Research Article

Impact of Subclinical and Clinical Mastitis on ALP & AP Changes in Sheep Milk

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Abstract

The present experiment was conducted to study the prevalence of mastitis and variations in milk Alkaline phosphatase and Acid phosphatase in relation to different udder health status of sheeps. A total of 170 quarter milk samples were collected on the basis of clinical examination of udder and CMT score. Out of these samples, 14 cases were found clinically affected on the basis of clinical examination. Remaining 156 milk samples were tested by CMT, from which 38 samples were found positive for CMT. Therefore, the prevalence rate of clinical and sub-clinical mastitis was 8.23% and 22.35%, respectively. The overall prevalence of mastitis was 30.58% from the total sample size. These milk samples were grouped as normal, subclinical (1+, 2+, 3+) and clinical. Whey was separated from these milk samples for the estimation of, ALP and AP. The average values of ALP and AP activity in milk-whey differed significantly ($P<0.01$) among various udder health status. The average values of milk ALP and AP enzymes estimated in milk-whey revealed direct relationship with CMT. The present study indicated that, the prevalence of sub-clinical mastits was higher than the clinical mastits and also increases in the average values of ALP and AP in milk-whey with the increase in severity of mastitis, thus suggesting these parameters may be of sensitive indicators for the assessment of udder health status in sheep.

Keywords: ALP, AP, CMT, Sheep, Milk.

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Introduction

India ranks sixth among the countries of the world in respect to sheep population. Sheep possesses a special ability to thrive on natural grasses and except during certain physiological stages of life, they do not need any supplemental feed. In fact, there is no substitute for sheep as class of livestock for utilizing waste lands or weeds from the field. Weeds, grasses, shrubs, roots, cereals, leaves, barks and even in times of scarcity, fish and meats all these furnishes a subsistence to this animal. Sheep are economical converter of grass into meat and wool. Sheep eat more different kinds of plants than any other kind of livestock. The production of wool, meat and manure, provides three different sources of income per year [1]. Sheep milk is highly nutritious, richer in vitamins A, B, and E, calcium, phosphorus, potassium, and magnesium than cow's milk. It contains a higher proportion of short- and medium-chain fatty acids, which have recognized health benefits. According to a German researcher, sheep milk has more conjugated linoleic acid (CLA) than the milk from pigs, horses, goats, cattle, and humans. CLA is a cancer-fighting, fat-reducing fat. The fat globules in sheep milk are smaller than the fat globules in cow's milk, making sheep milk more easily digested.

World milk production of sheep is 10,122,522 tones. (Source: FAO of United Nations, 2012) Mastitis is the term for a bacterial infection of the udder. It is most common in ewes raising multiple lambs or with high milk production. Most cases occur during the first weeks after lambing or immediately before weaning. The udder health is closely related to the milk production of the animal. And ill health of the udder was ascribed to decrease the milk production up to 55 per cent in ewes [2]**.** Udder health status particularly, of ewes has received a very limited scientific attention. Health of the udder not only alters the nutritional quality but also changes the biochemical profile of milk, affecting thereby the health of consumers, both lambs and human beings [3]. Mastitis is recognized as one of the most costly disease affecting the dairy animals. Upto 10 per cent of ewes used for milk production have subclinical mastitis. Clinical mastitis in pastured ewes averages only about 2 per cent per year but mastitis causes death up to 10 per cent. The forms of loss in milk, sheep are the same as those for dairy cattle. In meat and fiber sheep, the losses take the form of deaths, due to usually gangrenous mastitis, and to decreased growth in neonates [4]. In the recent years, although attention has been given for the diagnosis of subclinical mastitis by direct test like California Mastitis Test (CMT), Chloride test, Bromocresol purple test, however, their accuracy and sensitivity vary from person to person.

Milk of normal healthy sheep contains a wide variety of enzymes. These enzymes are secreted by the epithelial cells of mammary gland. In mastitis, muscle and tissues of mammary gland are damaged which may lead to increase in the release of these enzymes in milk [5]. Thus, determination of activity of such enzymes, Alkaline Phosphatase (ALP), Acid Phosphatase (AP) might serve as a possible method for detection of subclinical mastitis and udder diseases [6].

