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Relationship Among NAGase Enzyme Activity, CMT Score and Somatic Cell Count in Milk of Crossbred Dairy Cows with Sub-Clinical Mastitis in North-Western Province of Sri Lanka

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ABSTRACT

The assay procedure for N-acetyl-β-D-glucosaminidase (NAGase) was considered to be the most reliable, simple, and rapid enzymatic method for estimating the severity of mammary tissue damage. This study was carried out to assess the relationship of NAGase enzyme activity, California mastitis test score (CMTS) and somatic cell counts (SCC) in milk and the influence of breed, parity and age of the cow on NAGase activity. The results showed that prevalence of sub-clinical mastitis (SCM) was 74.5% (41/55) in the population of cows tested. The isolated pathogens were Staphylococcus spp. (34%, 14/41), Escherichia coli (34%, 14/41) and Streptococcus spp. (32%, 13/41). The SCC showed a significant ($p<0.05$) positive correlation with the activity of NAGase ($R2= 0.796$) and CMTS ($R2= 0.709$). A significant correlation was observed between the NAGase activity and SCC in SCM positive milk, where the correlation was stronger ($R2= 0.748$) in the former than in the latter. The parity ($R2= 0.843$) and age ($R2= 0.758$) of the cow also showed a significant ($p<0.05$) positive correlation with enzyme activity. The mean SCC and enzyme activity of different crossbreds of the study population did not show a significant correlation, but mean values of SCC and enzyme activity were higher when an animal was phenotypically more related to European breeds (779–1848 x103 SCC/ml;0.64 -0.89 µmole/min/ml) than tropical breeds (427–534x103 SCC/ml; 0.26-0.43 µmole/min/ml). It could be concluded that, the prevalence of SCM in crossbred cows in North-Western Province of Sri Lanka is considerably high. The determination of NAGase enzyme activity in milk could be used as a method for early detection of SCM more accurately than CMTS and SCC.

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INTRODUCTION

Sub-clinical mastitis (SCM) adversely affects milk production, milk quality and herd health (Schultz *et al*., 1978). Several studies conducted in the country have shown that the high prevalence of sub-clinical mastitis among lactating cows is noticeably contributing to the poor milk quality (Deshapriya *et al.,* 2007; Gunawardena *et al*., 2014; Abeygunawardena *et al.,* 2017; Sanotharan and Deshapriya, 2018; Deshapriya *et al*., 2019; Rahularaj *et al*., 2019).

The pathogens which cause SCM, mainly bacteria, damage the udder tissues leading to pathological changes in the udder, thus increasing the bacteria population in milk. Further, the milk quality is also affected due to the enzymes secreted from bacteria and the enzymes released into milk through the damaged epithelium of mammary tissues. These enzymes not only decrease the quality of raw milk but also reduce the keeping quality of processed milk products (Barbano *et al*., 1991). In response to the infection, udder tissue exerts an inflammatory response during which further leaking of blood components and infiltration of somatic cells into the milk take place. Studies have shown that somatic cells and somatic cell count (SCC) are good indicators of mammary infection (Barbano *et al*., 1991; Marcus *et al*., 1994; Ynte *et al*., 2003; Sharma *et al*., 2011; Li *et al*., 2014). The SCC is estimated directly by microscopic analysis or electronic counter method and using a number of other indirect methods. One of the conventional indirect methods is the California Mastitis Test (CMT) (Schalm and Noorlander, 1957). However, CMT is relatively an expensive and a subjective method and also might result false positive or false negative results (Viguier *et al*., 2009). The direct cell counting using cell counters are more accurate but the cost per sample is comparatively high. Hence, it is essential to develop rapid, sensitive and cost effective methods to detect SCM for the Sri Lanka dairy sector. A number of enzyme assays have been developed to quantify the extent of udder tissue damage that occurs during intra-mammary infections. The assay procedure for *N*-acetyl-β-D-glucosaminidase (NAGase) has been considered to be the most reliable, simple, and rapid enzymatic method (Kitchen, 1976). Therefore, this study was carried out with an aim of finding the relationship of SCC, CMTS and NAGase activity tests that are used to determine sub-clinical mastitis, and of selecting the most suitable method for early detection of SCM in crossbred cows.

