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Milk NAGase Activity as an Indicator of Subclinical and Clinical Mastitis in Sahiwal Cows

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A B S T R A C T

Present investigation was undertaken to evaluate the milk NAGase activity of Sahiwal cows suffering with subclinical or clinical mastitis. The incidence of occurrence of subclinical and clinical mastitis was evaluated by testing fresh milk samples by California Mastitis Test (CMT). A total of 73 Sahiwal cows in early lactation selected from the NDRI herd during different seasons. From this 12 clinical and 12 subclinical cases distributed over three different seasons were taken into account. Clinical mastitis cases had significantly higher ($P<0.05$) milk NAGase levels as compared to subclinical mastitis cases. Thus, it was found to be the most potential candidates in milk which could be used for detecting subclinical mastitis.

Introduction

Mastitis is an inflammation of mammary gland which result in reduction of milk yield and quality, high incidence of culling rate , mortality and the potential to serve as health risk to other cows in the farm (Boehmer *et al.*, 2008; Viguier *et al.*, 2009). Bovine mastitis causes significant economic problem in the dairy farm as well as the dairy industry (Vicente, 2014). Dairy cows are exposed to numerous physiological, environmental and genetic factors which compromise the host immune system and increase the incidence of mastitis (Sordillo, 2005). NAGase is an intracellular lysosomal enzyme that is released into milk from neutrophils during phagocytosis and cell lysis but also from damaged epithelial cells, indicating udder tissue destruction (Kitchen, 1981). Milk NAGase activity correlated very closely with SCC (Kitchen, 1981; Mattila *et al.*, 1986). NAGase activity accurately reflects the degree of inflammation, so that in mastitis caused by major pathogens milk NAGase activity is significantly higher than in mastitis caused by minor pathogens (Kitchen *et al.*, 1984; Mattila *et al.*, 1986; Pyorala and Syvajarvi, 1987, Miller and Paape, 1988). Kitchen *et al.*, (1984) applied NAGase assay

to estimate the somatic cell counts as a marker for mastitic milk. Mellor (1968) reported firstly the presence of NAGase in bovine milk and suggested that bovine milk NAGase is derived wholly from the leukocytes and their level in milk was helpful for detection of udder health. Ulberth *et al.*, (1984) reported that sensitivity and specificity of the NAGase assay did not differ from cell count determination. Differences in the lysosomal enzyme activities between the classes of animal leukocytes were reported by Healy, (1984). In cattle higher NAGase activity was found in granulocytes. Kitchen *et al., (*1978) reported that mammary gland secretory cells contained high levels of NAGase and appeared to be the major source of enzyme in milk whereas NAGase was from other sources (white blood cells and blood serum) contributed only 5-15% of the total activity in milk. A higher NAGase activity was found in serum, macrophage and neutrophils. From these results, it could be derived that most of NAGase activity in milk was from blood serum, macrophage and neutrophils and were associated with the inflammatory process. In normal quarter NAGase activity were 9.5nM/min/ml but in acute clinical mastitis it increased remarkably from 25 to 500nM/min/ml. In chronic mastitis this changes were small.

Aim of this study was to evaluate milk NAGase as a potential candidate to detect subclinical mastitis in Sahiwal cows

Materials and Methods

The animals were selected from the herd maintained at Livestock Research Centre (LRC) of NDRI, Karnal, Haryana. The animals were kept under normal routine management practice as followed at the institute's farm. All the cows were fed as per the standard feeding practices which consisted of concentrate mixture (mustard cake, maize, wheat bran, rice bran, mineral mixture and common salt) wheat straw and roughages (berseem, maize or jowar fodder). The feed and water was available adlibidum to these cows.

A total of 73 Sahiwal in early lactation selected from the NDRI herd during different seasons were included in this study. The incidence of occurrence of subclinical and clinical mastitis was evaluated by testing fresh milk samples by California Mastitis Test (CMT). which included 12 clinical and 12 subclinical cases distributed over three different seasons [4 cows of each breed with clinical and subclinical infection in each season i.e. thermo neutral (October-November), winter (December to January) and summer (April 15th -May)]. THI during the experimental period has been shown in the figure 1.

NAGase activity was evaluated both in milk and plasma using ELISA kit which was purchased from Bioassay Technology Laboratory. The samples of composite milk (representing all four quarters) were collected in tube (250ml) from SCM and CM affected cows during noon milking (12.00 Noon). Immediately after collection, the tubes were transported to the laboratory in ice box for further processing.