Therefore, early detection of the disease is important to facilitate its early treatment in order to minimize further udder damage and financial losses to the farmers. Sheep milk is relished and consumed more as compared to the milk from goats. About two per cent of the total milk consumed by the human population is from sheep [7]. In Western countries sheep milk is also processed for preparation of cheese and milk products [8]. Udder health status particularly, of ewes has received a very limited scientific attention. In the recent years, although attention has been given for the diagnosis of subclinical mastitis by direct test like California Mastitis Test (CMT). In the present work an attempt has been made to investigate the enzymatic changes i.e. ALP and AP in sheep milk related to udder health status for detection of subclinical mastitis and udder diseases.

Materials and Methods

The present investigation was carried out in the Department of Veterinary Biochemistry, at the Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola. The study comprised of 170 milk samples from normal, subclinical and clinical mastitic quarters of sheep of Khamgoan district.

Collection of samples: After thorough clinical examination of udder, fore-milk samples, each of about 30 ml, were collected quarter-wise separately in sterilized, clean, dry plastic bottles in the morning. Immediately, each milk sample was screened carefully by CMT using the reagents prepared [9] and stored in the ice bath for further physiochemical analysis. Likewise fore-milk samples were collected Milk sample were collected after through clinical examination of udder. Milk samples were categorized on the basis of CMT reaction, as normal, subclinical (grade 1+, 2+, 3+) and clinical group as per method [10]. Fifteen ml of each milk sample was centrifuged at 2500 rpm for 15 to 20 minutes in a centrifuge tube. The milk fat which formed the upper layer was hardened and made compact by keeping in freezer for about 15 minutes [11]. The fat free lower portion of milk was carefully collected into another clean centrifuge tube using a pasture pipette. Whey was prepared from 10 ml of such fat free milk by mixing thoroughly with 1 ml of 1N HCl in a centrifuge tube and centrifuging at 2500 rpm for 20 minutes. The casein settled at the bottom and the clear whey formed the supernatant. This clear whey was removed carefully by pasture pipette and used immediately for estimation of enzyme activity. The activity of ALP and AP in whey was estimated by using the laboratory made reagents as per the spectrophotometric method of [12] and [13]. The optical densities were read on a double beam systronic spectrophotometer at 520 nm. Standard statistical procedures like, completely randomized design, mean, standard error and regression coefficient laid down by [14].

Results and Discussion

Alkanine phosphatase

The **Table 1** showed average ALP activity was lowest (12.13 ± 0.52 KA U/dl) in normal group and highest (38.15 ± 1.07 KA U/dl) in clinical group. The average ALP activity in milk-whey for sub-clinical 1^+, 2^+ and 3^+ were 16.24 ± 0.47, 22.76 ± 0.77 and 27.30 ± 0.71 KA U/dl, respectively (**Figure 1**). The results indicated an increase in the activity of ALP with the increase in severity of mastitis.

Table 1 Mean & S.D. for ALP & AP of milk in different udder health status of sheep

Udder health status	Normal milk	Subclinical			Clinical
		1+	2+	3+	
ALP(KA U/dl)	12.13^a ± 0.52	16.24^b ± 0.47	22.76^c ± 0.77	27.30^d ± 0.71	38.15^e ± 1.07
AP(KA U/dl)	10.48^a ± 0.38	15.33^b ± 0.42	21.05^c ± 0.72	26.87^d ± 0.72	37.15^e ± 0.78
Different superscripts indicate significance between udder health statuses					

The average activity of ALP, 12.13 ± 0.52 KA U/dl in normal sheep milk-whey was lower than 137·24±19·62 [15]. The values of ALP activity in sub-clinical and clinical groups of the present study could not be compared, as the relevant report could not be traced in the literature searched. However, studies in cattle milk-whey showed the increasing trend of ALP activity with the increase in the severity of mastitis. [16] reported that the average ALP activity in normal goat milk-whey was 11.05 ± 0.7 KA U/dl which was significantly increased in sub-clinical 1+, 2+, 3+ and clinical mastitic milk-whey with the average values of 22.73 ± 0.61, 34.0 ± 0.59, 37.34 ± 0.7 and 39.99 ± 0.5

KA U/dl, respectively. A similar increasing trend of the ALP activities found in the present investigation supported the mentioned observations.