MATERIALS AND METHODS

A total of 55 crossbred milking cows (Friesian, Ayrshire, Jersey crosses with Sahiwal, Sindhi or Sahiwal crosses with Lankan cattle) were screened for SCM using CMT. Any cow showing a California Mastitis Test Score (CMTS) of above 2 at least in one quarter was classified as SCM positive.

A sample of milk (20ml) was collected aseptically from each positive quarter into sterilized glass bottles and sub-samples were taken for microbiological analysis and enzyme assay. The SCC was taken from the rest of the sample by using Lactoscan Somatic Cell Counter (Model- LQD kit4, Bulgaria) at cow side. The sub-samples were transported under cool conditions for further analysis to the laboratory of the Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka, and samples were cultured on nutrient agar enriched with 7% sheep blood within six hours of collection. The bacteriological and biochemical analyses were carried out according to the methods described by Jang *et al*. (1978).

The enzyme assay was carried out according to Kitchen (1976) where, citrate buffer (200mM) and ρ-nitophenyl-N-Acetyl-β-D-glucosaminide (Sigma Aldrich N9376 CAS:3459-18-5) substrate were used. A sample of 0.2 ml of milk and 0.3 ml of substrate were taken in to 5ml stoppered polypropylene tube, mixed well and incubated at 50˚C for 15 minutes. The reaction was stopped by adding 1ml of 1M-glycine adjusted to pH 10.5 with NaOH, and then 1ml of chloroform was added to the tubes, shaken vigorously for 5 seconds before centrifuging at 2000g for 10 minutes. The top aqueous layer was separated using a Pasteur pipette and absorbance was determined at

410 or 515 nm wave length using a V1000 digital VIS Spectrophotometer (Xi'an HEB Biotechnology Co. Ltd., China).

The correlation among SCC, CMTS and enzyme activity were analyzed using linear regression at 95% confidence interval. The comparison of means was carried out using one-way ANOVA (SPSS 19th version, IBM 2007).

RESULTS AND DISCUSSION

The results of both CMT and microbiological analysis showed that the prevalence of SCM of the study sample of crossbred cows was 74.5% (41/55). The biochemical characterization of specific pathogens revealed that there were three types of bacteria, namely *Staphylococcus* spp. (34%, 14/41), *Escherichia coli* (34%, 14/41) and *Streptococcus* spp. (32%, 13/41). *Staphylococcus* spp. is considered to be a contagious type of pathogen while *Escherichia coli* and *Streptococcus* spp. are considered to be environmental type of pathogens (Shaheen *et al*., 2016).

The SCC showed significant (P<0.05) positive correlations with NAGase enzyme activity (R^2= 0.796 and CMTS (R^2= 0.709). A strong correlation was observed between enzyme activity and SCC in SCM positive milk compared to normal milk as illustrated in Figures 1a and 1b. Similar observations have been reported by Chagunda *et al.* (2006) and Hovinen *et al*. (2016). According to Kitchen *et al*. (1978) the enzyme NAGase is released from both white blood cells and epithelial cells, and its concentration and activity are low in healthy quarters (Pyorala *et al*., 2011) and increase with SCM condition.

