ALTERATION OF ASPARTATE TRANSAMINASE (AST), ALANINE TRANSAMINASE (ALT) AND ACID PHOSPHATASE ACTIVITIES IN BUFFALO MILK RELATED TO UDDER HEALTH STATUS

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The present study was designed to correlate the aspartate transaminase (AST), alanine transaminase (ALT) and acid phosphatase activities in buffalo milk related to udder health status. A total of 60 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 these were categorized in normal, sub-clinical (1+), sub-clinical (2+), sub-clinical (3+), and clinical group containing 12 animals in each group. The AST, ALT and acid phosphatase activities were measured by spectrophotometric method. The AST, ALT and acid phosphatase activities were found proportional to the severity of the udder infection as detected by the CMT reactions.

Key words: Acid phosphatase, Alanine transaminase, Aspartate transaminase, CMT, Mastitis

The Indian sub-continent is the home tract of dairy buffaloes containing fifty eight percent of world's buffalo population (FICCI, 2013) which contribute a major share in nation's total milk production. But with day to day increase

in milk yield, there is a significant increase in "production diseases" like –mastitis.

Mastitis occurs in two forms: clinical and sub-clinical. Clinical mastitis is easily diagnosable

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in field, the incidence of which in buffaloes has been reported to be 1-10% where as in subclinical mastitis the incidence range is 5-20% (Joshi and Gokhale, 2006), and it requires laboratory examination and field tests as there are no apparent inflammatory changes in the udder. So, instances of subclinical mastitis usually go unnoticed which caused reduction in milk quality, its market value and consequently 70-80% of economic losses (Dua, 2001).

Subclinical mastitis causes considerable changes in milk and serum composition (Megalia *et al.*, 2001). Increased serum AST and ALT activities were found in buffaloes (Zaki *et al.*, 2008) and cattle (Gain *et al.*, 2015) in mastitis. The enzymes from blood leak into the milk due to an increased permeability (Pyorala, 2003). This was further reported by Andrei *et al.* (2009), regarding passage of proteins from blood to mastitic milk due to increased capillary permeability. Thus estimation of enzyme activity in the milk might serve as the most suitable probe for the diagnosis of udder infections (Zubeir *et al.*, 2005).

MATERIALS AND METHODS

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 the udder, four milk samples each of about 30 mL were collected in sterilized, clean, dry plastic bottles. Immediately each milk sample was screened

carefully by CMT (Durry and Reed, 1961) using the reagents prepared as mentioned by Chakarbarti (1997) and stored in the ice bath for further physiochemical analysis. Likewise, sixty samples were collected and categorized as normal, sub-clinical 1+, sub-clinical 2+, Sub-clinical 3+ and clinical containing 12 animals in each group.

About 15 mL of each milk sample was centrifuged at 2500 rpm for 15 minutes to get a layer of milk fat at the top leaving the fat free milk below. The upper milk fat layer was hardened and made compact keeping the centrifuge tubes in the freezer for 15 minutes (Olson *et al.*, 1981). The upper compact layer of 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 to separate the clear supernatant whey from the casein settled at the bottom. The clean whey was transferred carefully by pasture pipette into the dry clean vials for further estimation.

The spectrophotometric method of Reitman and Frankel (1957) was used to estimate the activity of AST and ALT in milk whey using the reagents prepared in the laboratory. The OD were observed on a digital spectrophotometer (E. I. model 301E) at 505 nm and a standard curve was plotted against AST equivalent units and ALT equivalent units of the standard prepared.

The acid phosphatase activity in milk whey was estimated using a digital spectrophotometer (E. I. model 301E) at 520 nm and the reagent prepared in the laboratory according to the

method described by Chawla (2006). The standard curve of Acid phosphatase units against OD observed at 520 nm was plotted.

The “t” test and mean differences were calculated as per the method described by Steel and Torrie (1960). The statistical analysis like mean, standard error, completely randomized design and regression coefficient of experimental data were carried out using the statistical procedure laid down by Snedecor and Cochran (1994).

