

Evaluation of Some Biochemical parameters in Clinically Normal lactating and Ovine Mastitis

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Summary

The aim of the present work was to determine the serum iron, total iron binding capacity (TIBC), unbound iron binding capacity (UIBC), transferrin saturation percentage (TS%) and Serum Copper in normal lactating and Ovine mastitis. The study was conducted on 50 clinically normal lactating and 50 ewes affected with *staphylococcal* mastitis (19 clinical and 31 subclinical), both groups aged 2-5 years in Salah Al-Din governorate. The blood samples were collected from jugular vein during the period October 2012 until April 2013. The separated sera were directly used for measurement of investigated biochemical tests. The results showed that the ranges and means \pm SE of normal lactating and Ovine mastitis were as follows; serum iron 29.81-39.63 $\mu\text{mol/L}$ and $33.38 \pm 0.4 \mu\text{mol/L}$, 8.76 - 34.11 $\mu\text{mol/L}$ and $20.42 \pm 0.98 \mu\text{mol/L}$ respectively, TIBC 44.7-79.89 $\mu\text{mol/L}$ and $62.03 \pm 1.65 \mu\text{mol/L}$, 43.24 - 125.72 $\mu\text{mol/L}$ and $91.24 \pm 2.49 \mu\text{mol/L}$ respectively, UIBC 14.71 - 40.27 $\mu\text{mol/L}$ and $28.57 \pm 1.31 \mu\text{mol/L}$, 13.41- 113.25 $\mu\text{mol/L}$ and $66.54 \pm 3.23 \mu\text{mol/L}$ respectively, TS% 47.92 -71.79 and 55.08 ± 1.01 , 14.1 - 65.76 and 26.73 ± 2.71 respectively, and serum copper 13.97 - 23.61 $\mu\text{mol/L}$ and $17.99 \pm 0.39 \mu\text{mol/L}$, 7.1 - 16.09 $\mu\text{mol/L}$ and $11.8 \pm 0.32 \mu\text{mol/L}$ respectively. However, significant ($P < 0.05$) differences between clinically normal and ewes affected with mastitis were recorded in all the measured parameters. The present data records reference ranges and means \pm SE of some biochemical parameters in normal lactating and Ovine mastitis with a significant differences between them.

Keywords: Serum Iron, TIBC, UIBC, TS%, Copper, Mastitis, Naemi ewes.

Introduction

Mastitis is the inflammation of mammary gland which is considered one of the important diseases affecting health and production of ewes (1). The economic loss in dairy sheep herd may be attributed to subclinical mastitis (2). However the higher prevalence of clinical cases and worldwide occurrence of subclinical mastitis leads to decrease in milk production; also, the economical importance of subclinical mastitis is especially significant in the Mediterranean countries because they are the highest sheep milk producers (3). The etiology is usually an infectious agent (4). The contagious pathogens which could spread during milking include *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Mycoplasma spp.* (5 and 6). *Staphylococcus aureus* is one of the most common cause of mastitis, the infection is usually spread at milking when the milk from

infected gland with *S. aureus* comes in contact with healthy glands (7 and 8). Blood is a complex fluid containing inorganic and organic substances of a different variety (9). Many authors studied complete blood picture and some biological constituents in sheep (10); iron was a major structural component of hemoglobin and directly required for erythropoiesis (11).

Serum iron, TIBC, TS %, UIBC and Ferritin are essential biochemical tests for the diagnosis of iron deficiency anemia (12), although a relation between anemia and staphylococcal toxins have been documented (13). However, serum iron, TIBC, UIBC, TS% and serum copper were studied in normal and infected with internal parasite sheep (14). While serum iron and Copper levels reported (15 and 16), these values were documented by (2 and 17), AL-Hadithy *et al.*(18) who have recorded Serum

iron, TIBC, UIBC, TS % and Copper of normal and anemic Awassi sheep. However, in lactating ewes there are few published articles, therefore this study was carried out on larger number in normal lactating and ewes with mastitis which have not been previously studied for measured parameters.

Materials and Methods

Blood samples were collected from the jugular vein into plain tubes of 50 normal lactating and 50 ewes infected with *S. aureus* mastitis (19 clinical and 31 subclinical) during the period from October 2012 until April 2013 both aged 2-5 years in Salah Al-Din governorate. The sheep affected with clinical staphylococcal mastitis were subdivided into acute and chronic subgroups. The blood samples were centrifuged for 5-10 minutes at 3000 rpm (19). The separated sera were used directly for measurement of iron, TIBC and copper. The serum iron and TIBC were measured according to colorimetric method by (20), serum copper which was assayed according to colorimetric method by (21). While TS and UIBC were measured according to the following formula: $TS\% = \text{serum iron} / \text{TIBC} \times 100$ and $UIBC = \text{TIBC} - \text{Serum iron}$ (22).

