

Research Article

Effect of Subclinical and Clinical Mastitis on AST & ALT Alteration in Sheep Milk

Sapana Paithane^{1*}, Mohini Khodke¹, Pravin Rathod², and Nagnath Bhojane²

¹Department of Veterinary Biochemistry, Post Graduate Institute of Veterinary & Animal Sciences Akola - 444104

²Department of Veterinary Pathology, Post Graduate Institute of Veterinary & Animal Sciences Akola - 444104

Abstract

The present experiment was conducted to study variations in milk Somatic Cell Count (SCC), pH and activity of whey enzyme Aspartate Transaminase (AST) & Alanine Transaminase (ALT) in relation to different udder health status of sheep. The average values of milk SCC, pH, AST and ALT differed significantly ($P < 0.01$) among various udder health status of sheep. The mean difference for SCC of milk showed significant ($P < 0.01$) differences varying from 2.89 to 35.45×10^5 . The average milk pH values ranged from 6.44 ± 0.006 to 7.22 ± 0.03 . Also, AST & ALT showed significant ($P < 0.01$) differences among various udder health status of sheep.

Keywords: pH, SSC, AST, ALT, Sheep, Milk

*Correspondence

Author: Sapana Paithane
Email: drmona_khodke@rediffmail.com, pravintoo@gmail.com

Introduction

Mastitis is a term which denotes inflammatory condition of the udder irrespective of cause. As such mastitis is recognized as one of the most costly disease affecting the dairy animals. 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. India ranks sixth among the countries of the world in respect to sheep population. 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 [1]. In Western countries sheep milk is also processed for preparation of cheese and milk products [2]. The udder health is closely related to the milk production of the animal. Udder of sick animals was ascribed to decrease the milk production up to 55% in ewes [3]. 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 [4]. In the recent years, although attention has been given for the diagnosis of subclinical mastitis by direct test like California Mastitis Test (CMT), 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].

In the present work an attempt has been made to investigate the enzymatic alteration i.e. AST, ALT, pH and SCC 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.

Milk sample are collected after through clinical examination of udder. For each freshly collected milk samples, the pH was measured using a digital pH meter (E.I. Model 101E). Following staining, SCC estimation done by the method in accordance with [6]. The activity of AST and ALT in whey was estimated by using the laboratory made reagents as per the spectrophotometric method of [7]. The optical 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 [8].

Table 1 Category-wise milk sample on the basis of clinical examination of udder and CMT reaction.

Group	Number of CMT negative milk sample	Number of CMT positive milk sample			Number of clinical mastitic milk samples	Total milk samples
		Normal	Subclinical (Grade)			
			+	++	+++	
No. of halves(Udder)	12	12	12	12	12	60
No. of Sheep	12	12	12	12	12	60

Results and Discussion

Estimation of somatic cell count and pH in milk along with whey AST and ALT obtained from healthy and mastitic quarters of sheep generated a sizable data, which were statistically analyzed to interpret the results.

Somatic Cell Count

The averages of SCC of milk with their standard errors for comparisons in different udder health status of sheep are presented in **Table 2**. The results indicated an increase in number of SCC of milk with the increase in severity of mastitis. The SCC is an indicator of the intensity of the cellular immune defence and it represents a marker of the sanitary state of the udder. During the course of intramammary infection, leucocytes migrate from the blood towards the mammary gland leading to increase somatic cells in the milk. SCC represents a valuable tool for prevalence assessment and screening mastitis [9]. The multiple factors (pathogens, toxins, mechanical damage to tissues) physiological factors (age, lactation stage) and pharmacological factors (different drugs) and stress factors (change in feeding, transport) and management factors were reported to affect SCC of milk [10].