A part of whole milk and skimmed milk (obtained after centrifugation at 3000 rpm for 20 minutes at 4^0C) were stored at -20°C for analysis of NAGase activity. Blood samples (~10ml) were drawn in sterile heparinised vacutainer from each cow by jugular vein puncture. Immediately after collection the tubes were transported to the laboratory in ice box for further processing. The plasma was separated out by centrifugation at 3000 rpm for 15 minutes and stored at –20^{o}C in different aliquots, for analysis of NAGase activity.

Results and Discussion

NAGase level was significantly higher ($p<0.05$) in clinical mastitis infected Sahiwal cows as compared to the subclinical mastitis in both plasma (except summer) and milk samples. The levels were highest during summers (38.18 ± 5.99 and 33.14 ± 4.94 ng/ml) followed by winter (34.02 ± 1.34 and 40.47 ± 8.28 ng/ml) and thermoneutral conditions (26.44 ± 1.61 and 22.13 ± 1.68 ng/ml) respectively in the plasma of the both clinical and subclinical mastitis infected Sahiwal cows. Clinical mastitis cases had significantly higher ($P<0.05$) milk NAGase levels as compared to subclinical mastitis cases. However, these differences were not significant for different seasons.

Fig.1 Temperature Humidity Index during experimental period

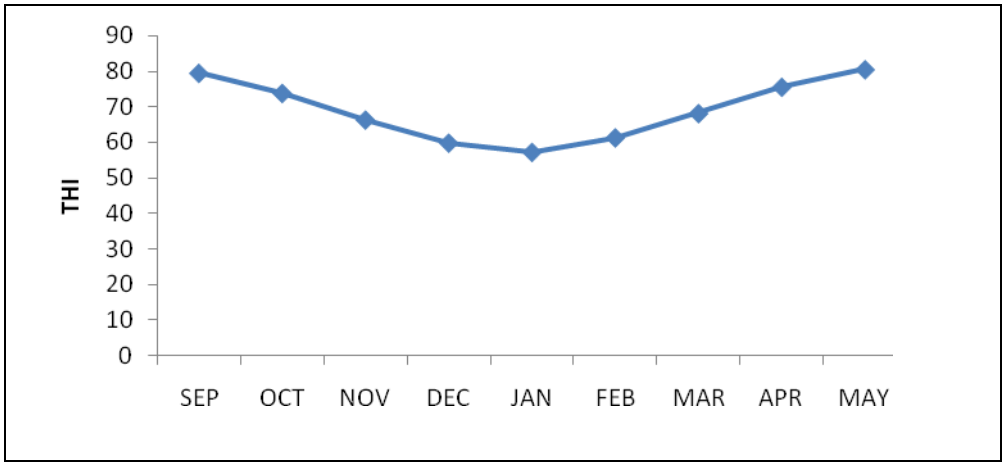


Fig.2 Variations in plasma N-acetyl –beta-D- glucosaminidase in Sahiwal cows suffering from clinical and subclinical mastitis in different seasons

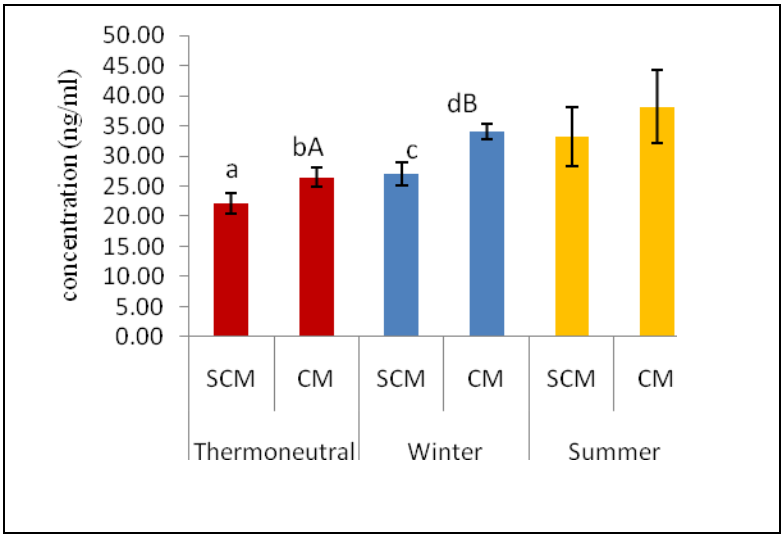
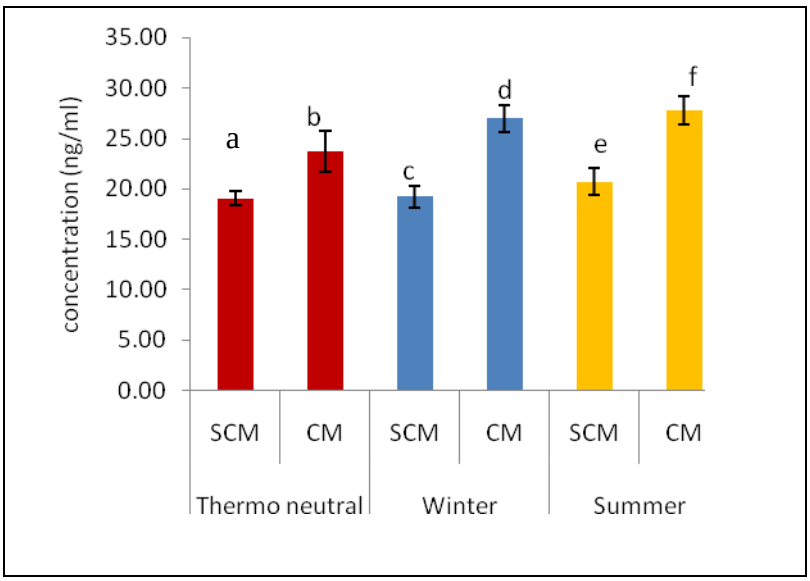


