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Original Article

Alteration of some enzymatic activities in whey of ewe's milk Suffered from *Staphylococcal* mastitis

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Abstract

The present experiment was conducted to study variation in milk California mastitis test(CMT) white side test(WST) and chloride test , pH test**,** along with activities of whey enzymes lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) in relation to staphylococcal mastitis in lactating ewes. A total of 310 milk samples were collected from the udder halves of 161 dairy ewes at mid period of lactation to determine the percentage of Staphylococcus mastitis. The overall percentage of infection with clinical and subclinical Staphylococcal mastitis was found to be 2.25 % and 12.22% respectively. All samples were subjected to bacteriological examination and the following staphylococcal species were isolated, *coagulase negative Staphylococcus* (1.29% & 27.8%) and *Staphylococcus aureus* (27.8% &12.22%) from clinical and subclinical mastitis respectively. The whey samples were divided into three groups: a non-infected group, subclinical infected group and clinical infected group for estimation of enzymes. Activities of LDH, ALP and AST were significantly higher in milk from the subclinical and clinical mastitis groups for S. aureus and coagulase negative *Staphylococcus*(CNS) (AST:222.09±31.54 ;194±27.15&271.82 ±30.50 ;201.0 ±49.51;ALP:837.08±63.57; 866.01±215.36& 884.22±26.08 ;807.45± 47.05LDH:332.95±5.67& 289.83±32.95;344.2 ±21.17 ;307.62± 72.77) respectively, than in non-infected group(AST: 38.84±2.71; ALP: 187.91±5.54; LDH: 142.59± 5.67).

In conclusions the results of the present study showed that the measurement of AST, LDH and ALP activities in milk samples could be used as reliable method and suitable for detection of ovine subclinical mastitis.

Keywords: Lactate dehydrogenase, Alkaline phosphatase, Subclinical mastitis, clinical mastitis

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Introduction

Mastitis namely, clinical and subclinical, is an economically damaging disease of the dairy industry, which causes physical, chemical and bacteriological alternation in the milk and blood along with morpho-pathological changes in the mammary gland (Guha*et al*.,2012).

Staphylococcus aureus is an opportunistic pathogen in dairy ruminant where it is found in healthy carriage and can be a major cause of mastitis (Seyffert *et al*., 2012). It is classified among the most serious pathogens causing clinical symptoms of various diseases not only in animals, but also in human (VASIĽ, 2007). De Santis *et al*.,(2005) found that the *S. aureus* isolates from sheep with subclinical mastitis are less enterotoxigenic (34.4%) than isolates from acute clinical mastitis (70–80%). Also the coagulase negative *Staphylococci*(CNS) are the most prevalent important pathogen which reported by most scientist (Pradiee *et al*.,2012; Gebrewahid *et al*.,2012).

Determination of enzymes activity might serve as a possible method for detection of subclinical mastitis and other udder diseases (Kitchen *et al*., 1970). It has been reported that the mean activity of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were higher in milk from subclinical mastitis (SCM) udders than in milk from health udders (Batavani, 2007).

The aim of this study was to investigate alteration of some enzymatic activities in whey of ewes milk suffered from clinical and subclinical Staphylococcal mastitis.

Materials and Methods

Ewes

One hundred sixty one lactating ewes at 2-6 years of age, from Al-Anbar province were used in this study, ewes were examined clinically to confirm infection with mastitis or apparently normal. The study was carried out over a 6 months period starting from October 2012 to March 2013.

Examination of ewes

Systemic reactions (temperature, pulse and respiratory rate) and local signs on the udder (hotness, redness and swelling) were recorded.

Collection of Samples

Three hundred ten milk samples from 161 ewes were collected aseptically, udder and teats were washed with water and then the teat end were disinfected with cotton soaked in 70% alcohol solution. The first three stripped milk were discarded and 20 ml of milk was collected. These samples transported immediately to the laboratory by cooling box then under aseptic condition (Radostits *et al*., 2007).

Examination of milk samples

Milk samples were examined for:-

1-Phyically, chemically and bacteriologically:

A- Physical Examination: Which include: Color, odor and consistency of the milk.

B-Chemical tests: Include White Side Test (Coles, 1986), California Mastitis Test (Schalmet al., 1971) and Chloride test (Coles, 1986) and pH test (Coles, 1986) performed on the normal apparent milk samples.

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C-Bacteriological examination: Isolation and identification of bacteria from milk samples were performed according to (Quinn *et al*.,2004), All milk samples were cultured on blood agar and nutrient agar, incubated at 37 Cº for 24 hrs. Diagnosis depends on morphological character (shape, color and size of colony) and type of hemolysis on sheep blood agar. Hemolytic colonies were subjected to Gram stain, then suspected isolates subculture on Staph-110 agar ,mannitol salt agar , and chrome agar(specific for *Staphylococcus aureus*) and biochemical tests(catalase, oxidase, Gelatin liquefaction, Urease, O/F test, Sugar fermentation and tube coagulase test) were used for identification of *Staphylococcus aureus* isolates.

