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Correlation Between Lactoperoxidase Activity and Somatic Cell Count for Diagnosis Subclinical Mastitis in Early Lactation of Dairy Cows

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Abstract: This study was conducted to determine the levels and relationship between Lacto Peroxidase (LP) activity and somatic cell count (SCC) for diagnosis subclinical mastitis in early lactation of dairy cows. Foremilk samples were obtained from quarters of 80 cows (August 2007 up to February 2008). Any cows had not evidence of clinical mastitis at time of sampling. The SCC ranged from 5.24×10^5 cells/mL in the first parity to 5.5×10^5 in the third parity with a mean value of 5.45×10^5. The mean LP activity in first, second and third parity were found 6.49, 4.63 U and 5.5 U mL^{-1}, respectively. No significant correlation ($r = 0$, $p>0.05$) was found between number of SCC and LP values in early lactation. Since, the measurement of LP activity of milk may not be used as a predictor subclinical mastitis in the early lactation period of dairy cows.

Key words: Lactoperoxidase, somatic cell counts, early lactation

INTRODUCTION

Mastitis is the most costly disease of dairy cattle due to economic loss from reduced milk production, treatment costs, increased labor, milk withheld following treatment, death and premature culling (Vliegher *et al*., 2001). Identifying and eliminating intramammary infection in early lactation may have significant economic benefits. The use of individual cow SCC to identify the presence of intramammary infections in early lactation cows is possible. However, a recent study in dairy cattle reported a maximum sensitivity of 60% for SCC for diagnosis intramammary infection in dairy cattle (Middieton *et al*., 2004). Therefore, in this study we want to use other test in order to diagnosis subclinical mastitis in early lactation period.

Lactoperoxidase (EC1. 11.1.7) is one of the most abundant milk enzymes in natural form and it represents approximately 1% of the proteins in whey (Reiter, 1985). Its activity can be affected by many factors, such as animal species (Pruit and Reiter, 1985), breed and lactation cycle (Zapico *et al*., 1991), in addition to the individual differences (Medina *et al*., 1989).

The activity of many milk enzymes such as lipase, lactaate dehydrogenase, lysozyme, plasmin, xanthine oxidase (Harmon, 1994), catalase, NAGase (Walstra *et al*., 1999) and lactoperoxidase (IDF, 1979) increases when SCC increases. Measurement of the activity of some enzymes in milk such as catalase, NAGase (Harmon, 1994; Walstra *et al*., 1999) has been used to monitor udder health in dairy cows. Since, LP is mainly synthesized by polymorphonuclear leukocytes (Korhonen, 1980), its activity is expected to increase with increase in SCC milk and thus may be used to detect mastitis in dairy cows. However, there is a little information available LP activity in bovine milk.

The aim of this study was to determine relationship between lactoperoxidase and SCC for diagnose subclinical mastitis in early lactation in a dairy cows.

MATERIALS AND METHODS

Animals: Animals were selected from a Holstein dairy farm included 680 milking cows located in Tabriz in East Azerbaijan province of Iran. In herd, cows were housed in free stall barns. Cows were in first to 8 lactation and were milked three times daily by machine milking. Cows were fed *ad libitum* by a total mixed ratio that had been formulated to meet the nutritional requirements of a 650 kg cow, yielding 20-45 kg of milk/day with about 3.5% milk fat

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and 3.4% protein. All cows were subjected to post-milking teat disinfection, those were dried off approximately 2 months before expected calving and all quarters of cows were infused with an dry cow antibiotic preparation following the last milking of lactation.

Milk sampling: Foremilk samples were obtained (August 2007 up to February 2008) from quarters of 80 cows. Any cows had not evidence of clinical mastitis at time of sampling. Teat ends were cleaned with ethyl alcohol 70% before sampling. First streams of foremilk were discarded and then 10 mL of milk was collected aseptically from each teat into sterile vials. The milk samples were stored at 4°C in a refrigerator until analysis. Milk samples intended for the SCC determination were preserved by potassium dichromate (0.2% w/v) (Seifu *et al*., 2007).

Determination of SCC: Composite milk samples were collected aseptically on 5-14 day post calving and were analyzed with the Fossomatic 5000 (Foss Electric, Hillerod, Denmark) (Vliegher *et al*., 2004).