Fig.3 Variations in milk N-acetyl –beta-D- glucosaminidase in Sahiwal cows suffering from clinical and subclinical mastitis in different seasons



NAGase is an intracellular lysosomal enzyme that is released into milk from lysis of mammary tissue and to some degree by damaged epithelial cells of mammary tissue (Kitchen *et al*., 1978, 1980; Fox *et al*., 1988) reflecting destruction of udder tissue and also during lysis of inflammatory neutrophils (Kitchen et al., 1978; Kaartinen *et al*., 1988). Milk somatic cells contribute less than 15% of the total milk NAGase activity (Fox *et al.*, 1988). To the best of our knowledge there are no comprehensive reports in blood and milk for evaluation of clinical or subclinical mastitis in different seasons in Indian context.

Hovinen *et al.*, (2016) indicated that milk NAGase activity could be used to detect both subclinical and clinical mastitis with a high level of accuracy (0·85 and 0·99) compared with other studies (Urech *et al*., 1999; Bansal *et al*., 2005; Nielsen *et al*., 2005). However, compared with the findings of Chagunda *et al*., (2006a), the performance of milk NAGase activity in this study was better than milk L-lactate dehydrogenase in their study, probably because of using quarter milk and not composite milk. Our data is in conformity to above references wherein we found significantly higher ($p<0.05$) levels of NAGase in clinical mastitis infected Sahiwal cows as compared to the subclinical mastitis in both plasma and milk samples. The levels were highest during summers followed by winter and thermoneutral conditions in the plasma of the both clinical and subclinical mastitis infected Sahiwal cows ($P<0.05$). The seasonal differences in the occurrence of CM were significant during winter and thermoneutral conditions. These variations could be accounted for differences in SCC during different seasons as suggested by Miller and Paape, (1988); Berning and Shook, (1992) who correlated its activity closely with SCC. Nagahata *et al.*, (1987) studied relationship between the CMT results of milk and their NAGase and B-Gase activities. The CMT scores of each group appeared to be well correlated with the levels of NAGase and B-Gase activity ($r = 0.86$ for NAGase and $r = 0.92$ for B-Gase). At CMT score 3, their activities increased remarkably and showed increments of 33-fold for NAGase and tenfold

for B-Gase, in comparison with those of normal milk at CMT score of 5. Pyorala *et al.*, (2011) reported Milk NAGase activity was significantly higher in the group with clinical mastitis than in the group with subclinical mastitis. Unlike milk SCC, a high NAGase activity was suggestive of likely destruction of epithelial cell in the udder, which might help to assess the prognosis for recovery from mastitis.

Due to epithelial origin of NAGase, this test could effectively be used to separate healthy quarters from those with subclinical (Urech *et al*., 1999; Bansal *et al*., 2005; Nielsen *et al*., 2005) or clinical mastitis (Chagunda *et al*., 2006; Larsen *et al*., 2010). Hovinen *et al.*, (2016) suggested that this test performed very well in separating quarters infected with major pathogens from those with minor pathogens. Overall, milk from quarters with clinical mastitis had much higher NAGase activity than in milk from quarters with subclinical mastitis.

It is concluded thus milk NAGase activity was found to be the most potential candidate which could be considered for detection of subclinical mastitis during different seasons.

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