ALTERATION OF MILK pH, SOMATIC CELL COUNT (SCC), LACTATE DEHYDROGENASE (LDH) AND ALKALINE PHOSPHATASE (ALP) ACTIVITIES IN BUFFALO MILK RELATED TO UDDER HEALTH STATUS

Sani Nandi

ABSTRACT

The present study was designed to correlate the milk pH, somatic cell count (SCC), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities in buffalo milk with the udder health status. A total of 60 (Sixty) buffaloes were randomly selected irrespective of their age, breed, feeding practice, stage and season of lactation from the herd belonging to Purnadhadi buffalo unit, PGIVAS, Teaching Veterinary Clinical complex, PGIVAS, Veterinary Polyclinic, Akola and also buffaloes of farmers in and around Akola. After thorough clinical examination of each udder, about 30 ml of milk was collected in sterilized, clean, dry plastic bottles and after careful screening by CMT, categorized in normal, sub- clinical (1+), sub- clinical (2+), sub- clinical (3+) and clinical group containing 12 animals in each group. The pH was measured using a digital pH meter (E.I. Model 101E) and SCC was calculated in freshly collected milk. LDH and ALP activities were measured by spectrophotometric method. The milk pH, SCC, LDH and ALP activities were found proportional to the severity of the udder infection as detected by the CMT reactions.

Keywords: *Bubalus bubalis*, buffaloes, alkaline phosphatase, lactate dehydrogenase, mastitis, pH, somatic cell count

INTRODUCTION

India stands first in the world for buffalo population and milk production (Borghese, 2005). The water buffalo population in India was over 97.9 million in 2003, representing 56.5% of the world buffalo population (Singh and Barwal, 2010) which contributed 51.06% of milk production share during 2014 to 2015 (DADF, 2016).

Mastitis is a multifactorial inflammatory reaction of mammary gland which can occur in two forms, clinical and subclinical and is considered as the costliest production disease in the dairy farm worldwide (Miller *et al*., 1993). Moreover, Buffaloes have some characteristics which make them more prone to mastitis. For example, the udder is more pendulous and teats are longer in comparison to that of cattle (Moroni *et al*., 2006) and also have an innate instinct of wallowing. The milk produced by the affected animals may harbor organisms which are particularly pathogenic not only to humans (Blumberg *et al*., 1992) are also equally harmful to suckling newborns (Batavani *et al*., 2007) and reduces the shelf life of fluid milk products (Oz *et al*., 1985). Along with the reduced and unhealthy milk production, mastitis also impels the premature culling of the genetically superior buffaloes.

Clinical mastitis shows all its cardinal signs (Bachaya *et al*., 2011), so it is easily diagnosable

Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry, West Tripura, India, E-mail: nandisani@gmail.com

on field but subclinical mastitis requires laboratory examination and field test as there are no inflammatory changes in the udder. Thus instances of subclinical mastitis usually go unnoticed with 3 to 40 times more prevalence than clinical mastitis (Bachaya *et al*., 2011) and responsible for 70% of economic losses (Heleili *et al*., 2012). Therefore, subclinical mastitis is more important than clinical one (Joshi and Gokhale, 2006).

The somatic cells rapidly increase during inflammation, so it can be used to predict the severity of the intra -mammary infection and be a useful tool to assess the milk quality, monitor herd health for mastitis and to decide milk quality based payment (Deshapriya *et al*., 2019).

Normal buffalo milk is slightly acidic which may be due to presence of phosphates, proteins, CO_2 and citrates in milk (Bilal and Ahmad, 2004). The milk pH shifted to alkalinity in mastitis because of the decreased lactose production and the increased selective transudation of alkaline salts by the udder tissue (Schalm *et al*., 1971). Thus the pH may serve as the best indicator to assess the udder health status of the animal (Ahmad *et al*., 2005).The damaged udder parenchyma due to inflammatory products results in the release of intra-cellular enzyme activity (Babaei *et al*., 2007). Increased permeability during inflammation leads the leakage of enzymes from the blood to the milk (Pyorala, 2003).

