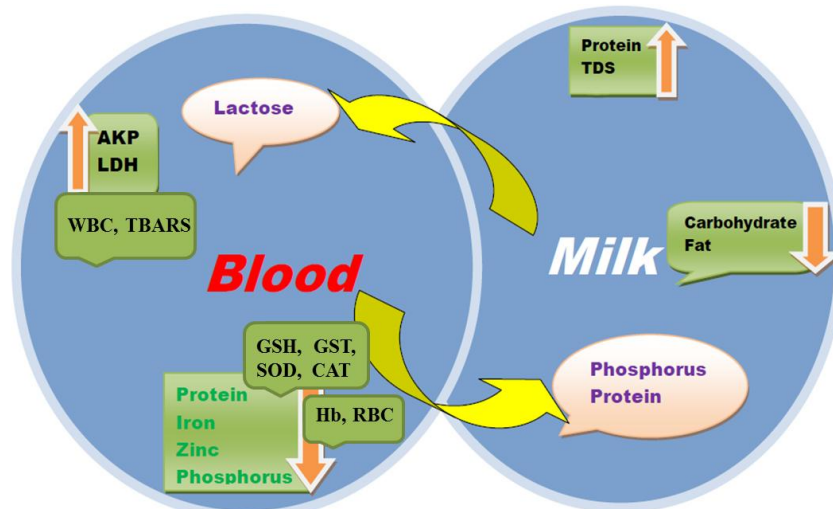


Figure 6: Overview of Bovine Mastitis Effect on Metabolic Parameters in Blood and Milk of the Cow

Finally, this investigation concludes that the oxidative stress parameters and biochemical mediators acts as a biomarkers in understanding physiology of cow during intramammary infection and disturbances in the balance of these markers can be used as diagnosis tool of bovine mastitis.

List of Abbreviations

Super Oxide Dismutase –SOD, Catalase –CAT, Glutathione-S-transferase-GST, Reduced glutathione-(GSH), Thiobarbituric acid reactive substances-(TBARS), Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis- (SDS PAGE), polymorphonuclear neutrophils- (PMN), Surf field mastitis test –

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SFMT, Lipid peroxidation- (LPO), Malondialdehyde -(MDA), Alkaline phosphatase activity- (AKP), Lactate dehydrogenase -(LDH), Total dissolved solids -(TDS), Poly unsaturated fatty acids- (PUFA), Reactive oxygen species -(ROS).

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