2-Biochemical analysis (Enzymes) in whey:

Ten milliliter of milk were centrifuged in cooled centrifuge high speed to separate whey of milk, after that the AST, ALP, LDH were measured by spectrophotometer by using commercial kits (Bio-Merieux, Laboratory reagents and Products, Marcy-I' Etoile, France).

Statistical analysis

All data are represented as means ± SE. One way analysis of variance (One-way ANOVA) by using SPSS program, followed by Least Significant Difference (LSD) test were used to determine differences among means of investigated groups. The level of statistical significant was set at ($P < 0.05$) (Snedecor and Cochran,1989).

Results

Clinical mastitis

Out of 161 ewes examined physically and bactiologically for mastitis, 5 ewes (10 halves) showed clinical mastitis (acute and chronic mastitis) after physical examination. Seven samples showed clinical Staphylococcal mastitis in a percentage of (2.25%) (Table, 1).

No.	No. of examined ewes	clinical mastitis	No with *Staphylococcal* spp.	+ve results for *Staph aureus*	+ve results for CNS
Ewes	161	5	4	**2**	**2**
Milk samples	310	10	7	3	4
%		3.22%	2.25%	0.96%	1.29%

Table (1) Percentage of *Staphylococcus aureus* and CNS in clinical cases of mastitis

Chemical tests

Relation between CMT and bacteriology

The percentage of *S. aureus* was 12.22% in a +ve samples for CMT, While the percentage of Coagulase negative staphylococci (CNS) was 27.8% (Table 2).

Table (3) showed the distribution of *S.aureus* and CNS isolates at different scores of CMT {±, +1, +2, +3}. The CMT +1 and +2 had the highest percent.

No of Milk samples examined	+ve *S. aureus* from all +ve CMT	+ve S.aureus from –ve CMT	+ve CNS from all +ve for CMT	+ve for CNS from all –ve CMT
300	11	2	40	13
%	12.22%	0.95%	27.8%	6.2%

Table (2).Relation between CMT and bacteriology

CMT scores	No of samples +ve to CMT	*+ve for S.aureus*	+ve for CNS mastitis
±	21	2	9
+	30	3	10
++	38	6	21
+++	1	0	0
Total	51	11	40
		21.57%	78.43%

Table (3) Relation between CMT scores and Staphylococcal spp. isolation

Relation between White side test (WST) and Bacteriology

The percentage of *S. aureus* in relation to White side test (WST) was 2.66%. While coagulase negative (CNS) isolates give higher percentage than *S. aureus* which reach a percentage of 12.66 %(table 4).

No of Milk samples examined	Samples +ve for *Staph aureus* from all +veWST	Samples +ve *Staph aureus* and -ve for WST	Samples +ve CNS and +ve for WST	Samples +ve CNS and -ve for WST
300	8	5	38	15
%	2.66%	1.66%	12.66%	5%

Table (4) Relation between White side test (WST) and Bacteriology.

Relation of mastitis with enzymes activities

The whey samples were divided into three groups: a non-infected group, subclinical infected group and clinical infected group for estimation of biochemical analysis. Milk serum(whey) activities of LDH, ALP and AST were significantly higher in the subclinical and clinical infected group thanfrom non-infected group in both *S.aureus*and CNS at ($P<0.05$) (Table 5).
Table (6) showed the efficacy of chemical tests and enzymatic activities used for detection of subclinical *Staphylococcal* mastitis, enzymatic activities revealed a higher percentage 100% than other chemical tests for detection subclinical mastitis in relation with isolation of bacteria .

Staphylococcus spp.	**AST**	**ALP**	**LDH**
	Means +SE*	Means +SE*	**Means +SE***
Non_ infected	38.84 B 2.71±	187.91 B 5.54±	142.59 B 5.67±
Subclinical mastitis	222.09 A 31.54±	837.08 A 63.57±	332.95 A 31.82±

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S. aureus(13)			
Subclinical mastitis CNS(53)	271.82 A 30.50±	807.45 A 47.05±	344.2 A 21.17±
Clinical mastitis S. aureus(3)	194.0 A 27.15±	866.01 A 215.36±	289.83 A 32.95±
clinical mastitis CNS(4)	201.0 A 49.51±	884.22 A 26.08±	307.62 A 72.77±

The different capital letters refer significant variations at (P<0.05)

Table (5) Relation of Staphylococcus spp. mastitis with enzymes

No of bacteria isolated	Chemical tests				+ve for enzymes activities
	+ve samples for CMT	+ve samples for WST	+ve samples for Chloride test	+ve for pH test	
S. aureus 13 %	11 **84.61%**	8 **61.53%**	7 **53.84%**	8 **61.53%**	13 **100%**
CNS 38 %	25 **65.78%**	23 **60.52%**	22 **57.89%**	**25** **65.78%**	38 **100%**

Table (6) Efficacy of chemical tests and enzymes activities in relation with isolated bacteria