The current study is aimed at assessing the relationship between pH, SCC, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities in buffalo milk and different form of udder health status.

MATERIALS AND METHODS

Sixty buffaloes were selected randomly irrespective of their age, breed, feeding practice, stage and season of lactation from the herd belonging to Purnadhadi Buffalo Unit, PGIVAS, Teaching Veterinary Clinical Complex, PGIVAS, Veterinary Polyclinic, Akola and also buffaloes of farmers in and around Akola. Fore milk samples each of about 30 ml were collected in sterilized, clean, dry plastic bottles after thorough clinical examination of the udder. Immediately each milk sample was screened carefully by CMT (Durry and Reed, 1961) using the reagents prepared as mentioned by Chakroborti (1997) and stored in the ice bath for further physiochemical analysis. Likewise sixty samples were collected and categorized as normal, subclinical 1+, subclinical 2+, subclinical 3+ and clinical containing 12 animals in each group.

For each freshly collected milk sample, the pH was measured using digital pH meter (E.I. Model 101 E).

For the estimation of SCC a drop (0.01 ml) of each freshly collected milk sample was spread in 1 cm^2 area with the pasture pipette on a clean glass slide. The smear was air dried and stained by Livowitz Weber solution for 5 to 10 minutes. The smear was washed with distilled water and again air dried. This stained smear was then examined under oil immersion objective of microscope to count the somatic cells in at least 10 different fields. The average of theses counts was then multiplied with the microscopic factor of 50,000 in accordance with Schalm *et al.* (1971) to calculate the direct microscopic SCC for each sample of milk. Each milk sample of about 15 ml was centrifuged at 2500 rpm for 15 minutes to get a milk fat layer on the top leaving below the fat

free milk. Then the centrifuge tubes were kept in a freezer for 15 minutes to make the upper milk fat layer hardened and compact. (Olson *et al*., 1981). The upper compact milk fat layer was punctured and the lower fat free milk was collected carefully into another centrifuge tube. Each 10 ml of such fat free milk sample was mixed thoroughly with 1 ml of 1 N HCl and centrifuged at 2500 rpm for 20 minutes. The casein settled at the bottom and the clear supernatant whey was separated and transferred carefully by pasture pipette into the dry clean vials for further estimation.

The LDH and ALP activities in each sample of milk whey were estimated using the reagents prepared in the laboratory and the digital spectrophotometer (E.I. model 301 E) according to the methods described by Cabaud *et al.* (1958); Chawla (2006) respectively. The standard curve for LDH units (U) was plotted against OD 520 nm and for ALP units the standard curve was plotted against OD of 510 nm.The statistical analysis of experimental data were carried out using the statistical procedure laid down by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Milk pH

The average pH values of milk samples with their standard errors for comparison of different udder health status of buffaloes are presented in Table 1. The result indicated an increase in the pH values with the increase in the severity of mastitis. The pH values were also significant ($P<0.01$) for the comparison of all the groups.

Damage to the blood-milk barrier in mastitis leads to the leakage of epithelial tight junctions (Nguyen and Neville, 1998) and as a result blood and extracellular fluid components mix with secreted milk (Zhao and Lacasse, 2008) and showing the trend of increased milk pH with the increase in the severity of inflammatory process (Qayyum *et al*., 2016). Increased permeability of blood bicarbonates and other alkaline salts into the milk together with the decreased production of lactose by the gland may result in milk pH became 7.0 (Rao, 1990). Pernoud *et al.* (2004) opined that the increase in pH value of mastitic milk could be due to higher pK value of ammonia produced on hydrolysis of urea in milk. Thus, the pH could serve as the best indicator to assess the udder health status of the animals as well as the food value of the milk (Haggag, 1991).

Somatic cell count (SCC)

The averages of SCC of milk with their standard errors for comparison in different udder health status of buffaloes are presented in Table 2. The results indicated the increase in number of SCC of milk with the increase in the severity of mastitis.