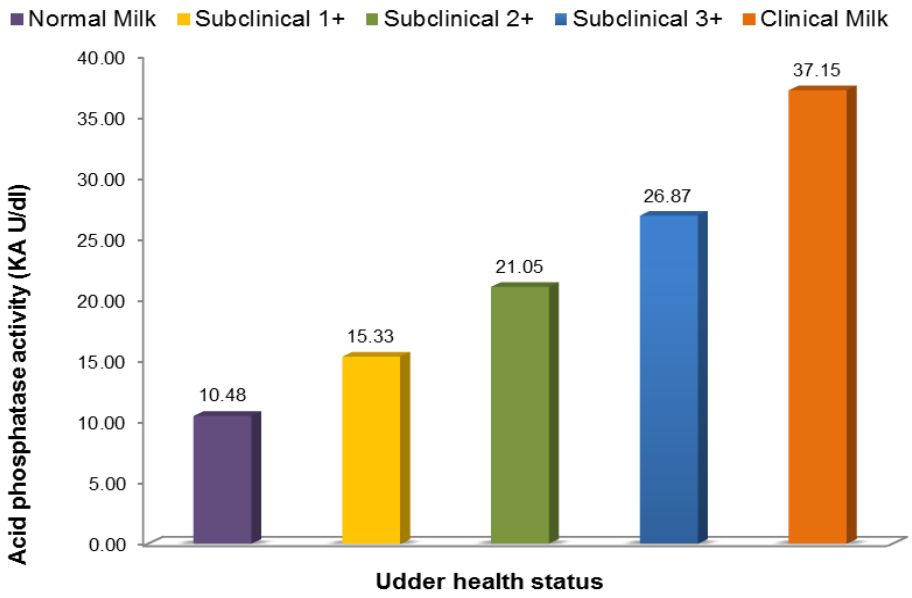

Figure 1 Comparison of whey acid phosphatase activity among various udder health status

The statistical analysis of the data for average ALP activity indicated significant (P<0.01) increase in the activity with the increase in the severity of the infection. The increased activity of ALP enzyme in udder might be due to marked aggregation of neutrophils and also due to damage to udder tissues [17]. Although ALP activity is increasing in the present study, according to [18] mentioned in the literature, that alkaline phosphatase enzyme requires an alkaline medium for its activity which is increasing in the mastitic milk, therefore its measurement can constitute an indicator for mammary gland infection i.e. mastitis. The variations in the ALP activity recorded in milk-whey could be due to the difference in method of whey separation, breed, management, method of estimation, stages of lactation, etc. However, 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

Acid phosphatase

The **Table 2** average AP activity was lowest in normal group (10.48 ± 0.38 KA U/dl). Further, the average AP activity in subclinical 1+, subclinical 2+ and sub clinical 3+ mastitic milk-whey was 15.33 ± 0.42, 21.05 ± 0.72 and 26.87 ± 0.72 KA U/dl, respectively (**Figure 2**). The average AP activity in clinical mastitic milk was highest (37.15 ± 0.78 KA U/dl). The results indicated significant increase in the AP activity with the increase in the severity of mastitis. The AP activity in normal group ranged from 8.93 - 13.34 KA U/dl, where as for sub clinical 1+, 2+, 3+ and clinically mastitic milk ranged from 13.03 – 17.82, 17.63 – 25.2, 23.2 – 30.91 and 34.18 – 42.98 KA U/dl, respectively. The statistical analysis of the data on average AP activity showed a trend of significant (P<0.01) increase from normal to clinical group. Table 2 shows significant (P<0.01) increase in ALP activity by 6.31 KA U/dl for each unit rise in CMT score.