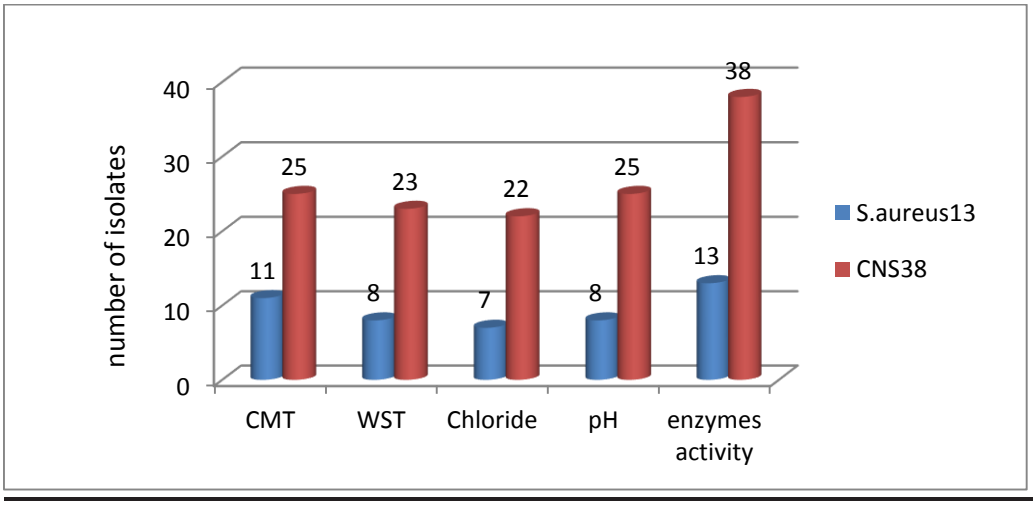

Figure (1) Chemical tests and enzymes activities in relation with isolated bacteria

Discussion

In this study, we found that the percentage of subclinical mastitis was higher than clinical mastitis. McDougall et al (2002) reported a prevalence of SCM 19.0% and a similar result obtained by Contreras et al (2007) who noticed a prevalence of SCM 5-30% in goats.

In this study CMT test showed higher prevalence rate of subclinical mastitis than other tests (WST, chloride & pH tests), California mastitis test indirectly detect increased number of leukocytes in mammary secretion there for can be considered as a good test and more accurate diagnostic technique for detection of subclinical mastitis (Schalm *et al*., 1971). CMT scores values in our result was compatible with those obtained by other authors (De la Cruz*et al*.,1994; Fthenakis,1994) and according to these studies the predictive value of positive result is mainly influenced by the prevalence of mammary infections in the flocks. Also our result revealed that scores +1 &+2 of CMT had the highest diagnostic accuracy. This result is in agreement with (Fthenakis,1994) which recorded that score +2 of CMT was appropriate threshold value for detection of subclinical mastitis.

Intramammary infections caused by *S. aureus* warrant special attention because this bacterium is responsible for both acute clinical mastitis and subclinical mastitis as recorded by (Contreras *et al*.,2007). Our results for CNS isolation agree with a result of Rahim *et al*., (2010) which found that Coagulase-negative staphylococci (CNS) were the most prevalent species. Also similar to results of (Dadkhah,2012) who found that the most prevalent species were Coagulase-negative staphylococci (CNS) (71%), followed by *Staphylococcus aureus* (12%).

Our results of bacterial isolation seem to be lower that results of other researchers,(Watson *et al*.,1990),(Tormod *et al*.,2007), (Yousif,1982), who recorded a percentage (65.3) ,(90%)(57.60%) respectively.

The enzymes (AST,ALP, LDH) are secreted by the epithelial cells of mammary gland. In mastitis, muscle, tissues of mammary gland are damaged which may lead to increase in the level of these enzymes. (Khodke *et al*.,2009). The results of the present study showed that the means of AST ,ALP & LDH activities in milks from ewes with clinical & subclinical mastitis were significantly ($P< 0.05$) higher than those from healthy normal ewes. this indicate that using of determination of enzymes activities in serum milk is a sensitive and reliable method for detection of ovine subclinical mastitis. The results is in agreement with (Batavani *et al*., 2007) who found that the increased in milk enzymes including lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase in mastitic animals might be linked with tissue damage occurring in mammary tissue. It is also in agreement with result of (Hussain *et al*., 2012) who concludes that the enzymes including lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase were significantly higher in mastitis than healthy buffaloes. (Katsoulos *et al*., 2009) conclude that the determination of LDH activity in milk serum is a sensitive and reliable method for the detection of subclinical IMI in dairy sheep and goats.

Moreover, (Fruganti *et al.*,1986) found that the increase in LDH and ALP activities were associated with clinical mastitis and to lesser extent with subclinical mastitis. In contrast, we don't agree with a study of (Yang *et al*., 2011) who found that milk AST activity was not significantly different between normal and sub clinical infected udders.

Conclusion

Alteration in enzymatic activity can be used as reliable method for detection of subclinical mastitis in dairy ewes. Early diagnosis of subclinical mastitis in dairy animals may be important in reducing production losses and enhancing prospects of recover herds in order to avoid the development of clinical mastitis.

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