The major increase in the SCC during mastitis is due to the influx of neutrophills into the milk to fight infection (Miller and Paape, 1985) which is related to the immunological status of the udder (Leitner *et al*., 2000) and modulated by the inflammatory mediators (Harmon, 1994). In buffalo as well as bovine mastitis, there is increase in somatic cell count (SCC), which varies according to the intensity and length of inflammation (Hamza and Choudhuri, 1994). Thus SCC can be considered as a golden standard to measure inflammation of udder (Hamann, 2002) in cytological investigation.

As such, increased levels of SCC found in the present investigation irrespective of the causative agents and influencing factors could be used as the clinical indicator of predictive and

Table 1. Mean and standard error for milk pH values in different udder health status of buffalo.

Udder health status	Normal milk	Sub-clinical milk			Clinical milk
		1^+	2^+	3^+	
pH	$6.64^{a}\pm0.02$	$6.80^{b}\pm0.01$	$6.92^{c}\pm0.01$	$7.08^{d}\pm0.01$	$7.31^{e}\pm0.03$
Range	6.54-6.75	6.76-6.84	6.86-6.99	7.01-7.14	7.2-7.46

Different superscripts indicate significance (P<0.01) between udder health statuses.

Table 2. Mean and standard error for somatic cell count of milk in different udder health status of buffalo.

Udder health status	Normal milk	Sub-clinical milk			Clinical milk
		1^+	2^+	3^+	
SCC (X 10^5 cells/ml)	$1.99^{a}\pm0.03$	$4.02^{b}\pm0.06$	$6.43^{c}\pm0.14$	$8.21^{d}\pm0.12$	$10.13^{e}\pm0.19$
Range	1.86-2.12	3.72-4.32	5.63-7.01	7.56-8.76	9.23-11.25

Different superscripts indicate significance (P<0.01) between udder health statuses.

Table 3. Mean and standard error for milk whey lactate dehydrogenase (U/ml) activity in different udder health status of buffalo.

Udder health status	Normal milk	Sub-clinical milk			Clinical milk
		1^+	2^+	3^+	
LDH (U/ml)	$411.74^{a}\pm8.28$	$642.33^{b}\pm10.83$	$796.38^{c}\pm11.85$	$963.88^{d}\pm12.92$	$1250.07^{e}\pm26.01$
Range	372.57-455.43	598.29-699.71	746.86-855.43	891.14-1028.29	1126.86-1386.86

Different superscripts indicate significance (P<0.01) between udder health statuses.

Table 4. Mean and standard error for milk whey alkaline phosphatase (KA U/dl) activity in different udder health status of buffalo.

Udder health status	Normal milk	Sub-clinical milk			Clinical milk
		1^+	2^+	3^+	
ALP (KA U/dl)	$16.39^{a}\pm0.80$	$25.96^{b}\pm0.73$	$31.87^{c}\pm0.60$	$37.07^{d}\pm0.64$	$42.14^{e}\pm0.59$
Range	12.96-20.34	22.98-30.83	29.83-36.02	34.04-40.35	38.22-44.43

Different superscripts indicate significance (P<0.01) between udder health statuses.

diagnostic values, respectively for the detection of early and advanced stages of mastitis in buffaloes.

Lactate dehydrogenase (LDH) activity

The average LDH activities in milk whey with their standard errors for the comparison in different udder health status of buffaloes are presented in Table 3. The results indicated that there were significant ($P<0.01$) increases of LDH activity with the increase in the severity of different forms of mastitis in buffaloes.

The enzymes are the cellular constituents and rupture of the cells or tissues spill them into the milk (Shahani, 1966). Being larger molecular size, enzymes might be concentrated by active transport only from blood to milk (Folley, 1956). The increased LDH activity in mastitic milk was reported to be caused by the liberation from parenchyma cells of the udder and disintegrating leukocytes or both and from other sources like, serum (Bogin and Ziv, 1973) and the increasing trend of LDH activity with the increase in the severity of mastitis was reported by Batavani *et al.* (2007). Thus the LDH activity was described as an important indicator of bovine mastitis based on its estimation in individual sample of milk (Larson, 2005; Chagunda *et al*., 2006). The same potential was reflected by the LDH activity in the present study too.

