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Effect of Extra-mammary Diseases on Udder Health and Biochemical Changes in Crossbred Cows with Subclinical Mastitis

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ABSTRACT

Background: Infectious diseases and metabolic disorders are common in crossbred cows and adversely affect optimum production as well as quality of milk. The quality of milk plays a significant role in the production of high-quality dairy products. High somatic cell count (SCC) in the milk significantly decreases the potential of such milk for the production of high quality dairy products. In this study, it is hypothesized that the extra-mammary infections and metabolic disorders increase probability of intra-mammary infections thereby increasing somatic cell count in the milk making it unsuitable for dairy industry.

Methods: Cows were grouped based on the diseases or disorders like Teat Stenosis (Gp SCM-TS), Ruminal Acidosis (Gp SCM-RA), Nonspecific Diarrhea (Gp SCM-ND), Respiratory Tract Infections (Gp SCM-RTI) and Repeat Breeder Syndrome (Gp SCM-RD). Diagnosis of subclinical mastitis was made on the basis of California Mastitis Test (CMT) scores and by Somatic Cell Count (SCC) by automatic somatic cell counter. Biochemical parameters analyzed in automatic biochemical analyzer using commercially available kits.

Result: The mean SCC values significantly higher in cows with sublinical mastitis and with concurrent infectious and metabolic diseases. Similarly, the concentrations of serum alkaline phosphatase (ALP), aspartate amino transferase (AST) and alanine amino transferase (ALT) were higher in affected cows. Concentrations of serum total proteins (TP) and blood urea nitrogen (BUN) in all the groups of affected animals were higher. The changes in the calcium (Ca) and phosphorus (P) levels were not observed in cows with subclinical mastitis and with other diseases under study. The SCC values did not correlate with the values of ALP, AST, ALT, TP, BUN, Ca and P among the studied groups. It can be concluded that animals with concurrent infections and metabolic disorders increase the SCC and influence the alteration in the biochemical parameters of subclinical mastitic animals.

Key words: Biochemical parameters, California mastitis test, Infectious diseases, Metabolic diseases, Somatic cell count.

INTRODUCTION

Infectious diseases and metabolic disorders of dairy animals are important risk factors affecting optimum production and the quality of milk. The quality of milk is paramount feature for the production of high quality dairy products and essentially for food safety. The previous studies indicated that the presence of cells in the milk at the lower or higher levels can harm its hygienic quality also (Kaouche-Adjlane and Mansour, 2020). Mastitis is usually caused by pathogenic bacteria in the mammary gland however, existence of aseptic or non- specific mastitis is possible (International Dairy Federation 1987). Moreover, the extra-mammary infections and metabolic disorders increase the probability of intra-mammary infections thus affecting the composition and manufacturing properties of milk. The leucocytes, lymphocytes, plasmacytes and macrophages increase in number in the milk under such circumstances and are responsible for the high somatic cell count (SCC) leading to the changes in the milk composition. SCC in milk is influenced by many factors, such as species of animal, milk production level, lactation stage, environmental factors and management practices (Malik *et al*., 2018). The supply of milk with higher SCC is a constant risk for the diary industry considering the current system of dairy management (Jadhav *et al*., 2016). Quantification of SCC during illness of a lactating cow helps in determination of quality of milk as well as for early identification of subclinical and clinical mastitis. An automatic and portable somatic cell counter as cow side test is suitable for quantification of SCC and thus facilitating the supply of good quality milk form the dairy farms.

Many of the research efforts are directed at understanding the nature of pathogenic bacteria that are responsible for the most intra-mammary infections. There are many studies on predisposing factors such as herd, breed, parity, periparturient period and stage of lactation on the incidence of clinical and subclinical mastitis. (Sordillo, 2005, Subramanian *et al*., 2019; Hiitio *et al*., 2017). The

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defense mechanisms of the mammary gland is affected by genetic differences and pathophysiological stressors such infectious and metabolic diseases. The extra-mammary disorders like ruminal acidosis, diarrhea with dehydration and loss of appetite, respiratory tract infections and repeat breeder syndrome in crossbred cows are supposed to influence the udder health and on the occurrence of subclinical mastitis which is characterized by the increase in the SCC. Increased incidence of clinical mastitis and high SCC are reported in animals with reproductive disorders (Svensson *et al*., 2006). A better understanding of various disease risk factors is essential in consideration of producing high quality milk and of planning an improved mastitis prevention strategies.