Table 2 Mean and standard error for somatic cell count, pH, AST and ALT of milk in different udder health status of sheep

Udder health status	Normal milk	Subclinical			Clinical
		1+	2+	3+	
SCC ($\times 10^5$ cells/ml)	2.89 \pm 0.03	6.59 \pm 0.03	15.33 \pm 0.07	20.54 \pm 0.14	35.45 \pm 0.17
pH	6.44 \pm 0.006	6.5 \pm 0.44	6.62 \pm 0.37	6.85 \pm 0.30	7.22 \pm 0.03
AST	19.09 ^a \pm 0.60	22.93 ^b \pm 0.42	26.04 ^c \pm 0.50	30.51 ^d \pm 0.56	35.57 ^e \pm 0.60
ALT	15.66 ^a \pm 0.37	19.07 ^b \pm 0.43	24.02 ^c \pm 0.52	27.48 ^d \pm 0.53	33.57 ^e \pm 0.83

Different superscripts indicate significance between udder health statuses

pH

The averages of pH of milk with their standard errors for comparisons in different udder health status of sheep are presented in Table 2. The results indicated an increase in pH of milk with the increase in severity of mastitis. The pH values for normal group ranging between 6.4 to 6.49 with a mean of 6.44 \pm 0.006. The statistical analysis of variance of the data generated for the average pH of sheep milk indicated an increasing trend from normal to clinical mastitic groups (Table 2). Inflammation [11] of the udder may be responsible for the increased permeability of mammary gland to the components of blood, particularly bicarbonates and other alkaline salts into the milk together with the decreased production of lactose by the gland so that the milk pH became above 7.0.

Aspartate Transaminase

The average activity of AST in the whey of normal milk was lowest (19.09 \pm 0.60 U/ml) and that for sub-clinical 1⁺, 2⁺, 3⁺ groups were 22.93 \pm 0.42, 26.04 \pm 0.50 and 30.51 \pm 0.56 U/ml respectively. The average AST activity, 35.57 \pm 0.60 U/ml was highest in clinical mastitic milk whey. The results indicated a significant (P<0.01) increase in the milk-whey ASAT activity with the increase in the severity of mastitis. The average activity of ASAT, 19.09 \pm 0.60 U/ml in normal sheep milk-whey was higher than 17.32 \pm 0.46 [12]. However, studies in goat milk-whey showed the increasing trend of AST activity with the increase in the severity of mastitis. Khodke [13] reported the average

activity of ASAT in normal goat milk was 146.44 ± 4.37 U/ml which was significantly higher in sub-clinical 1+, 2+, 3+ and clinical milk with the average values of 173.19 ± 2.34 , 188.32 ± 0.89 , 195.40 ± 0.70 and 205 ± 1.29 U/ml respectively. Same trend of increasing the AST activity was observed in the present investigation. The results in the present study regarding the AST activity showed almost comparable trends for all the groups. The increased activity of AST in milk with the severity of mastitis, observed as in the present work has also been reported by [14].

Alanine Transaminase

The average activity of ALT in the normal milk whey was lowest (15.66 ± 0.37 U/ml) and that for sub-clinical 1+, 2+ and 3+ groups were 19.07 ± 0.43 , 24.02 ± 0.52 and 27.48 ± 0.53 U/ml, respectively. The average ALT activity 33.57 ± 0.83 U/ml was highest in clinical mastitis milk-whey. The results indicated that there was significant ($P < 0.01$) increase in the milk-whey ALT activity with the increase in the severity of different forms of mastitis. The statistical analysis of data on average ALT activity in milk-whey indicated a trend of significant ($P < 0.01$) increase from normal to clinical groups. However, the findings of the present work showed a trend similar reported by Khodke [13] in goat milk-whey. She reported that the average ALT activity in normal goat milk was 145.45 ± 3.07 U/ml which was significantly increased with the severity of mastitis with the mean values of 171.77 ± 3.08 , 186.79 ± 0.9 , 196.55 ± 1.01 , 206.99 ± 0.8 U/ml in sub-clinical 1+, 2+, 3+ and clinical milk-whey, respectively. Same increasing trend ALT activity can be seen in the present investigation too. The increase in ALT activity might be due to the lysis of leukocytes and its granules, releasing variety of enzymes into the milk [15]. A major source of enzymes in normal and mastitic milk was the mammary glands [16] and the increase in the enzyme activity found in the mastitic milk samples could be primarily due to the increased neutrophils into the infected udder [17]. Other than these all the factors that contributed the increase in LDH activity could also influence the increase of ALAT activity.

Conclusion

The statistical analysis and interpretation of data led to conclusions that the somatic cell count of milk can be used as reliable indicator of diagnostic importance for clinical and subclinical conditions of udder inflammation in sheep. The alterations in the somatic cell count, pH and activity of AST & ALT in milk whey are proportional to the severity of the udder infection as detected by CMT reaction.

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