Alkaline phosphatase (ALP) activity

The average ALP activities in milk whey with their standard errors for the comparison in different udder health status of buffaloes are presented in Table 4. The results indicated an increase in the activity of ALP with the increase in severity of mastitis.

Subclinical mastitis causes considerable changes in milk and serum composition (Meglia *et al*., 2001). Gain *et al.* (2015) reported significant increase in serum ALP activity in mastitis. The enzymes from blood leak into the milk due to an increased permeability (Pyorala, 2003). The increased enzyme activity in mastitic milk sample could be due to inflammation (Bogin *et al*., 1977) resulting in the migration of neutrophills into the infected udder (Mohammed *et al*., 1981). Leukocytes were one of the major sources of phosphatases in milk particularly when there was infection in the udder causing their lysis leading to the release of the lysosomal enzymes (Pathania and Seth, 1995).

The variation in the milk-whey ALP activity of various udder health status groups studied in the present investigation indicated the significance of this enzyme as a potential indicator of mastitis.

ACKNOWLEDGEMENTS

The author is highly thankful to Dr. N. S. Mangle, Ex Professor & Head, Department of Veterinary Biochemistry, PGIVAS, Akola, Maharashtra for his valuable guidance and advice which made the current study possible. The author is also thankful to the authorities of PGIVAS for providing necessary research facilities for this work.

REFERENCES

Ahmad, T., M.Q. Bilal, S. Ullah and G. Muhammad. 2005. Effect of severity of mastitis on pH and specific gravity of buffalo milk. *Pak. J. Agric. Sci*., **42**(3-4): 64-67. Available on: http://pakjas.com.pk/papers/438.pdf

Babei, H., L.M. Mansouri, M.M. Molaei, A. Kheradmand and M. Sharifan. 2007. Assessment of lactate dehydrogenase, alkaline phosphatase and aspartate amino transferase activities in cow's milk as an indicator of subclinical mastitis. *Vet. Res. Commun*., **31**(4): 419-425. DOI: 10.1007/s11259-007-3539-x

Bachaya, H.A., M.A. Raza, S. Murtaza and I.U.R. Akbar. 2011. Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan). *J. Anim. Plant Sci*., **21**(1): 16-19.

Batavani, R.A., S. Asri and H. Naebzadeh. 2007. The effect of subclinical mastitis on milk composition in dairy cows. *Iran. J. Vet. Res*., **8**(3): 205-211. Available on: https://ijvr.shirazu.ac.ir/article_925_8c286f02d8fb54b4bf530539cc1f950c.pdf

Bilal, M.O. and A. Ahmad. 2004. *Dairy Hygiene and Disease Prevention*. Usman and Bilal Printing Linkers, Faisalabad, Pakistan.

Ahmad, T., M.Q. Bilal, S. Ullah and G. Muhammad. 2005. Effect of severity of mastitis on pH and specific gravity of buffalo milk. *Pak. J. Agric. Sci.*, **42**(3-4): 64-67. Available on: http://pakjas.com.pk/papers/438.pdf

Blumberg, H.M., D.S. Stephens, C. Licitra, N. Pigott, R. Facklam, B. Swaminathan and I.K. Wachsmuth. 1992. Molecular epidemiology of Group B Streptococcal infections: Use of restriction endonuclease analysis of chromosomal DNA and DNA restriction fragment length polymorphisms of ribosomal RNA genes (ribotyping). *J. Infect. Dis*., **166**(3): 574-579. DOI: 10.1093/infdis/166.3.574

Bogin, E. and G. Ziv. 1973. Enzymes and minerals in normal and mastitic milk. *Cornell Vet*., **63**(4): 666-676.

Bogin, E., Z.V. Zager, B. Rivertz, S. Gordin and A. Saran. 1977. Distribution of lactate dehydrogenase isoenzyme in normal and inflamed bovine udder and milk. *Res. Vet. Sci*., **22**(2): 198-200.