RESULTS

The average of aspartate transaminase (AST), alanine transaminase (ALT) and acid phosphatase activities in milk whey with their standard errors for the comparison in different udder health statuses of buffaloes are presented in Table 1.

The statistical analysis of data on average AST, ALT and acid phosphatase activities in the milk whey showed a trend of significant increase ($P < 0.01$) from normal to clinical group.

DISCUSSION

The same increasing trend of milk-whey AST activity with the severity of mastitis was observed by Bogin and Ziv (1973). The results of the present work with respect to AST and ALT activity in different udder health status in buffalo milk could not be compared as the relevant report could not be traced in the literature searched. However, the increased activity of AST and ALT with the increase in the severity of mastitis was observed by Paithane *et al.* (2017) in sheep milk.

Due to larger molecular size, enzymes might be concentrated by active transport only from blood to milk, rather than penetrating passively into the milk (Folley, 1956). The secretion of the normal components along with the increased concentration of granulocytes released together with the tissue debris should reflect the severity of mastitis in bovines (Macadam, 1958). The increase in ALT activity might be due to the lysis of leukocytes and its granules, releasing variety of enzymes into the milk (Kitchen and Middleton, 1976). Further

Table 1. Mean and standard error for milk whey aspartate transaminase (U/mL), alanine transaminase and acid phosphatase activities in different udder health statuses of buffalo

Udder health status	Normal milk	Sub-clinical milk			Clinical milk
		1 ⁺	2 ⁺	3 ⁺	
AST (U/mL)	36.83 ^a ±1.6 (26.05-43.06)	55.26 ^b ±1.37 (48.00-63.06)	77.75 ^c ±1.71 (70.98-89.04)	103.16 ^d ±2.1 (93.06-116.05)	134.80 ^e ±2.42 (120.99-148.00)
ALT (U/mL)	26.57 ^a ±1.35 (19.95-33.97)	41.16 ^b ±1.33 (35.92-48.91)	64.48 ^c ±2.36 (52.03-78.00)	98.33 ^d ±2.44 (86.05-109.95)	129.47 ^e ±3.05 (116.96-151.9)
Acid phosphatase (KA U/dL)	14.75 ^a ±0.92 (11.16-21.42)	22.55 ^b ±0.74 (18.96-28.16)	29.25 ^c ±0.66 (26.22-33.82)	33.75 ^d ±0.71 (29.38-37.24)	38.39 ^e ±0.57 (34.86-40.84)

Different superscripts indicate significance ($P < 0.01$) between udder health statuses for a particular enzyme

the damage to the udder tissue due to inflammation (Bogin *et al.*, 1977) might have contributed to the increase enzyme activity in mastitic milk. The increase in the enzyme activity found in the mastitic milk samples could be primarily due to the increased neutrophils into the infected udder (Mohamed *et al.*, 1981) and the damaged condition of mammary tissue during mastitis resulting in the physiological disturbance of blood milk barrier (Chaudhary *et al.*, 1995).

The exact value of acid phosphatase activity in buffalo milk both in normal and mastitic conditions could not be compared for the lack of relative information in the recorded literature. Kitchen *et al.*, (1970) and Pathania and Seth (1995) found the increased level of acid phosphatase activity in cattle mastitic milk.

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).

Besides the bacterial infection could have

caused tissue inflammation leading to the influx of granulocytes into the area of infection (Schalm and Lasmanis, 1968). Kitchen (1976) reported that cytoplasmic granules in the polymorphonuclear leukocytes contained a wide variety of enzymes, which on lysis were released into the milk resulting in the increased acid phosphatase activity in the mastitic milk.

On the basis of statistical analysis and critical perusal of the results suggested that the activities of milk whey AST, ALT and acid phosphatase can serve as sensitive indicators for assessing the udder health status in buffaloes.

Conflict of interest: Authors declare that there is no conflict of interest regarding the present research work.

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