

## Evaluation of Buffalo Milk With Reference to Somatic Cell Count and Antitrypsin

Syed.A.M, S.U. Digraskar and K.B.Awaz

Dept. of Veterinary Medicine,  
College of Veterinary and animal Sciences, MAFSU, Parbhani – 431 402

### Abstract

The present study was carried out for the assessment of buffalo milk quality by assessing the somatic cell count and antitrypsin of milk. Thirty buffalo milk samples collected directly from udder were subjected to the detection of somatic cell count and antitrypsin of the milk. The mean value of somatic cell count was  $223.46 \times 10^3 \pm 26.522$  cells/ml and the mean value of antitrypsin of raw buffalo milk was  $6.87 \pm 0.054$   $\mu\text{g/ml}$ . The result showed that there was an increased somatic cell count and antitrypsin which indicated that there was inflammation of udder suggestive of underlying mastitis.

**Keywords:** somatic cell count, antitrypsin, Milk quality, mastitis.

### Introduction

Assessment of milk quality is very important to know the health status of the udder as well as the suitability of milk as per the public health aspect is concerned. The present study was carried out on the assessment of buffalo milk quality under existing managemental practices. Thirty milk samples for the present study were collected directly from quarters of buffaloes and subjected to the detection of somatic cell count and antitrypsin of milk.

### Materials and Methods

Somatic cell count was calculated according to method described by Schalm *et.al* (1971). The dried smears of freshly collected milk samples were made on clean grease free glass slide and stained by "Newman Lampart stain". A total of twenty five randomly selected fields of microscope were counted under oil immersion and average microscopic factor for Somatic cell count per milliliter of milk was calculated. Somatic cell count were graded as low (<1, 00,000), moderate (1, 00,000-250,000) and high (>250,000). A count less than 250,000 cells/ml of milk was taken as insignificant for presence of mastitis.

For measurement of antitrypsin, the Spectrophotometric method is said to be quite sensitive (Church *et.al* 1985). For the estimation of antitrypsin by Spectrophotometric method, the solution should be clear therefore milk whey was prepared by adding 10-15mg citric acid to 10ml of milk and then centrifuged at 1500 rpm for 15minutes (Mulkalwar *et.al* 1999) and from middle portion of tube the whey was collected. The clear watery whey was used to estimate antitrypsin

as per the method described by Sandholm *et.al* (1984) and Mattila *et.al* (1985). The pH of the whey was adjusted to 7.0 by adding Sodium Hydroxide to maintain the stability. Clearing solution was prepared by mixing 1% dimethyl formamide and 16.7% polyethylene glycol-6000 in 0.1M calcium chloride and pH 8.2 buffer. Trypsin working solution was prepared by dissolving 1mg trypsin in 10ml boric acid borate buffer (pH 7.4) to get final concentration of 100 $\mu\text{g/ml}$  (microgram per milliliter) of trypsin. BAPNA substrate (N-Benzoyl Arginine-P-Nitroanilide) 50mg dissolved in 50 ml distilled water to get final BAPNA concentration 1mg/ml.

The optical density of whey should be between 0.2 to 0.8 so 1:8 dilution of whey was used for the assay which has 0.458 optical density at 405nm. The plates were incubated at 37°C for one hour. The colour absorbance of each dilution was measured at 405nm. The absorbance reading for each dilution was obtained by subtracting readings of respective whey control from that of the test. The antitrypsin activity in test sample was calculated by subtracting reacted trypsin value from trypsin added and then this value was multiplied with the dilution factor *i.e.* 1:8 (it means multiplied by 8). Protocol for estimation of antitrypsin in the milk whey samples is as follows.

The data on milk was analyzed by completely randomized design (CRD) as per the method described by Panse and Sukhatme (1988).

### Results and Discussion

Somatic cell count is widely used to predict the udder health status and the suitability of milk for human consumption. Antunac *et.al* (1997) showed that somatic

cell count is internationally recognized parameter for assessing milk quality and udder health. In the present study, Somatic cell count ranged between  $0.4 \times 10^4$  cells/ml to as high as  $5 \times 10^5$  cells/ml with a mean of  $223.46 \times 10^3 \pm 26.522$  cells/ml. Mahendra and Dang (2001) recommended that if somatic cell count less than  $1 \times 10^5$  cells/ml udder was considered healthy while somatic cell count  $2.5 \times 10^5$  cells/ml and above then considered udder was having infection. The quality of milk was poor in nine buffaloes out of thirty buffaloes studied which showed higher somatic cell count i.e  $4.98 \times 10^5$  cells/ml and above which was in agreement with Mahendra and Dang (2001) . The higher values for somatic cell count reported in the present study has underlined the need for improving managerial practices to reduce the milk contamination with microorganism and judicious use of antibiotics in lactating animals.

The estimation of antitrypsin in milk samples collected directly from udder in the present study indicated elevated antitrypsin concentration. Awaz and Samad (1993) reported that the normal milk contains undetectable level of antitrypsin, mastitic milk has 2-64mg/ml and colostrum (on first day) 64mg/ml of antitrypsin. The elevated level of antitrypsin were found in close agreement with Sandholm *et.al* (1984) who showed milk antitrypsin activity was high in the beginning of lactation (colostrums) but after first month of lactation only blood derived antitrypsin was present in the milk in case of mastitis. An average antitrypsin level in present study was  $6.87 \pm 0.054 \mu\text{g/ml}$ . In few individual cases there was high somatic cell count associated with corresponding high values of

antitrypsin in milk and its detection provides a reliable tool for diagnosis of mastitis.

#### Acknowledgement

Authors are thankful to the Associate Dean, College of veterinary and animal sciences, MAFSU, Parbhani (Maharashtra) for providing necessary laboratory facilities.

#### References

1. Antunac, N, Lukac Havranek and D. Samarazia (1997): Somatic cells and their effect on the quality and processing of milk. *Mijekastvo*, 47(3):183-193.
2. Awaz.K.B and Abdul Samad (1993): A rapid enzyme inhibition assay for detection and quantitation of trypsin inhibitors in biological materials. *J.Bombay.Vet.Coll*,4 (1&2):25-31.
3. Church.F.C, D.H Porter, G.L.Cargon and H.E. Swassigood (1985): Anophthalaldehyde spectrophotometric assay for proteinases. *Ana.Biochem*.146:343-348.
4. Mahendra Singh and A.K.Dang (2001): Range of somatic cell count of milk. National Dairy Research Institute, ICAR, Karnal (Haryana) India.
5. Mattila.T, S.Saari, H.Vartiala and M.Sandholm (1985): Milk antitrypsin as a marker of bovine mastitis correlation with bacteriology. *J.Dairy Sci*, 68(1):114-122.
6. Mulkalwar.D.B, C.P.Parkhi, N.S.Mangle, D.R.Kalorey and V.P.Pathak (1999): Effect of udder health on pH, lactose and citric acid levels in sheep milk. *Indian vet. J*, 76(12):1088-1090.
7. Panse.V.G and P.V.Sukhatme (1988): Statistical methods for agricultural workers, 4<sup>th</sup> ed<sup>n</sup>, Indian Council of Agric.Res, New Delhi.
8. Sandholm.M, Buzalski.T.H, and Kangasniemi.R (1984): Milk antitrypsin capacity as an indicator of bovine mastitis a novel principle which can be automated. *J.Dairy.Res*.51:1-9.

\* \* \* \* \*