Table 2 Regression of milk-whey Alkaline Phosphatase activity on CMT score

Milk constituent	Regression	S.E. (b)	t (b)
ALP	6.31	0.27	22.95**
**= Significant at 1 % level			

Table 3, shows significant (P<0.01) increase in the AP activity by 6.49 KA U/dl for each unit rise in CMT score. [16] and [19] in buffalo milk-whey during mastitis. Same increasing trend of AP activity is seen in the present investigation too. Although AP is having limited serum activity, in certain pathological conditions its activity could be markedly increased [11]. Thus, its high activity might serve as a possible method for detection of udder diseases and other disorders [12]. The increase in the activity of this enzyme might be due to the reason already mentioned while discussing the activity of ALP, besides the bacterial infection could have caused tissue inflammation leading to the

influx of granulocytes into the area of infection [20]. Cytoplasmic granules in the polymorphonuclear leukocytes [12] obtained a wide variety of enzymes, which on lysis were released into the milk resulting in the increased AP activity in the mastitic milk. However, he did not find AP as a reliable indicator of mastitis when applied to a random selection of individual quarter milk samples.

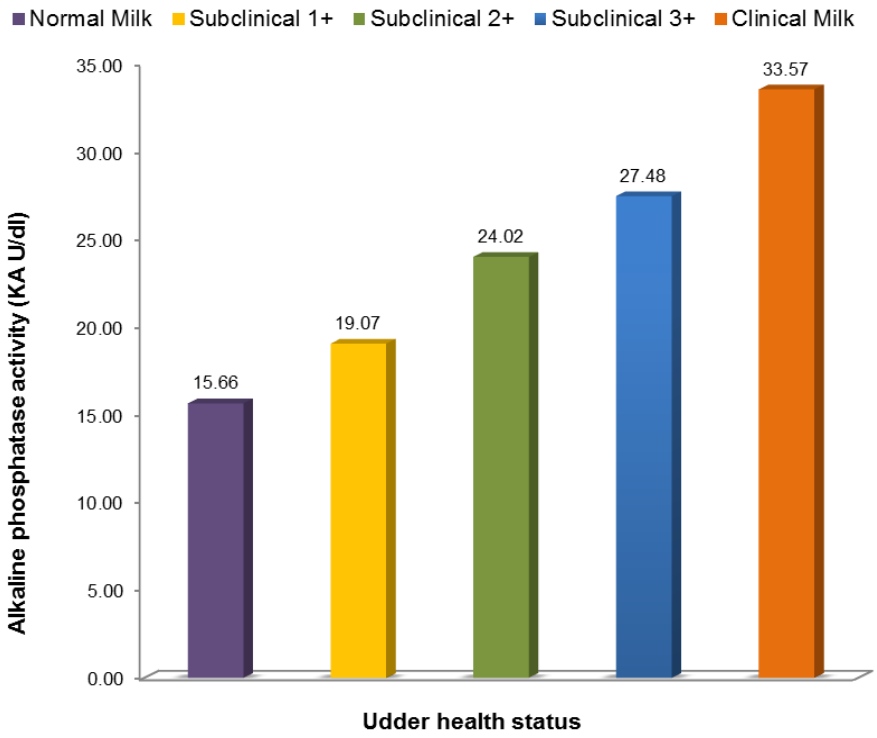

Figure 2 Comparison of whey alkaline phosphatase activity among various udder health status

Table 3 Regression of milk Acid Phosphatase on CMT score

Milk constituent	Regression	S.E. (b)	t (b)
Acid phosphatase	6.49	0.24	27.58**
**= Significant at 1 % level			

The findings of the present study indicated that, the variation in AP activity in various udder health status of sheeps could be used for detection of mastitis, as there was significant increase in activity of AP with the severity of mastitis. However, it would be too early to conclude without detailed study, that the AP would alone guarantee detection of mastitis, yet its importance in combination with other parameters can be used for detection of mastitis in sheeps.

Conclusion

The findings of the present study indicated that, the variation in AP activity in various udder health status of sheeps could be used for detection of mastitis, as there was significant increase in activity of AP with the severity of mastitis. However, it would be too early to conclude without detailed study, that the AP would alone guarantee detection of mastitis, yet its importance in combination with other parameters can be used for detection of mastitis in sheeps. The statistical analysis and interpretation of data led to conclusions that conditions of the alterations in activity of ALP & AP in milk whey are proportional to the severity of the udder infection as detected by CMT reaction.

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