SAS program was used for statistical analysis. Data were subjected to Analysis of Variance (ANOVA) and significant means were compared by T-test at a level ($P < 0.05$).

Results and Discussion

Serum iron in normal lactating ewes ranged 29.81- 39.63 $\mu\text{mol/L}$ with a mean \pm SE of $33.38 \pm 0.4 \mu\text{mol/L}$, while in ewes with mastitis was 8.76 - 34.11 $\mu\text{mol/L}$ and $20.42 \pm 0.98 \mu\text{mol/L}$ with a significant ($P < 0.05$) differences between

them (Table, 1). It was $22.23 \pm 1.31 \mu\text{mol/L}$ in clinical and $19.3 \pm 1.34 \mu\text{mol/L}$ in subclinical with no significant differences between them nor between acute and chronic subgroups (Tables, 2 and 3). TIBC in normal lactating ewes was 44.7 - 79.89 $\mu\text{mol/L}$ and $62.03 \pm 1.65 \mu\text{mol/L}$, TIBC in Ovine mastitis ranged 43.24 -125.72 $\mu\text{mol/L}$ and a mean of $91.24 \pm 2.49 \mu\text{mol/L}$ with a significant increase (Table, 1). It was $88.5 \pm 2.84 \mu\text{mol/L}$ in clinical and $92.92 \pm 3.62 \mu\text{mol/L}$ in subclinical, with no significant differences between clinical, subclinical, acute and chronic subgroups (Tables 2 and 3).

In normal lactating and Ovine mastitis UIBC was 14.71 - 40.27 $\mu\text{mol/L}$ and $28.57 \pm 1.31 \mu\text{mol/L}$, 13.41 - 113.25 $\mu\text{mol/L}$ and $66.54 \pm 3.23 \mu\text{mol/L}$ respectively with significant differences between them (Table, 1). It was $65.23 \pm 3.6 \mu\text{mol/L}$ in clinical and 63.08 ± 5.71 in subclinical with no significant differences between different groups (Tables, 2 and 3). TS% in normal lactating ewes was 47.92 - 71.79% and $55.08 \pm 1.01\%$, on other hand TS% in Ovine mastitis was 7.17- 69.7 % and $24.9 \pm 2.11\%$ with a significant difference between them (Table, 1). It was $26.73 \pm 2.71 \%$ in clinical and $23.78 \pm 2.99 \%$ in subclinical with no significant differences between them nor between acute and chronic (Tables, 2 and 3). Serum Copper in normal lactating ewes was 13.97- 23.61 $\mu\text{mol/L}$ and $17.99 \pm 0.39 \mu\text{mol/L}$, and in Ovine mastitis it ranged 7.1- 16.09 $\mu\text{mol/L}$ and $11.8 \pm 0.32 \mu\text{mol/L}$ with significant differences between them (Table, 1). It was $12.6 \pm 0.37 \mu\text{mol/L}$ in clinical and $11.31 \pm 0.45 \mu\text{mol/L}$ in subclinical with no significant differences between clinical, subclinical, acute and chronic subgroups (Tables, 2 and 3).

Table, 1: Serum Iron, TIBC, UIBC, TS % and Copper in normal lactating and Ovine mastitis (Ranges and Means \pm SE)

Groups	Parameters				
	Iron $\mu\text{mol/L}$	TIBC $\mu\text{mol/L}$	UIBC $\mu\text{mol/L}$	TS%	Copper $\mu\text{mol/L}$
Normal lactating ewes n = 50	29.81-39.63 33.38 ± 0.4 a	44.7-79.89 62.03 ± 1.65 b	14.71-40.27 28.57 ± 1.31 b	47.92-71.79 55.08 ± 1.01 a	13.97-23.61 17.99 ± 0.39 a
Ewes with Mastitis n =50	8.76-34.11 20.42 ± 0.98 b	43.24-125.72 91.24 ± 2.49 a	13.41-113.25 66.54 ± 3.23 a	7.17-69.7 24.9 ± 2.11 b	7.1-16.09 11.8 ± 0.32 b

Different small letter (a, b) vertically means significantly different at ($P < 0.05$).