Lactoperoxidase activity of the milk samples: Milk samples were diluted to 40- fold using PBS (0.1 M, pH 7.0) and analyzed for peroxidase activity. Lactoperoxidase activity of the milk samples were measured in phosphate buffer (0.1 M, pH 6.0) with one-step ABTS (2,2-azino-bis-(3-ethyl-benzthiazoline-6-sulphonic acid solution as substrate using the method described by Seifu *et al*. (2007) and Kumar and Bhatia (1999). 3 mL of ABTS solution (1 mM in 0.1 M phosphate buffer, pH 6.0) and milk sample (0.1 mL) were added together in cuvette. The reaction was initiated by the addition of 0.1 mL hydrogen peroxide solution (3.2 mM) and immediately the measurement of absorbance started at 412 nm as a function of time for 2 min at 15 sec intervals using an UNICO UV-2100 PC spectrophotometer. One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of ABTS per min at room temperature (~22-25°C).

Statistical analysis: The data for SCC was transformed to logarithmic value ($\log_{10}$) prior to statistical analysis. The correlation between SCC and LP activity were analyzed by the SPSS software (SPSS version 11.5 for windows; SPSS Inc., Chicago, IL, USA). Effect of parity on SCC and lactoperoxidase values were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered to be statistically significant at ($p<0.05$).

Table 1: Mean values of LP enzyme activity within parity (n = 80)

Parameter parity	n/cow	Mean LP activity
1	26	6.49[a]
2	27	4.63[b]
3	27	5.50[ab]

Mean values with different superscript letters (a, ab with b) in a column are significantly different ($p<0.05$)

Table 2: Effect of Parity on SCC and LP activity values

		Mean square	
Parameter	df	SCC	LP activity
Between parity	2	661[ns]	20.01[*]
F	77	426	6.245

RESULTS

All milk samples from dairy cows in early lactation had SCC ranged from 5.24×10^5 cells mL^{-1} in the first parity to 5.5×10^5 in the third parity with a mean value of 5.45×10^5. All milk samples had SCC greater than the threshold value of 500,000 cells mL^{-1} for subclinical mastitis. Whereas 100% of cows had subclinical mastitis. No significant differences were observed ($p>0.05$) in SCC values between parities.

The mean values of LP activity were 6.49 units mL^{-1} for the first parity, 4.63 units mL^{-1} for the second parity and 5.5 units mL^{-1} for Third parity with a total mean value of 5.51 units mL^{-1}. Differences were considered to be statistically significant at $p<0.05$ (Table 1).

The regression analysis showed no significant correlation between LP activity and SCC ($r = 0$). Effect of parity on number of SCC and by one-way ANOVA were not significant($p>0.05$). However, effect of parities on LP value were significant ($p<0.05$) (Table 2).

DISCUSSION

Inflammation of the mammary gland leads to a variety of compositional changes in milk either because of local effects or because of serum components entering the milk and the movement of some normal milk components out of the alveolar lumen into the perivascular space (Harmon, 1994). Theoretically, all changes in mammary secretion during inflammation might be used to measure the effects of mastitis, but problems of instrumentation and standardization have hampered farm application of most tests.

The international dairy federation recommended classification of quarter cow milk as subclinical mastitis or non mastitis using a SCC threshold of 5×10^5 cells mL^{-1} (IDF, 1979). Based on this recommendation and our results the mean SCC 5.45×10^5 cells mL^{-1} observed in this study for cow milk in early lactation. Our finding showed that most of the lactating cow in early lactation had

subclinical mastitis. The results in this study are to be interpreted with caution as data on bacteriological were not available.

The mean value of the LP enzyme activity in our investigation was (5.51 units mL^{-1}). The LP value in this study higher than to those reported for other species. For example 0.31 (Shindler *et al*., 1976) and 0.19 U mL^{-1} (Kumar *et al*., 1994) in buffalo milk and 0.95-2.15 U mL^{-1} (Zapico *et al*., 1991) in goat milk. In our investigation much greater average LP activity value observed in the first parity (6.49 U mL^{-1}) followed by a gradual decrease in the second parity (4.63 U mL^{-1}). The reason of this reduction not considered, but might be related to decrease of SCC and infection or increase of age.

