Receiver operating characteristic analysis of milk lactose for identification of mastitis in buffaloes


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ABSTRACT

Receiver operating characteristic (ROC) analysis is a simple statistical tool used to classify a diagnostic indicator in terms of area under a ROC curve (AUC) and to develop potential threshold values of a diagnostic indicator. Milk lactose was analyzed by ROC analysis to see its accuracy to discriminate infected and healthy udder quarters, and to develop an optimum threshold value along with corresponding sensitivity (Se), specificity (Sp) and positive likelihood ratio (LR+) value. Data for the present study comprised of 1516 milk samples collected from Jaffrabad buffalo. Milk lactose was estimated by milk analyzer ‘LACTOSCAN’ and further samples were checked for sub-clinical mastitis by California mastitis test (CMT). The threshold values of milk lactose for identification of moderate and severe infection were found to be 5.31g% (Se, 58.82%; Sp, 58.28%) and 5.23g% (Se, 70.97%; Sp, 64.41%), respectively by ROC analysis. Milk samples with lactose content below 5.31g% were 1.41 times more likely come from moderately infected quarters (LR+ = 1.41); whereas, below 5.23g% were 1.99 times more likely come from severely infected quarters (LR+ = 1.99). The overall accuracy of milk lactose for discrimination of normal quarters from moderately infected quarters was 64% (AUC=0.64) and from severely infected quarters was 72% (AUC=0.72) (P<0.001). Thus, the present study indicated that milk lactose classified mastitic and healthy udder quarters in Jaffrabad buffalo with moderate accuracy.

Key words: Buffaloes, Milk lactose, ROC analysis, Mastitis.

INTRODUCTION

Receiver operating characteristic (ROC) analysis is an important statistical tool originally developed in 1950s (Zou et al., 2007). ROC analysis has been used in medical science for the first time to assess the imaging devices. Since, then it has been used for the evaluation of accuracy of clinical laboratory diagnostic tests as well as diagnostic indicators or markers for classification of diseased and non diseased cases (Hughes and Bhattacharya, 2013). Additionally, it is being used for development of optimum threshold value of diagnostic indicators in medical and veterinary sciences (Fan et al., 2006; Hughes and Bhattacharya, 2013; Patbandha et al., 2012, 2013). The ROC analysis is considered as a simple statistical tool used to characterize a diagnostic variable in terms of area under a ROC curve (AUC) and further provides range of potential threshold values along with their corresponding Sensitivity (Se), Specificity (Sp) and positive likelihood ratio (LR+) values (Patbandha et al., 2012, 2013). Milk constitutes major components such as fat, protein and lactose which are most important to the dairy farmers, producers and consumers (Reis et al., 2013). However, these traits are affected by environmental and nutritional factors as well as physiological and pathological condition of animals. Further, fat is considered as the most variable milk component, followed by protein and lactose is the least variable (Ravikala et al., 2014). On the other hand, during mastitis, alteration of milk lactose occurs markedly compared to other traits (Bansal et al., 2007; Sharif et al., 2007; Tripaldi et al., 2010; Hussain et al., 2012; Reis et al., 2013). Hortic and Seegees (1998) reviewed the alteration of milk fat and protein during mastitis and observed that the alterations were contradictory to several authors. Recently, Reis et al. (2013) also reported contradictory alteration of milk fat and protein during mastitis. Moreover, milk lactose showed little variation within lactation as well as from one lactation to the next, and consistently decreased during udder inflammation (Pyorala, 2003). Therefore, milk lactose content could be used as mastitis indicator in dairy animals. Though, few studies reported threshold values of milk lactose for mastitis identification in cows (Pyorala, 2003; Bansal et al., 2005; Tripaldi et al., 2010) and buffaloes (Bansal et al., 2007), the threshold values for identification of mastitis in Jaffrabad buffalo is not available. Hence, the present study aimed to see the overall accuracy of milk lactose to classify...
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Negative (un-exposed) Positive (exposed)

Test outcome  (based on diagnostic indicator) Gold standard test outcome

<table>
<thead>
<tr>
<th>Test outcome (based on diagnostic indicator)</th>
<th>Positive</th>
<th>Negative</th>
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<tr>
<td>Positive (exposed)</td>
<td>a [TP]</td>
<td>b [FP]</td>
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<tr>
<td>Negative (un-exposed)</td>
<td>c [FN]</td>
<td>d [TN]</td>
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TP, true positive; FP, false positive; FN, false negative; TN, true negative
et al., 2007; Tripaldi et al., 2010; Hussain et al., 2012). Lower lactose content may be due to impairment of synthetic activity of mammary tissues or decomposition by leucocytes or mastitis causing pathogens or leakage from milk into blood (Hussain et al., 2012; Reis et al., 2013). Hence, lactose could be used as an indicator due to consistent change during intra-mammary infections (Tripaldi et al., 2010; Reis et al., 2013).