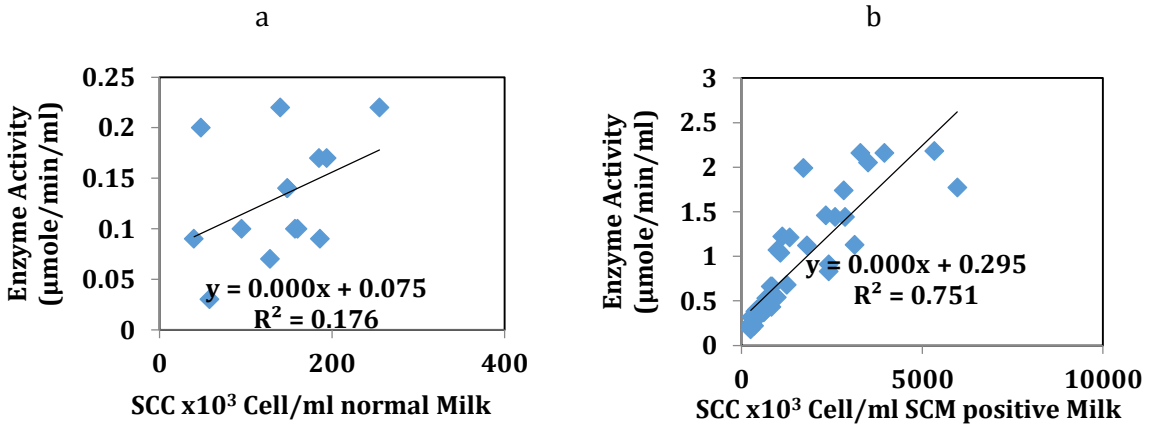

Figure 1: Relationship between Enzyme Activity and SCC in (a) normal and (b) SCM positive milk.

Figures 2 and 3 show the relationship of SCC with NAGase enzyme activity and CMTS. The correlation between the enzyme activity and SCC was stronger (R^2= 0.796; p<0.05) than that between SCC and CMTS. CMT is not sensitive at low somatic cell count for detection of subclinical infections (Kitchen, 1981). Parity and age of SCM positive cows showed a strong (P<0.05) positive correlation with enzyme activity, with R^2= 0.843 and R^2= 0.758, respectively. When the age of a cow increases, the occurrence of SCM also increases because the teat canal of older animals is more dilated and permanently open due to years of repeated milking (Sudhan and Sharma., 2010). Higher the parity, more the chances to be positive for SCM because teat canals become tender (Alemu *et al*., 2013).

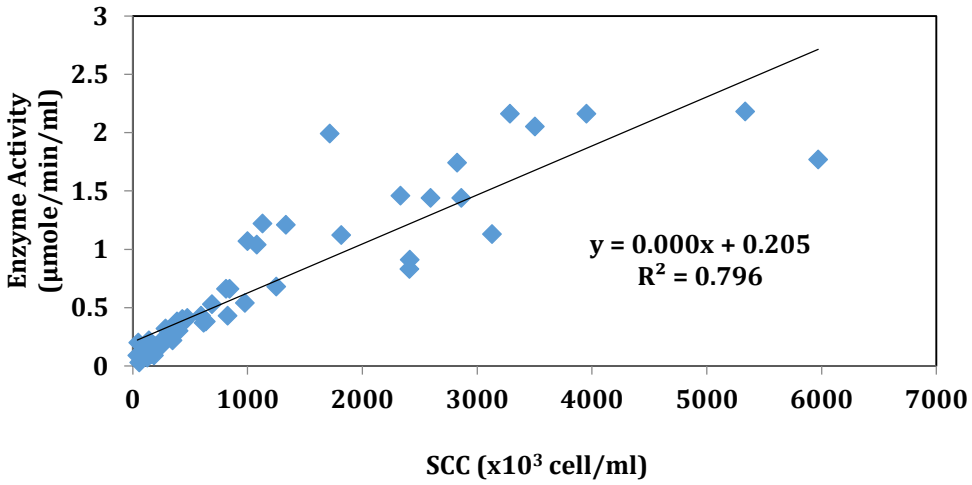

Figure 2: Relationship between Enzyme activity and Somatic Cell Counts.

Even though the correlation between mean SCC and enzyme activity of different crossbreds was not significant, the mean values of SCC and enzyme activity were comparatively high when an animal phenotypically resembled more of European breeds (Friesian, Ayrshire, Jersey) (779–1848 x10³ SCC/ml; 0.64 -0.89 µmole/min/ml) than towards tropical crosses (427–534x10³ SCC/ml; 0.26-0.43 µmole/min/ml). This could be due to the fact that the European crosses are high yielding animals, and thus they are more susceptible to mastitis than tropical breeds (Sudhan and Sharma, 2010). Shittu *et al.* (2012) stated that teats of high yielding European breeds are anatomically weak to resist the disease causing bacteria compared to tropical breeds and the latter has a narrow teat canal or a firmer sphincter at the end of the teat tip.

