

## Relationship among clinical mastitis, vitamin E and selenium in Awassi ewes

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### Abstract

The study was conducted to investigate the correlation between ovine clinical mastitis with the ovine serum concentration of selenium and vitamin E. Blood and milk samples were collected from 50 ewes with clinical mastitis and another 50 healthy animals (controls), aged 1-6 years during the period from October 2012 to March 2013 from three locations at Al-Qadisiya province; Serum selenium and vitamin E concentration were measured, and microbiological examination of milk samples were done. The percentage of clinical mastitis was found (13.15%) and the *Staphylococcus* spp. was the dominant bacteria. The percentage of gram positive bacteria was (86.75%) which included *Staphylococcus aureus* (66.17%) coagulase negative staphylococci (10.29%) and *Actinomyces pyogenes* (10.29%) from total bacterial isolates. The percentage of gram negative bacteria was (13.23%) which included *Escherichia coli* (4.41%) and *Pseudomonas aeruginosa* (8.82%) from total bacterial isolates. Estimation of vitamin E and selenium levels in serum revealed a lower levels in mastitic ewes than in healthy ewes with significant differences at ( $P<0.05$ ). Estimation of selenium and vitamin E concentration in serum revealed a lower levels in mastitic sheep in first month than in the other months with significant differences at ( $P<0.05$ ).

**Key words:** Sheep, mastitis, selenium and vitamin E.

### العلاقة بين فيتامين هـ والسلينيوم مع التهاب الضرع السريري في النعاج العواسية

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### الخلاصة

اجريت الدراسة لبحث العلاقة بين التهاب الضرع السريري في الاغنام مع تركيز السلينيوم وفيتامين هـ في مصل الدم مقارنة مع الاغنام السليمة. جمعت خمسون عينة دم من نعاج مصابة بالتهاب الضرع السريري وخمسين عينة اخرى من نعاج سليمة وبعمر 1-6 سنة ، تم قياس السلينيوم وفيتامين هـ في مصل الدم واجري العزل الجرثومي لعينات الحليب. كانت نسبة الاصابة بالتهاب الضرع السريري (13.15%) من مجموع الحيوانات المفحوصة وكانت المكورات العنقودية هي النوع الغالب اما النسبة المئوية للجراثيم الموجبة لصبغة جرام (86.75%): (*Staphylococcus aureus* (66.17%) & *Coagulase Negative Staphylococci* (10.29%) & *Actinomyces pyogenes* (10.29%) العزلات الجرثومية. انتشار الجراثيم سالبة الجرام (13.23%): (*Escherichia coli* (4.41%) & *Pseudomonas aeruginosa* (8.82%) من مجموع العزلات الجرثومية. اظهرت النتائج فرقا " معنويا" بين النعاج المصابة بالتهاب الضرع بالنسبة لمستوى السلينيوم وفيتامين هـ عند مستوى احتمالية ( $p<0.05$ ). اظهرت نتائج تقدير عنصر السلينيوم في مصل الاغنام المصابة بالتهاب الضرع وجود انخفاضاً في مستوى هذا العنصر عند الشهر الاول من فترة الرضاعة عنه في بقية الاشهر مع وجود فروقات معنوية عند مستوى احتمالية ( $P<0.05$ ). وكذلك فيتامين هـ فقد اظهر انخفاضاً في مصل الدم عند الشهر الاول عنه في بقية الاشهر مع وجود فروقات معنوية عند مستوى احتمالية ( $p<0.05$ ). بينت النتائج ان انخفاض مستوى السلينيوم وفيتامين هـ له علاقة واضحة مع زيادة نسبة حصول التهاب الضرع في الاغنام.

**الكلمات المفتاحية:** التهاب الضرع ، السلينيوم ، فيتامين هـ ، الاغنام

## Introduction

Mastitis is an inflammatory condition of the mammary gland, characterized by changes in the physical characteristics of the udder or milk (1). It can be classified into three major types: clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (2). Selenium (Se) and/or vitamin E can increase antibody production against different antigens (3) and also can improve the cellular immune response (4). Vitamin E ( $\alpha$ -tocopherol) and selenium (Se) have complementary role, as antioxidants, in the protection of cells against the damaging effects of lipid peroxides and free radicals produced during normal metabolism. The multiple functions of both nutrients, at cellular and molecular levels, extend beyond antioxidant protection, as their inclusion in the diet at concentrations above requirements is associated with variable improvements in sheep performance and immune function (5). The aim of this study was to investigate serum concentrations of vitamins E and selenium in ewes with clinical mastitis compared to healthy ewes in order to investigate any correlation between these indices and mastitis in lactating ewes.