Borghese, A. 2005. *Buffalo Production and Research*, *Reu Technical Series 67.* Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. Available on: http://www.fao.org/3/a-ah847e.pdf

Cabaud, P.G., F. Wroblewski and V. Reffiero. 1958. Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am. J. Clin. Pathol*., **30**(3): 324-326. DOI: 10.1093/ajcp/30.3.234

Chagunda, G.G.M., T. Larsen, M. Bjerring and K.L. Ingvartsen. 2006. L-lactate dehydrogenase and N-acetyl-β-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. *J. Dairy Res*., **73**(4): 431-440. DOI: 10.1017/S0022029906001956

Chakarbarti, A. 1997. *A Text Book of Preventive Veterinary Medicine*, 2nd ed. Kalyani Publishers, New Delhi, India. p. 405-423.

Chawla, R. 2006. *Practical Clinical Biochemistry: Methods and Interpretations*, 3rd ed. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, India. p. 110-114.

DADF. 2016. *Buffalo Milk Production Estimates in India during 2014-15.* Ministry of Agriculture and Farmers Welfare, Government of India, India.

Deshapriya, R.M.C., R. Rahularaj and R.M.S.B.K. Ransinghe. 2019. Mastitis, somatic cell count and milk quality: An overview. *Sri Lanka Veterinary Journal*, **66**(1): 1-12. DOI: 10.4038/slvj.v66i1.32

Durry, A.R. and G.W. Reed. 1961. A herd irritation

index using the California mastitis test. *Vet. Med*., **56**: 147-150.

Folley, S.J. 1956. *The Physiology and Biochemistry of Lactation*. Oliver and Boyd, Edinburgh, UK. p. 34-44.

Gain, S., J. Mukherjee, S. Chattopadhyay, S. Batabyal and C. Guha. 2015. *In vitro* activity of blood and milk leukocytes together with the serum enzyme profile during subclinical mastitis in cross bred cows. *Indian J. Anim. Sci*., **85**(8): 856-860.

Haggag, H.F., L.F. Harmzawi, G.A. Maharam and M.M. Ali. 1991. Physicochemical properties of colostrums and clinical and subclinical mastitic buffalo milk. *Egyptian Journal of Dairy Science*, **19**(1): 55-63.

Hamann, J. 2002. Milk quality and udder health in relation to modern milking. In recent development and perspectives in bovine medicine, p. 334-345. *In The 22nd World Buiatrics Congress*, Hannover, Germany.

Hamza, P.A. and P.C. Choudhuri. 1994. Chemoprophylactic studies on mastitis in buffaloes. *Indian J. Dairy. Sci*., **47**: 723-726.

Harmon, R.J. 1994. Physiology of mastitis and factors affecting somatic cell count. *J. Dairy Sci*., **77**(7): 2103-2112. DOI: 10.3168/jds.S0022-0302(94)77153-8

Heleili, N., A. Ayachi, M. Melizi, A.L. Kassah and B. Mamache. 2012. Prevalence of subclinical bovine mastitis and the in vitrosensitivity of bacterial isolates in Batna Governorate, East of Algeria. *Journals of Animal Science Advances*, **2**: 576-582.

Joshi, S. and S. Gokhale. 2006. Status of mastitis as an emerging disease in improved and periurban dairy farms in India. *Ann. N. Y. Acad. Sci*., **1081**: 74-83. DOI: 10.1196/annals.1373.007

Larson, T. 2005. Determination of lactate dehydrogenase (LDH) activity in milk by a fluorometric assay. *J. Dairy Sci*., **72**(2): 209-216.

Leitner, G., E. Shoshani, O. Krifucks, M. Chaffer and A. Saran. 2000. Milk leukocyte population patterns in bovine udder infection of different etiology. *J. Vet. Med. B*., **47**(8): 581-589. DOI: 10.1046/j.1439-0450.2000.00388.x

Meglia, G.E., A. Johannisson, S. Agenäs, K. Holtenius and K.P. Waller. 2001. Effects of feeding intensity during the dry period on leukocyte and lymphocyte sub-populations, neutrophil function and health in periparturient dairy cows. *Vet. J*., **169**(3): 376-384. DOI: 10.1016/j.tvjl.2004.02.003

Miller, R.H. and M.J. Paape. 1985. Relationship between milk somatic cell count and milk yield, p. 60. *In Proceeding Annual Meeting National Mastitis Council*, Kentucky, USA.