Assessment of biochemical parameters such as plasma proteins, serum enzymes, minerals and blood urea nitrogen in the blood and milk are useful in determining general health status of animals as well as for identification of biomarker. There are no studies on the correlation of SCC and blood metabolic parameters. Blood serum proteins are the indicators of the immune status in dairy cows. Total plasma proteins including acute phase proteins, pro-inflammatory cytokines, immunoglobulins are known to increase in subclinical mastitis thereby increasing SCC (Kadry Sadek *et al*., 2017). Some earlier studies showed relationships between milk yield with level of cholesterol and vitamin C in milk and blood (Ling *et al*., 2003; Chládek *et al*., 2004; Sordillo *et al*., 2009; Strzałkowska *et al*., 2009a, b). There are no studies on the associations between biochemical parameters in blood and the SCC. Therefore, the present work is taken up to study the existence of relationship between SCC, metabolic disorders and infectious diseases with the dynamics of mammary gland.

MATERIALS AND METHODS

Animal selection

The cross bred lactating cows (n=42) from various organized farms in central Karnataka were selected based on the history of previous illness and grouped as detailed below for the study. The cows (Gp-C, n=6) which were considered as controls, had history of no co-infections or previous illness for a period of 3 months. The cows (Gp SCM, n=6) with subclinical mastitis without any co-infections or previous illness for a period of 3 months were considered as positive controls. The experimental groups (n=6 in each group) were considered as following. Gp SCM-TS consists cows affected with stenosis of teat, Gp SCM-RA with ruminal acidosis, Gp SCM-ND with diarrhea along with dehydration and loss of appetite, Gp SCM -RTI with coughing, fever, decreased appetite, varying degrees of dyspnea, Gp SCM -RB with a history of failure to conceive after at least two successive inseminations.

California Mastitis Test (CMT)

CMT was performed on milk sample from each quarter of the udder. In the paddle with 4 portions, 3 ml of milk and 3 ml of the commercial CMT reagent was rotated ten times quickly by hand and depending upon the degree of gel formation, the grades were assigned.

Interpretation and scoring of the CMT test

Depending upon the degree of gel formation, the grades were assigned according to Faroult *et al*., (2003). Negative for CMT indicates no precipitation. Score-1 is assigned for appearance of fine floc which disappears after shaking paddle. Score-2 for appearance of clear floc without tendency to gelation. Score 3 for the appearance of thick floc with gel formation (white egg consistency) which, when swirled, quickly moves toward the centre. Score 4 for the appearance of thick gel (spit consistency).

Milk samples

Milk samples from selected animals were collected into sterilized tubes maintained at 4°C and subjected to SCC.

Somatic Cell Counts (SCC)

SCC was performed using a DeLavel Cell Counter . DeLaval cassettes were loaded with 1µl of milk sample by inserting tip into the milk and pressing the piston. Readings were taken by placing the cassette into the cell counter. Number of cells present in 1 mL of milk sample is obtained by multiplying DeLaval Cell counter reading in cells/µl by 1000.

The SCC of less than 200,000 cells/ ml were considered as normal milk and animal is said to be uninfected. The cows with SCC greater than 200,000 cells/ml are considered as being subclinically infected.

Blood samples

Blood samples were collected in a heparinsed tubes for plasma and in test tubes without anticoagulant for serum from all the selected animals.

Estimation of biochemical parameters

The estimation of alkaline phsophotase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), blood urea nitrogen (BUN), total plasma proteins (TPP), serum calcium and serum phosphorus were done by using commercial kits in a biochemical analyzer.

Statistical analysis

The data obtained from the various experiments were expressed as Mean $\pm$ S.E. Data obtained were statistically subjected to one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* multiple comparison test. Means were compared by paired t test. Pearson Correlation coefficients were calculated using Graph Pad Prism software programme (GraphPad® software Inc., Version 5.0; San Digo, CA, USA). Differences were considered significant at $p<0.05$ or lower.

RESULTS AND DISCUSSION

Present study involved identification of lactating cows which had a history of co-infections and metabolic disorders along with subclinical mastitis. The most frequently encountered diseases in the farms considered for the study are teat

stenosis (TS), ruminal acidosis (RA), non specific diarrhea (ND), respiratory infections (RTI) and repeat breeding (RB). The crossbred cows with subclinical mastitis were identified through CMT scores and SCC by Delaval cell counter. Evaluation of the udders health status is routinely done through SCC and the Californian Mastitis Test (CMT) in dairy farms the CMT is a rapid and reliable test for determining of somatic cell concentration in milk (Anderson *et al.*, 2010; Bastan *et al*., 2015). Therefore, the CMT is used as preliminary screening test before the sample of milk is taken for SCC by automatic cell counter. Interpretation of CMT score in relation to SCC is represented in (Table 1). The results are in accordance with Kaouche-Adjlane and Mansour (2020) who have evaluated udders health status through SCC and the CMT in Algerian dairy farms.