## Materials and methods

Three hundred eighty lactating ewes (aged 1-6 years, weighing approximately 40-45 kg)

## Results

*Staphylococcus aureus* was found to be the more common organism isolated from clinical mastitis in sheep, *S. aureus* isolates were (66.17%), Coagulase - negative staphylococci was isolated from (10.29%), *Actinomyces pyogenes* isolated in a percent of (10.29%), *E. coli* was isolated from ewes with clinical mastitis at a percent of (4.41%) and *Pseudomonas aeruginosa* was isolated from (8.82%) of ewes with clinical mastitis. Blood samples which were collected from effected dairy sheep with mastitis showed lower serum selenium levels and vitamin E levels that reached ( $0.19 \pm 0.00$  ppm) and ( $1.99 \pm 0.05$  mg/L), respectively than that in ewes in control group which reached ( $0.47 \pm 0.01$  ppm) and ( $2.68 \pm 0.04$  mg/L),

from different Awassi sheep flocks in Al-Qadisiya province were followed up during the period from October 2012 to March 2013 for developing of mastitis. Blood and milk samples were collected during different lactation stages (1,2,3,4,5 and 6 months post lambing) from 50 ewes with clinical mastitis and another 50 clinically healthy animals as a controls. All milk samples were submitted into physical examination (color and consistency) as well as pH measurement, while the healthy milk samples were examined by California Mastitis Test (CMT) according to (6) in addition to physical and pH testing. Milk samples were cultured on blood agar containing 5% sheep blood and MacConky agar, incubated aerobically at 37 C° for 24-48 hrs., then suspected isolates purified on the same first media. Diagnosis of bacteria depends on the morphological character (shape, color and size of colony), Gram stain, and also cultured on slant nutrient agar for biochemical testing (7). Blood sample (10 ml) were taken from jugular vein in sterile vacutainer tubes for separation of serum. Serum Se concentration was measured by using the flameless atomic absorption method (8) and Assessment of serum vitamin E concentration by using spectrophotometer (9). Data were statistically analyzed using SPSS software (10).

respectively (Table 1). Blood samples which were collected from mastitic sheep within month after lambing gave a lower serum selenium levels that reached ( $0.13 \pm 0.01$  ppm)

**Table (1) Relationship between selenium and vitamin E levels of healthy and infected ewes by clinical mastitis**

Parameter \ Group	No. of animals	Serum Selenium (ppm) Mean $\pm$ S.E	Serum vitamin E (mg/L) Mean $\pm$ S.E
Infected	50	$0.19 \pm 0.00$ a	$1.99 \pm 0.05$ A
Control	50	$0.47 \pm 0.01$ b	$2.68 \pm 0.04$ B

Different letters means significant differences between two groups at ( $P < 0.05$ )

than that in ewes with other periods within months after lambing and a lower serum vitamin E levels which reached ( $1.58 \pm 0.15$  mg/L). Serum selenium and vitamin E levels of ewes showed significant differences at ( $P < 0.05$ ) between different lactation periods (Table 2). The present study revealed a significant difference at ( $P < 0.05$ ) in selenium and vitamin E in serum levels in ewes with clinical mastitis in comparison with healthy ewes (Table 1). In the present study, serum selenium levels in ewes with clinical mastitis were ( $0.19 \pm 0.00$  ppm) and was significantly different at ( $P < 0.05$ ) and lower than that in healthy ones ( $0.47 \pm 0.01$  ppm). In our study, serum vitamin E levels in ewes with clinical mastitis were ( $1.99 \pm 0.05$  mg/L) significantly