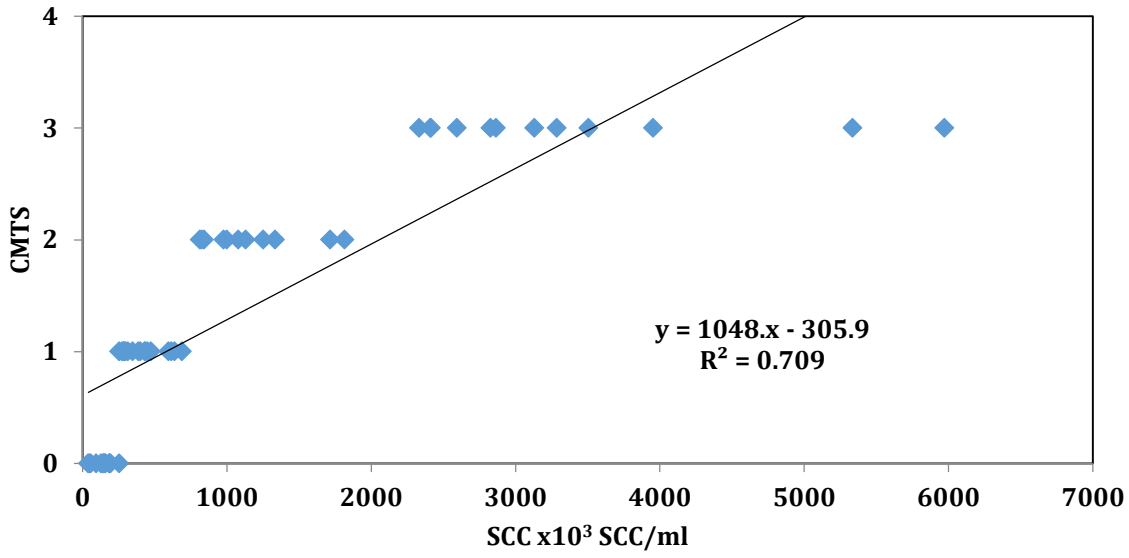

Figure 3: Relationship between California Mastitis Test Score and Somatic Cell Counts.

Adequate supply of irrigation water is one of the most limiting factors determining the yield of paddy and thus the nutrient removal from the soil. Further, irrigation water is a source of K supply for the paddy crop (Buresh *et al*., 2019). Majority of farmers in Polonnaruwa and Kurunegala districts are relying on irrigation water from major irrigation systems (command area is greater than 80 ha) in which the irrigation water supply is more reliable during the dry *yala* season. But, in Anuradhapura district equal proportions of farmers depend on major and minor irrigation facilities. Minor irrigation systems depend on village tanks which are often abandoned during *yala* season and shortages of adequate supply of water are experienced in the *maha* season. Thus, this heterogeneity of the supply of irrigation water also has an influence on the variability of soil available K content.

The oxidation and reduction processes governed by the alternate flooding and drying of paddy soils directly influence the P availability (Ponnamperuma, 1972). Thus, the spatial-temporal variation of oxidation-reduction process resulted by the heterogeneity of the availability of irrigation water could contribute for the spatial variability of soil P levels observed in study areas.

CONCLUSIONS

The prevalence of sub-clinical mastitis in crossbred lactating cows in North-Western Province of Sri Lanka was considerably high (74.5%). The most common pathogens found were *Staphylococus* spp., *E. coli* and *Streptococcus* spp. Even though the California mastitis test is available through milk collecting agencies, routine checking for sub-clinical mastitis has not happened mainly due to the high cost. The assay of NAGase activity in sub-clinical mastitis positive milk is an accurate method for assessing the mammary tissue damage compared to the somatic cell counts and California mastitis test score. Therefore, the determination of NAGase activity could be suggested as an accurate method for early diagnosis of sub-clinical mastitis.

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