The threshold limit of SCC for the detection of subclinical mastitis was kept at 200,000 cells/ ml of milk. The cows with SCC greater than 200,000 cells/ml are considered as being subclinically infected. Similar assumptions were made by Sharma *et al*. (2010) and reported that the animals are said to be suffering from subclinical mastitis with no gross abnormalities in milk or udder but the SCC of more than 2 lakh cells/ml of milk is seen. However, Jha *et al*. (1993) suggested that the cell count more than 5 lakh cells/ml was considered as the positive indication of mastitis. Das *et al*. 2008 reported SCC value of 7 lakh cells/ml of milk would be the cut off value for detection of subclinical mastitis. Therefore, there is lot of variation in the definition of the SCC limits by various researchers in India to detect subclinical mastitis in cows. The values reported by various researchers show a wide variation ranging from 1.22 lakh cells/ml to 15.51 lakh cells/ml of the milk however the major observations revolve around 4 lakh cells/ml to 2 lakh cells/ml (Jadhav *et al*., 2016). This variation may be attributed to the pathophysiological and immunological status of an animal.

In the present study SCC is significantly higher in groups Gp SCM, Gp SCM-TS, Gp SCM-RA, Gp SCM-ND, Gp SCM -RTI and Gp SCM-RB, compared to Group C ($p<0.05$). Moreover, the mean SCC values were higher in the groups Gp SCM -TS, Gp SCM -RA, Gp SCM -ND, Gp SCM -RTI, RB in comparison to group Gp SCM (Table 1). An elevated SCC in the milk of animals with subclinical mastitis and with concurrent extra-mammary diseases indicates that the extra-mammary diseases might influence increased shedding of somatic cells. This is similar to studies associating decreased milk production and significantly increased bulk tank SCC in the herds affected with skin diseases (Yeruham *et al*., 2000). However, there are no much studies relating SCC and mastitis with the other extra mammary diseases in dairy cows.

In most of animal farms, monitoring of subclinical and clinical mastitis is usually performed through indirect test such as pH, electrical conductivity, culture test and biomarker tests apart from SCC, CMT (Sinha *et al*., 2018). Estimation of biochemical parameters help not only in an assessment of the physiological status of the animals but identification of biomarkers for early identification of subclinical mastitis. The present study revealed changes in biochemical parameters of affected cows with concurrent subclinical mastitis. However, the changes in the concentrations were not noticeable enough to consider any parameter under study as biomarker for detection of subclinical mastitis. The mean serum ALP concentrations were significantly higher in groups Gp SCM, Gp SCM -TS, Gp SCM -RA, Gp SCM -ND, Gp SCM -RTI and Gp SCM -RB, than in group Gp C. The mean values in groups Gp SCM -RA and SCM -ND differed significantly with the groups Gp SCM, Gp SCM TS, Gp SCM RTI and Gp SCM RB (Table 2). This can be attributed to the quantum of metabolic stress on the animals. There are many experimental studies that indicate an increase of serum alkaline phosphatase from cows with mastitis, which may suggest that this enzyme plays a role in the pathogenesis of the disease (Vangroenweghe *et al*., 2004). The mean concentrations of ALT (U/dL) did not differ

Table 1: Mean Somatic Cell Counts (SCC) and CMT score.

Groups	SCC (cells/mL)	Interpretation of californian mastitis test (CMT)
Gp C	$53000\pm08574^{a\alpha}$	No change in consistency
Gp SCM	$1470000\pm69150^{\beta}$	2
Gp SCM- TS	1252000 ± 222881^{b}	3
Gp SCM- RA	1495000 ± 176925^{b}	3
Gp SCM- ND	1883000 ± 189352^{b}	4
Gp SCM- RTI	1407000 ± 193640^{b}	3
Gp SCM- RB	1676000 ± 183330^{b}	3

Note: Values are mean ± SE. Means with different superscripts differ significantly within the column ($p<0.05$).

Table 2: Mean values of serum enzymes.