Miller, R.H., M.J. Pape., L.A. Fulton and M.M. Schutz. 1993. The relationship of milk and somatic cell count to milk yields for Holstein heifers after first calving. *J. Dairy Sci*., **76**(3): 728-733. DOI: 10.3168/jds.S0022-0302(93)77396-8

Mohamed, A.H., M.I. Dessoukey, M. Abdel Ghani and A.M. Bakeer. 1981. Contributors of alkaline phosphatase in milk of mastitic goats. *Egyptian Journal of Dairy Science*, **9**(2): 105-112.

Moroni, P., C.S. Rasso, G. Pisori, V. Bronzo, B. Castiglioni and P.J. Baettcher. 2006. Relationships between somatic cell count and inflammatory infection in buffaloes. *J.*

Dairy Sci., **89**(3): DOI: 998-1103. 10.3168/jds.S0022-0302(06)72165-8

Nguyen, D.A.D. and M.C. Neville. 1998. Tight junction regulation in the mammary gland. *J. Mammary Gland Biol*., **3**(3): 233-246. DOI: 10.1023/a:1018707309361

Olson, D.P., R.C. Bull, L.F. Woodard and K.E. Kelly.1981. Effects of maternal nutritional restriction and cold stress on young calves: Absorption of colostral immunoglobulins. *Am. J. Vet. Res*., **42**(5): 876-880.

Oz, H.H., D.J. Hillmann and R.J. Farnsworth. 1985. Bulk tank milk analysis for isolating mastogenic bacteria. *Dairy and Food Sanitation*, **5**(7): 248-251.

Pathania, V. and R. Seth. 1995. Effect of mastitis on the milk, fat globule membrane enzyme. *Indian J. Dairy Sci*., **48**(1): 249-250.

Pernoud, S., C. Fremaux, A. Sepilche, G. Corrieu and C. Monnet. 2004. Effect of the metabolism of urea on the acidifying activity of *Streptococcus thermophilus*. *J. dairy Sci*., **87**(3): 550-555. DOI: 10.3168/jds.S0022-0302(04)73196-3

Pyorala, S. 2003. Indicators of inflammation in the diagnosis of mastitis. *Vet. Res*., **34**(5): 565-578. DOI: 10.1051/vetres:2003026

Qayyum, A., J.A. Khan, R. Hussain, M. Avais, N. Ahmad and M.S. Khan. 2016. Investigation of milk and blood serum biochemical profile as an indicator of sub-clinical mastitis in Cholistani cattle. *Pak. Vet. J*., **36**(3): 275-279.

Rao, K.R.S. 1990. Milk formation-alternation in mastitis milk composition. *Indian Dairyman*, **42**(7): 314-316.

Schalm, O.W., G. Caroll and N.C. Jain. 1971. *Bovine Mastitis.* Lea and Febriger Publisher, Philadelphia, USA. p. 95-127.

Shahani, K.M. 1966. Milk enzymes: Their role and significance. *J. Dairy Sci*., **49**(8): 907-920. DOI: 10.3168/jds.S0022-0302(66)87980-8

Singh, C.V. and R.S. Barwal. 2010. Buffalo breeding research and improvement strategies in India. *Revista Veterinaria*, **21**(Suppl. 1): 1024-1031.

Snedecor, G.W. and W.G. Corchran. 1994. *Statistical Method*, 8th ed. East West Press Pvt. Ltd. New Delhi, India. p. 217-232.

Zhao, X. and P. Lacasse. 2008. Mammary tissue damage during bovine mastitis: Causes and control. *J. Anim. Sci*., **86**(Suppl. 13): 57-65. DOI: 10.2527/jas.2007-0302