## Discussion

*Staphylococcus aureus* was found to be the more common organism isolated from clinical mastitis in sheep, this was in agreement with (11) who indicated that *Staphylococcus aureus* to be the major causative agent of mastitis. *S. aureus* isolates were (66.17%) and this result was in agreement with (12), as they showed that *S. aureus* had been reported as the most recovered pathogen from dairy sheep with clinical mastitis. Coagulase-negative staphylococci was isolated from (10.29%) and was considered higher than had found by (13) who isolated Coagulase-negative staphylococci (2.9%) with clinical mastitis. *Actinomyces pyogenes* isolated in a percent of (10.29%) and this was in agreement with the results of (14) who isolated *Actinomyces pyogenes* from clinical mastitis at percent of (7.6%). *E. coli* was isolated from ewes with clinical mastitis at a percent of (4.41%), this result was in agreement with (15) who found that the percent of *E. coli* was (4.5%) and higher than (14) who isolated *E. coli* from (2.94%) of ewes with clinical mastitis. *Pseudomonas aeruginosa* was isolated from (8.82%) of ewes with clinical mastitis, this result was in agreement with result of (16) who isolated *Pseudomonas aeruginosa* (9.09%) from clinical mastitis. Blood samples which were collected from effected dairy sheep with mastitis showed lower serum selenium levels and vitamin E levels

difference at ( $P < 0.05$ ) lower than that in healthy ones ( $2.68 \pm 0.04$  mg/L).

**Table (2) Relationship between selenium and vitamin E with lactation period in mastatic ewes.**

Parameter Lactation period (month)	No. of animals	Serum selenium (ppm) Mean $\pm$ S.E	Serum vitamin E (mg/L) Mean $\pm$ S.E
1st	9	0.13 $\pm$ 0.01 a	1.58 $\pm$ 0.15 a
2nd	9	0.18 $\pm$ 0.00 b	2.03 $\pm$ 0.10 b
3rd	10	0.19 $\pm$ 0.00 cb	2.01 $\pm$ 0.08 b
4th	7	0.21 $\pm$ 0.00 dc	2.14 $\pm$ 0.08 b
5th	6	0.21 $\pm$ 0.00 dc	2.10 $\pm$ 0.17 b
6th	9	0.21 $\pm$ 0.00 dc	2.15 $\pm$ 0.08 b
Total	50	0.19 $\pm$ 0.00	1.99 $\pm$ 0.05

Different small letters means significant differences between results of different groups at ( $P < 0.05$ ).

than that in ewes in control group, this result was in agreement with (17), who was reviewed numerous studies have linked low selenium to increased susceptibility of dairy cows to mastitis. Also (18) found that high serum selenium concentrations were associated with reduced rates of clinical mastitis and low bulk tank milk somatic cell counts. The results of this study were also in line with (19) who indicated a possible predisposing role of selenium deficiency in ovine mastitis. Selenium status of ewes may possibly be used to indicate animals at risk to develop clinical mastitis. Selenium deficiency has been shown to result in immunosuppression and decreased antibody production, whereas administration of selenium increases antibody production according to (20). In dairy cows, many clinical and experimental studies have been assessed the correlation of antioxidant status with mammary health and pointed a positive association between Se and/or vitamin E with mammary health (18, 21). In our study, serum vitamin E levels in ewes with clinical mastitis were lower than that in healthy ones, these results were in disagreement with (19) who reported that flocks with a reduced incidence rate of the disease had higher, but still non-significant, vitamin E concentrations compared to flocks with increased incidence. The present results indicate that increasing levels of oxidative stress markers in ewes

with clinical mastitis might have an essential role in the process of inflammation and tissue damage. Mastitic sheep within month after lambing gave a lower serum selenium levels than that in ewes with other periods within months after lambing and a lower serum vitamin E levels. This result was in agreement with (22), who showed that selenium and vitamin E administration in selenium-deficient pregnant ewes 45 days before parturition lead to reduce somatic cell counts post-lambing and there were a positive association between selenium and/or vitamin E with reduced incidence of clinical mastitis and lower numbers of somatic cell counts in dairy cow (21, 18 and 23). Deficiency of vitamin E and selenium, increase the incidence of mastitis, these

nutrients are known to be important in ensuring immune function and deficiencies have been associated with increased mastitis in dairy goats (24) and this result was in agreement with (25) who showed that concentration of  $\alpha$ -tocopherol was low during the peripartum period. Otherwise, (26) pointed  $\alpha$ -tocopherol concentrations in serum are not greatly affected by stage of lactation. Our results agreed with (27) as they reported that stage of lactation, may interfere with the immune response, the transition period is a good example, as hormonal changes and stresses lead to depression of the non-specific immune system. This observation suggests that lactation might have an important influence on the concentrations of blood selenium.

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