

The Effect of Subclinical Mastitis on Lactate Dehydrogenase in Dairy Cows

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Abstract: Diagnosis of Subclinical Mastitis (SCM) is of increasing importance and appropriate detection methods are needed. The present study aimed at assessing the relationship between lactate Dehydrogenase (LDH) activity and (SCM) occurred naturally on dairy herds. Milk samples were collected from quarters of 25 cows with (SCM) as well as from 35 healthy controls. Blood specimens were also collected from jugular vein of mentioned cows for the assay. According to results the mean activity of (LDH) was higher in milk from udders than in milk from healthy udders. There were no significant differences in blood serum (LDH) of healthy and (SCM) cows ($p < 0.01$). The increment in (LDH) in milk of udders shows the presence of tissue damage provoked by (SCM). Thus, this parameter might be suitable for use in the early diagnosis of (SCM) in cows.

Key words: Cow, lactate dehydrogenase level, milk, serum, mastitis

INTRODUCTION

Bovine mastitis, the most significant disease of dairy herds, has huge effects on farm economics due to reduction in milk production and treatment costs. Mastitis can be either environmental or infectious and also some types of mastitis pathogens are more virulent than others. The severity of the inflammation can be classified into subclinical, clinical and chronic forms (Bramley and Dodd, 1984; Grohn *et al.*, 2004). Subclinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications (Viguier *et al.*, 2009). Reneau and Packard (1991) summarized that 70 to 80% of mastitis losses were due to subclinical mastitis. Kivaria and Noordhuizen (2007) concluded that California Mastitis Test (CMT) is the best cut off to correctly identify SCM mastitis especially by *S. aureus* in low yielding cows (Zaki *et al.*, 2008). Traditionally, methods of detection have included estimation of somatic cell counts, an indication of inflammation, measurement of biomarkers associated with the onset of the disease (e.g., the enzymes N-acetyl-b-D-glucosaminidase and lactate dehydrogenase) and identification of the causative microorganisms (Viguier *et al.*, 2009).

As the enzymic activities of milk are influenced by different factors, such as stage of lactation, season, species, race, food, mastitis and play a role as indicators for certain technological changes, this study was conducted to evaluate changes of LDH in milk and blood of cow as a result of SCM in the udder.

MATERIALS AND METHODS

During the year 2009 animals were selected from three Holstein dairy herds located in Urmia in West Azerbaijan province of Iran.

Sampling: Milk samples were collected from quarters of 25 cows with SCM, as well as from 35 healthy controls. Before sampling, the first strippings of milk were discarded, teat ends were then disinfected with 70% alcohol and allowed to dry. Then approximately 10 mL foremilk sample were collected from each quarter of cow. CMT was carried out in all milk samples, using the method by Schalm *et al.* (1971). It is based on the principle that the addition of a detergent to a milk sample with a high cell count will lyse the cells, release nucleic acids and other constituents and lead to the formation of a 'gel-like' matrix consistency. According to the visible reactions the results were classified in four scores: 0 = negative or trace, 1 = weak positive, 2 = distinct positive and 3 = strong positive. Blood specimens were also collected from jugular vein for the LDH assay. All samples were kept cold during transportation and delivered to the laboratory for examination within 2-4 h after collection. Blood serum and defatted milk (centrifuged at 3000g for 10 min) were used for enzyme activities estimations.

Enzyme analysis: Milk samples were centrifuged at 3000 rpm for 10 min to remove their creams and cells. LDH activity was measured by spectrophotometer, using

commercial kit (Pars Azmun Co. Inc., Karaj, Iran) by the method of Bergmeyer (1974) at wavelength of 340 nm.

Statistical analysis: Data were analyzed by SPSS (version10). Student's t-test was carried out to find the differences between the results of mastitic and non-mastitic milk and serum.

RESULTS AND DISCUSSION

The mean LDH activity was significantly higher in milk from inflamed SCM quarters than in normal milk ($p<0.01$); no significant difference was in blood enzyme values (Table 1 and 2). The origin of LDH in mastitic milk is attributed to leucocytes (Kato *et al.*, 1989) and also epithelial cells from the udder (Bogin *et al.*, 1977; Zank and Schlatterer, 1998). Bogin and Ziv (1973) have suggested that LDH in milk was a sensitive indicator of epithelial cell damage and subsequently proposed that LDH originated mainly from the damaged udder epithelial cells and from the elevated numbers of leucocytes.

In the context of milk lactate being a potential diagnostic measurement for mastitis, Mayer *et al.* (1988) reported that milk oxygen concentrations were reduced during mastitis suggesting that, given a favorable supply of substrates, the metabolism of somatic cells in milk and/or the mammary epithelium may become anaerobic leading to release of lactate into milk (Davis *et al.*, 2004). Mackie *et al.* (1977) found a relatively slow but substantial increase in lactate in milk during mammary involution with concentrations reaching approximately 5 mM, 5 d after the last milking.

Kato *et al.* (1989) reported highest LDH activity in milk samples infected with *S. aureus*. Lower LDH activities were found in samples being positive for *C. bovis* and CNS. Higher LDH activity in milk serum of inflamed udders has been previously reported in cows (Kovac and Beseda, 1975; Grun *et al.*, 1992) and sheep (Nizamliglu and Erganis, 1991; Batavani *et al.*, 2003). Chagunda *et al.* (2005) developed a statistical model for the detection of mastitis based on LDH activity. Sensitivity and specificity were 76.5 and 97.7%, respectively for diagnosing clinical mastitis. The higher level of LDH in mastitic milks than blood serum shows that blood serum was not the sole source of this enzyme in mastitic milk and it was probably also liberated from disintegrated leukocytes and the parenchymal cells of the udder (Bogin *et al.*, 1977; Michel, 1979; Kato *et al.*, 1989).

In this study the mean activities of LDH was significantly higher in the milk from udders with SCM than in the milk from healthy udders ($p<0.01$), no significant difference was in blood enzyme values. It seems that the origin of the elevated LDH in mastitic milk

Table 1: Changes in the level of LDH in milk samples

Enzyme	Normal	Sub clinical mastitis
LDH	485.94±13.66	1524.04±111.74
IU/L	(35)	(25)

Parenthesis shows the number of samples

Table 2: Changes in the level of LDH in serum samples

Enzyme	Normal	Sub clinical mastitis
LDH	867.16±31.31	809.32±21.67
IU/L	(35)	(25)

Parenthesis shows the number of samples

is the leucocytes and the parenchyma cells of the udder. In conclusion; milk L-lactate has potential as an indicator of clinical and subclinical mastitis in dairy cows.

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