A correlation between LP activity and SCC has been reported in some species of domestic animals such as goat. Seifu *et al*. (2007) reported that high LP activity observed in goat milk may be attributed to the higher levels of somatic cells in the Saanen goat milk at the time of the experiment since the number of somatic cells in a goat milk increases with the occurrence of mastitis in goat and science LP is synthesized mainly by polymorphonuclear leukocytes is logical to expect that the LP activity increase with the increase in SCC an this with the occurrence of subclinical mastitis. However, our results do not supports in this claim, because we not found significant correlation between LP activity value and SCC in early lactation (r = 0). However, the results in this study are to be interpreted with caution as effect of time sampling and all lactation cycle were not considered. In conclusion, our results showed that LP activity value could not be used for detection subclinical mastitis in early lactation in dairy cows.

REFERENCES

Harmon, R.J., 1994. Physiology of mastitis and factors affecting somatic cell count. J. Dairy. Sci., 77: 2103-2112.

IDF, 1979. Somatic cells in milk: their significance and recommended methods for counting. Bull. Int. Dairy Fed., 114: 4-20.

Korhonen, H., 1980. Potential Role of the Lactoperoxidase System ($LP/SCN^-/H_2O_2$) in Mastitis Resistance. In: Bassalike-Chabielska, L. and Z. Ryniewicz (Eds.). Proceeding of international Conference on Resistant factors and Genetic Aspects of mastitis Control. Jablonna, Poland, pp: 421-438.

Kumar, S., B.N. Mathur and S. Kumar, 1994. Lactoperoxidaseactivity in raw buffalo milk. Indian. J. Dairy Sci., 47: 806-807.

Kumar, R. and K.L. Bhatia, 1999. Standardization of method for lactoperoxidase assay in milk. Lait, 79: 269-274.

Medina, M., P. Gaya and M. Nunez, 1989. The lactoperoxidase system in ewe milk: levels of lactoperoxidase and thiocyanate. Lett. Applied Microbiol., 8: 147-149.

Meddieton, J.R., D. Hardin, B. steevens, R. Randle and J.W. Tyler, 2004. Use of somatic cell counts and California mastitis test result from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somtic cell count. J. Am. Vet. Med. Assoc., 224: 419-423.

Pruitt, K.M. and B. Reiter, 1985. Biochemistry of Peroxidase System: Antimicrobial Effect. In the Lactoperoxidase System: Chimisty and Biological Significance. Pruitt, K.M. and J.O. Tenovuo (Eds.). Immunol. Marcel Dekker Inc. New York, 27: 143-178.

Reiter, B., 1985. The Lactoperoxidase System of Bovine Milk. In the Lactoperoxidase System: Chemistry and Biological Significance. Pruitt, K.M. and J.O. Tenovuo (Eds.). Immunol. Ser .27, Marcel Dekker Inc., New York, pp: 123-141.

Seifu,E., E.F. Donkin and E.M. Buys, 2007. Potential of lactoperoxidase to diagnose subclinical mastitis in ats. Small Rumin. Res., 69: 154-158.

Shindler, J.S., R.E. Childs and W.G. Bardsley, 1976. Peroxidase from human cervical mucus. The isolation and characterization. Eur. J. Biochem., 65: 325-331.

Vliegher, S. De., H. W. Barkema, H. Stryhn, G. Opsmer and A. de kruif, 2004. Impact of early lactation somatic cell count in heifers on somatic cell counts over the first lactation. J. Dairy Sci., 87: 3672-3682.

Vliegher, S.D., H. Laevens, G. Opsomer, E. DeMuelenaere and A. De Kruif, 2001. Somatic cell counts in dairy heifers during early lactation. Flemish. Vet. J., 70: 212-215.

Walstra, P., G.T.J. Geurts, A. Noomen, A. Jellema and M.A.J.S. Vanboekel, 1999. Dairy Technology: Principles of milk properties and processing, Marcel Dekker Inc., NewYork.

Zapico, P., P. Gaya, M. DePaz, M. Nunez and M. Medina, 1991. Influence of breed, animal and days of lactation on lactoperoxidase system components in goat milk. J. Dairy Sci., 74: 783-787.