Group	ALP (mg/dL)	ALT(U/dL)	AST (U/dL)
Gp C	59.28 ± 5.064^{a}	31.82 ± 1.449^{a}	85.45 ± 2.645^{a}
Gp SCM	$159.7\pm15.34^{b\alpha}$	51.73 ± 2.342^{b}	167.7 ± 13.45^{b}
Gp SCM- TS	$138.5\pm15.93^{b\alpha}$	63.08 ± 7.609^{b}	110.6 ± 7.3^{b}
Gp SCM- RA	$108.1\pm15.15^{b\beta}$	58.78 ± 6.772^{b}	105.8 ± 11.24^{b}
Gp SCM- ND	$106.7\pm20.14^{b\beta}$	50.32 ± 7.672^{b}	108.4 ± 17.46^{b}
Gp SCM- RTI	$138.9\pm23.93^{b\alpha}$	36.36 ± 6.308^{b}	202.7 ± 33.19^{b}
Gp SCM- RB	$148.2\pm20.67^{b\alpha}$	39.03 ± 6.488^{b}	170.2 ± 35.02^{b}

Note : Values are mean ± SE. Means with different superscripts differ significantly within the column ($p<0.05$).

Table 3: Mean values of blood biochemical parameters.

Group	Total protein (g/dL)	BUN (mg/dl)	Calcium (mg/dl)	Phosphorous (mg/dl)
Gp C	5.911±0.1103^{a}	21.23±1.77$^{a\alpha}$	10.41±0.332	6.746±0.49
Gp SCM	9.616±0.4688^{b}	39.43±1.605$^{b\beta}$	9.474±0.5029	5.312±0.5029
Gp SCM- TS	8.835±0.987 ^{b}	36.66±5.99^{b}	11.15±0.7711	7.163±0.3569
Gp SCM- RA	11.18±0.8997^{b}	40.16±2.973^{a}	10.8±0.7557	5.497±0.5673
Gp SCM- ND	9.314±1.27^{b}	32.79±3.836^{a}	9.799±1.454	5.799±1.171
Gp SCM- RTI	8.265±1.448^{b}	41.32±1.469^{c}	9.939±1.297	5.111±0.6619
Gp SCM- RB	8.342±1.181^{b}	34.27±4.776^{a}	12.05±1.552	6.32±0.593

Note : Values are mean ± SE, Means with different superscripts differ significantly within the column ($p<0.05$).

Table 4: Correlation coefficients data of somatic cell count and biochemical parameters.

Group / Parameter	Gp (SCM-TS)	Gp (SCM-RA)	Gp (SCM-ND)	Gp (SCM- RTI)	Gp (SCM- RB)	Gp (SCM)
ALP	0.8911*	0.05069	0.7674*	-0.4888	0.1317	0.08707
ALT	0.7898	-0.6048	-0.3732	0.5052	0.1402	0.008053
AST	-0.902*	-0.5411	-0.4772	-0.3144	-0.4504	-0.2836*
BUN	0.5586	-0.3943	-0.1762	0.08242	-0.6256	-0.198
Total protein	0.6549	0.182	-0.3673	0.3573	0.7322*	0.02594
Calcium	0.4528	0.1423	0.5364	-0.8191*	-0.1388	0.07406
Phosphorous	0.6089	-0.0343	0.01441	0.5422	0.4488	0.2536

Note: NS – Non significant,* - Significant weak correlation,** - Significant moderate correlation, *** - Significant uphill moderate correlation, *** - Highly significant strong correlation.

significantly among groups having affected animals but concentrations were higher in comparison to control group. The results of serum AST concentrations in control and affected animals are similar to ALT concentrations (Table 2). The enzyme estimation is useful for identification of affected organs in relation to udder health.

The higher concentrations of serum total proteins were found in all the groups in the study (Table 3). This may be due to increased concentration of immunoglobulin and acute phase proteins. The variations in BUN concentrations occur due to numerous extra-renal causes such as fasting, or sepsis, which increase protein catabolism. Cows suffering from mastitis with Gram negative organisms have higher level of blood urea nitrogen as compared to Gram positive infected cows (Smith *et al*., 2001). The calcium and phosphorous concentrations of healthy and mastitis affected cows appear to be similar to the study reported by Yildiz and Kaygusuzoglu (2005). The present findings indicate that Ca levels (mg/dL) in cows with subclinical mastitis were lower (9.474±0.5029) compared to healthy cows (10.41±0.332), but decrease is statistically non- significant ($p<0.05$).

The results of correlation of SCC with other biochemical parameters like ALP, ALT, AST, TPP, BUN, calcium, phosphorous revealed no correlation between the healthy animals and the subclinical mastitis affected animals with concurrent infectious diseases and metabolic disorders (Table 4). However, no biochemical parameter showed characteristics by which a early detection of subclinical mastitis is possible in presence of extra-mammary diseases.

CONCLUSION

The increased SCC of milk samples from the crossbred cows with subclinical mastitis significantly associated with the occurrence of concurrent extra-mammary diseases. The biochemical changes occur in a less significant level in relation to extra-mammary affections in lactating cows with subclinical mastitis.

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