The threshold value having highest combining Se and Sp of a test is called an optimum threshold value indicates the point of coincidence of Se and Sp. The threshold values of milk lactose for identification of moderate and severe infection and their corresponding Se and Sp are presented in Figure 2 and 3, respectively. The threshold value of milk lactose for identification of moderate infection was 5.31g% (Se and Sp values were 58.82 and 58.28%, respectively). Similarly, for identification of severe infection, the threshold values of milk lactose was 5.23g% (Se and Sp values were 70.97 and 64.41%, respectively). The optimum threshold value suitable for identification of mastitis observed in Jaffrabadi buffaloes are more or less comparable with earlier studies in dairy bovines (Pyorala, 2003; Bansal et al., 2005; Bansal et al., 2007; Tripaldi et al., 2010). Several authors reported threshold value of milk lactose for mastitis identification to be 4.7g% (Pyorala, 2003; Tripaldi et al., 2010) and 4.8g% (Bansal et al., 2005) in cows and 5.5g% (Bansal et al., 2007) in buffaloes. However, more practical experience is required to determine the usefulness of the threshold values (Pyorala, 2003; Tripaldi et al., 2010). The Se and Sp reflects the proportion of TP and TN cases of a test, respectively. The Se of 58.82% indicated that optimum threshold value of milk lactose (5.31g%) could correctly identify 58.82% of moderately infected quarters (TP cases) but 41.18% cases of moderately infected quarters may go unidentified (FN cases). Similarly, Sp of 58.28% indicated that optimum threshold value of milk lactose could correctly identify approximately 58.28% normal udder quarters (TN cases), but 41.72% normal quarters may be incorrectly identified as moderately infected (FP cases). Further, while considering identification of severely infected udder quarters, the Se and Sp values were comparatively higher than moderate infection, indicated that milk lactose was more accurate to identify severely infected udder quarters. We observed comparable Se but lower Sp compared to Pyorala, (2003), who reported 43.8 to 60.8% Se and 80.6 to 94.7% Sp for threshold value of milk lactose to identify sub-clinical mastitis in composite milk samples.

The LR+ value indicated the number of times a sample would as likely come from infected quarters if milk lactose content remained below the threshold values. In the present study, if milk lactose test value remained below 5.31g%, then the sample was 1.41 times (LR+ = 1.41) as likely come from moderately infected udder quarter. Similarly, a sample was said to be 1.99 times (LR+ = 1.99) as likely come from severely infected udder quarter, if milk lactose test value remained below 5.23g%. Thus, LR+ increases with decrease in milk lactose content, indicates negative relationship of milk lactose with probability of mastitis. Further, decrease threshold value of milk lactose indicates the severity of infection, that is, lower the lactose threshold value, higher the severity of udder infection.

The value of AUC of milk lactose for discrimination of normal and infected quarters was 0.64 (Figure 4) and 0.72 (Figure 5) during moderate and severe infections, respectively (P<0.001). The AUC in ROC analysis reflects the overall accuracy of diagnostic indicator (that is, milk lactose) for classification or discrimination of infected and non infected udder quarters. Further, as AUC does not depend on threshold value or cut off values or prevalence of mastitis, considered as a good measure of accuracy to classify healthy and infected quarters (Kumari et al., 2014). The overall accuracy of classification power of milk lactose was 64% during moderate infection and 72% during severe infection. Previous studies also reported that milk lactose is a good measure to distinguish infected and non infected quarters in dairy cows (Pyorala, 2003; Bansal et al., 2005; Bansal et
al., 2007) and buffaloes (Bansal et al., 2007). Milk lactose could classify infected and non-infected udder quarters correctly in cows with 81% accuracy (Bansal et al., 2005) and in buffaloes with 83.76% accuracy (Bansal et al., 2007). Further, Pyorala (2003) reported that milk lactose could be used to differentiate infected quarters from healthy quarters by 73.9 to 77.1% accuracy. These authors also reported more accuracy to classify infected and healthy udder quarters using milk lactose compared to milk SNF (55.08% accuracy), pH (59% accuracy) and EC (62.94 to 69.0% accuracy). The value of AUC (0.64 to 0.72) reflected that milk lactose is moderately accurate to classify infected and healthy udder quarters in Jaffrabadi buffaloes.

CONCLUSION
Results of the present study indicated that milk lactose decreased with increase in severity of infection. Milk samples with lactose content below 5.31g% were more likely to come from moderately infected quarters; whereas, below 5.23g% were more likely come from severely infected quarters. However, the milk lactose is moderately accurate to classify mastitic and healthy udder quarters in Jaffrabadi buffaloes.

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