Table, 2: Serum Iron, TIBC, UIBC, Ts % and Copper in clinical and subclinical mastitis (Ranges and Means \pm SE)

Groups	Parameters				
	Iron $\mu\text{mol/L}$	TIBC $\mu\text{mol/L}$	UIBC $\mu\text{mol/L}$	TS%	Copper $\mu\text{mol/L}$
Clinical mastitis n=19	12.96-27.25 22.23 \pm 1.31 a	46.14-106.56 88.5 \pm 2.84 a	15.8-83.18 65.23 \pm 3.6 a	14.1-65.76 26.73 \pm 2.71 a	9.25-15.5 12.6 \pm 0.37 a
Subclinical mastitis n=31	8.76-34.07 19.3 \pm 1.34 a	43.24-125.72 92.92 \pm 3.62 a	13.41-113.25 73.03 \pm 4.69 a	7.17-69.7 23.78 \pm 2.99 a	7.1-16.09 11.31 \pm 0.45 a

Different small letter (a, b) vertically means significantly different at ($P < 0.05$).

Table, 3: Serum Iron, TIBC, UIBC, Ts % and Copper in acute and chronic mastitis subgroups (Means \pm SE)

Groups	Parameters				
	Iron $\mu\text{mol/L}$	TIBC $\mu\text{mol/L}$	UIBC $\mu\text{mol/L}$	TS%	Copper $\mu\text{mol/L}$
Acute mastitis n=11	23.09 \pm 1.73 a	87.97 \pm 4.82 a	63.08 \pm 5.71 a	28.81 \pm 4.25 a	13.09 \pm 0.48 a
Chronic mastitis n=8	21.12 \pm 1.73 a	89.24 \pm 1.86 a	68.18 \pm 3.58 a	23.87 \pm 2.68 a	11.92 \pm 0.53 a

Different small letter (a, b) vertically means significantly different at ($P < 0.05$).

In normal lactating ewes of the present study the means of serum iron, TIBC and Ts % increased, serum UIBC very close to this, while serum copper was higher in comparison with (14). While the range of serum iron was almost similar to that reported by (2 and 17). However, Kargin et al (23) reported a significant increase in serum copper compared to that of this work. The lower limit in serum copper range of this study was higher than that reported by (17 and 24), while the upper limit of the present range was similar to that reported by (24) and slightly lower than that recorded by (17). Serum iron TIBC, Ts% and copper revealed a significant increase in normal lactating ewes of the present study, while there was reported a significant decrease in UIBC (25). The difference in serum iron, TIBC, UIBC, TS% and copper of this study compared to other researchers may be attributed to one or more of the followings: absence of scientific feeding program, type of feed, physiologic status, living in hot areas or genetic factors (18). The trace elements are essential for health and are important in functioning of various components of the immune system, their deficiency reduce disease resistance and increase the susceptibility, the concentration of trace elements change during different infections or inflammations, which reflect changes in action binding of plasma proteins, and alterations in cellular uptake mechanisms (26 and 27). Kushner (28) reported

a decrease in plasma iron and Copper concentrations during the acute phase in response to immunological challenges. The results of present work are in agreement with findings by (26-28). Moreover, high TIBC in ovine mastitis confirm the findings by (17) of high or normal level in low serum iron, although, infection by bacteria can cause bone marrow suppression, resulting in thrombocytopenia and anemia (29 and 30). Also, anemia possibly due to the staphylococcus aureus hemolysins which cause lysis of red blood cells by damaging their cell membrane (13). However, one or more of the observations by (13, 17, 26-30) may explain the differences between values of Ovine mastitis and normal ewes in the present study. In conclusion the present work records reference ranges and means \pm SE of studied biochemical parameters in normal lactating ewes. Furthermore, the results reveal significant differences between normal lactating and ewes affected with mastitis in all measured parameters, while there is no significant difference between clinical and subclinical nor between acute and chronic mastitis.

References

1. Saratsis, P.; Leontides, L.; Tzora, A. and Fthenakis, G. C. (1998). Incidence risk and etiology of mammary abnormalities in dry ewes

- in 10 flocks in Southern Greece. *Prev. Vet. Med.*, 37:173-183.
2. Radostits, O. M.; Gay, C. C.; Hinchclift, K.W. and Constable, P. D. (2007). *Veterinary Medicine*, 10th edition. London, W.B. Saunders Company Limited. PP: 453-454, 1711, 1717, 2043-2050.
 3. Las Heras, A.; Dominguez, L. and Fernandez, J. F. (1999). Prevalence and aetiology of subclinical mastitis in dairy ewes of the Madrid region. *Small Rum. Res.*, 32: 21-29.
 4. Seifu, E. and Tafesse, B. (2010). Prevalence and etiology of mastitis in traditionally managed camels (*Camelus dromedarius*) in selected pastoral areas in eastern Ethiopia. *Ethiopian Vet. J.*, 14(2):103-113.
 5. Zhao, X. and Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: Causes and control. *J. Anim. Sci.*, 86: 57-65.
 6. Oliver, S. P. and Murinda, S. E. (2012). Antimicrobial resistance of mastitis pathogens. *Vet. Clin. North Am. Food An. Pract.*, 28: 165-185.
 7. Roberson, J. R.; Fox, L. K.; Hancock, D. D.; Gay, J. M. and Besser, T. E. (1994). Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. *J. Dairy Sci.*, 77(11): 3354-3364.
 8. Schukken, Y. H.; Gonzalez, R. N.; Tikofsky, L. L.; Schulte, H. F.; Santisteban, C. G.; Welcome, F. L.; Bennett, G. J.; Zurakowski, M. J. and Zadoks, R. N. (2009). CNS mastitis: Nothing to worry about? *Vet. Microbi.*, 134(1-2): 9-14.
 9. Stewart, M. (1991). *Animal Physiology*. Publ. The Open University, U.S.A., Pp: 132-133.
 10. Jawasreh, K.; Awawdeh, F.; Bani Ismail, Z.; Al-Rawashdeh, O. and Al-Majali, A. (2010). Normal Hematology and Selected Serum Biochemical Values in Different Genetic Lines of Awassi Ewes in Jordan. *The Internet J. Vet. Med.*, 7: 2 online.
 11. Mullallya, A. M.; Vogelsangb, G. B. and Moliterno, A. R. (2004). Wasted sheep and premature infants: the role of trace metals in hematopoiesis. *Blood Rev.*, 18: 227-234.
 12. Munoz, M.; Garcia- Erce, J. A. and Remacha, A. F. (2011). Disorders of iron metabolism. Part II: iron deficiency and iron overload. *J. Clin. Pathol.*, 64: 287-296.
 13. Stipcevic, T.; Piljac, T. and Isseroff, R. R. (2005). Di-rhamnolipid from *Pseudomonas aeruginosa* display differential effect on human keratinocyte and fibroblast culture. *J. Dermatol. Sci.*, 40(2):141-143.
 14. Kozat, S.; Yuksek, N.; Goz, Y. and Keles, I. (2006). Serum Iron, Total Iron-Binding Capacity, Unbound Iron-Binding Capacity, Transferrin Saturation, Serum Copper, and Hematological Parameters in Pregnant Akkaraman Ewes Infected with Gastro-Intestinal Parasites. *Turk. J. Vet. Anim. Sci.*, 30: 601-604.
 15. Abdelrahman, M. M.; Abo-Shehada, M. N. and Mukbel, R. M. (2006). Effect of Stage of Gestation on the Accumulation of Copper, Manganese, Zinc, Iron and Calcium in Fetal Tissue of Awassi Ewes in Northern Jordan. *Jordan J. Agric. Sci.*, 2(2): 38-54.
 16. Abdelrahman, M. M. and Aljumaah, R. S. (2012). Metabolic Blood Profile and Milk Compositions of Peri -Parturient and Early Lactation periods in Sheep. *Asian J. Anim. Vet. Adv.*, 7(11):1130-1138.
 17. Kaneko, J. J. (2008). "Veterinary Clinical Biochemistry of Domestic Animal". 6th ed. Elsevier Inc. Pp: 883-88.
 18. AL-Hadithy, H. A. and AL- Badawi, N. M. (2012). Evaluation of Specific Biochemical Values in Clinically Normal and Anemic Awassi Sheep. *International J. Sci. Nature*, 3(3): 688-691.
 19. Coles, E. H. (1986). *Veterinary Clinical Pathology* 4th ed. W.B. Saunder, Philadelphia: Pp: 11-41, 114-121.
 20. Tietz, N. W. (2006). *Fundamentals of Clinical Chemistry*, Saunder, Philadelphia 4th Edit., Pp: 984.
 21. Abe, A.; Yamashita, S. and Noma, A. (1989). Sensitive, direct colorimetric assay for copper in serum. *Clinic. Chem.* 35(4): 552-554.
 22. Weiss, D. J. and Wardrop, K. J. (2010). *Schalms Veterinary Hematology*. 6th ed. Wiley-Blackwell-USA. Pp: 168-170, 593-595, 1162-1163.

23. Kargin, F.; Seyrek, K.; Bildik, A. and Aypak, S. (2004). Determination of the levels of Zinc, Copper, Calcium, Phosphorus and Magnesium of Chios Ewes in the Aydin Region. Turk. J. Vet. Anim. Sci., 28: 609-612.
24. Aitken, I. D. (2007). Diseases of Sheep. 4th ed. Blackwell Garsington Road, Oxford. Pp: 602-603.
25. AL-Badawi, N. M. and AL-Hadithy, H. A. (2012). Diagnosis of Iron Deficiency Anemia In Awassi Sheep. M.Sc. Thesis, College of Veterinary Medicine, University of Baghdad, Iraq.
26. Andrieu, S. (2008). Is there a role for organic trace element supplements in transition cow health? Vet. J., 176 :77-83
27. Terpiłowska, S. and Siwicki, A. K. (2011). The role of selected microelements: selenium, zinc, chromium and iron in immune system. Centr. Eur. J. Immunol., 36 (4): 303-307.
28. Kushner, I. (1982). The phenomenon of the acute phase response. Annals of the New York Academy of Sci., 389: 39 – 48.
29. Cole, J. L.; Marzec, U. M.; Gunthel, C. J.; Karpatkin, S.; Worford, L.; Sundell, I. B.; Lennox, J. L.; Nichol, J. L. and Harker, L. A. (1998). Ineffective platelet production in thrombocytopenic human immunodeficiency virus-infected patients. Blood, 9: 3239- 3246.
30. Kulkosky, J.; Laptev, A.; Shetty, S.; Srinivasan, A.; BouHamdan, M. Prockop, D. J. and Pomerantz, R.J. (1999). Human Immunodeficiency Virus Type 1 Vpr alters bone marrow cell function. Blood, 93:1906-1915.

تقييم بعض المعايير الكيموحيوية في النعاج الطبيعية والمصابة بالتهاب الضرع

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الخلاصة

اجريت الدراسة لتقدير تركيز حديد المصل ، القدرة الكلية للحديد المرتبط، القدرة للحديد غير المرتبط ، نسبة تشبع للحديد الناقل ونحاس المصل في النعاج الحلوب الطبيعية والمصابة بالتهاب الضرع . اجريت الدراسة على 50 نعجة مرضعة طبيعية و 50 نعجة مصابة بالتهاب الضرع (19 حالة حادة و 31 تحت السريري) ببيكتريا المكورات العنقودية الذهبية في محافظة صلاح الدين. جمعت عينات الدم من الوريد الوداجي خلال الفترة بين تشرين الاول 2012 و نيسان 2013. استعمل المصل مباشرة لقياس الصورة الكيموحيوية. اشارت النتائج الى ان المدى والمعدل والخطأ القياسي للنعاج الطبيعية والمصابة بالتهاب الضرع كما يلي: حديد المصل 29.81 – 39.63 مايكرو مول / لتر و 33.38 ± 0.4 مايكرو مول / و 8.76 – 34.11 مايكرو مول / لتر و 20.42 ± 0.98 مايكرو مول / لتر على التوالي ، القدرة الكلية للحديد المرتبط 44.7 – 79.89 مايكرو مول / لتر و 62.03 ± 1.65 مايكرو مول / لتر و 43.24 – 125.72 مايكرو مول / لتر و 91.24 ± 2.94 مايكرو مول / لتر على التوالي و القدرة للحديد غير المرتبط ، 14.71 – 40.27 مايكرو مول / لتر و 28.57 ± 1.31 مايكرو مول / لتر و 13.41 – 113.25 مايكرو مول / لتر و 66.54 ± 3.23 مايكرو مول / لتر على التوالي، نسبة تشبع الحديد الناقل 47.92 – 71.79 % و 55.08 ± 1.01 % و 14.1 – 65.76 % و 26.73 ± 2.7 % على التوالي ونحاس المصل 13.97 – 23.61 مايكرو مول / لتر و 17.99 ± 0.39 مايكرو مول / لتر و 7.1 – 16.09 مايكرو مول / لتر ، و 11.8 ± 0.32 مايكرو مول / لتر على التوالي . اظهرت النتائج فروقات معنوية (P < 0.05) بين كل من النعاج الطبيعية والنعاج المصابة بالتهاب الضرع. تستنتج الدراسة وجود فروق معنوية بين النعاج الطبيعية والمصابة بالتهاب الضرع في المدى والمعدل لبعض المعايير الكيموحيوية.

الكلمات المفتاحية : حديد المصل، القدرة الكلية للحديد المرتبط، القدرة للحديد غير المرتبط، نسبة تشبع الحديد الناقل، نحاس المصل، التهاب الضرع، النعاج النعمية.