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THE ADDING EFFECT OF VITAMIN E-SELENIUM ON SUBCLINICAL MASTITIS TREATMENT IN BUFFALOES

(With 8 Tables)

By

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التأثير الإضافي لفيتامين هـ – سيلينيوم في علاج التهاب الضرع دون الإكلينيكي في الجاموس

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أجريت هذه الدراسة على عدد 30 رأس من الجاموس ببعض المزارع الخاصة بمحافظة الشرقية. تم تقسيم تلك الحيوانات إلى ثلاث مجموعات تتكون كل مجموعة من عدد 10 رأس جاموس. المجموعة الأولى سليمة ظاهريا وإكلينيكيا ولم تعالج وتستخدم كضابط للتجربة. المجموعتين الثانية والثالثة مصابة بالتهاب الضرع دون الاكلينيكى. تم علاج المجموعة الثانية بالانروفلوكساسين لمدة 5 أيام، كما تم علاج المجموعة الثالثة باستخدام الانرو فلوكساسين بالإضافة إلى فيتامين هـ والسيلينيوم لمدة 5 أيام. تم تجميع عينات من اللبن والدم من كل الحيوانات قبل العلاج و بعد 3 ، 8 و 15 يوم من بداية العلاج. أظهرت النتائج أن نسبة الإصابة بالتهاب الضرع دون الإكلينيكي تصل إلى 44,89% وبالفحص البكتيري لعينات اللبن تم تحديد المسببات البكتيرية لالتهاب الضرع وكانت كالاتى: الميكروب القولوني بنسبة 36.36% وميكروب العنقود الذهبي 20.45% والميكروب السبحي 11.36% ميكروب السيدوموناس 4.54%. كما تم عزل الميكروب العنقودي مختلطا مع القولوني بنسبة 18.18% والسبحي مختلطا مع القولوني بنسبة 9%. وتم إجراء اختبار الحساسية للمعزولات البكتيرية فكانت أكثر حساسية لمركب الانروفلوكساسين. بينت نتائج الدراسة أن التهاب الضرع دون الاكلينيكى كان له العديد من التغيرات السلبية والتي تمثلت في زيادة معنوية في عدد الخلايا الجسدية ونقص نشاط الالتهام ومعيار الالتهام في اللبن. كما سجلت العديد من التغيرات في صورة الدم للحيوانات المصابة قبل العلاج مثل حدوث الأنيميا والتي كانت مصاحبة لزيادة عدد كرات الدم البيضاء الكلى والخلايا المتعادلة وكذلك سرعة الترسيب مع نقص معنوي في الخلايا الحمضية والليمفاوية. وبفحص مصل الدم وجد نقص معنوي في معدلات الكالسيوم والبوتاسيوم مع زيادة معنوية في مستوى الكورتيزول ، حمض السياليك الكلى والمرتبط بالدهون وذلك في الحيوانات المصابة مقارنة بالمجموعة الضابطة. كما

أوضحت نتائج التجربة أن العلاج بالانروفلوكساسين بالإضافة إلى فيتامين هـ والسيلينيوم في المجموعة الثالثة قد احدث نقصا معنويا في عدد الخلايا الجسدية وزاد من نشاط الالتهام ومعدل الالتهام في اللبن مقارنة بالمجموعة الثانية والتي عولجت بالانروفلوكساسين فقط. كما لوحظ أن صورة الدم والقياسات البيوكيميائية ودلالات الالتهاب قد عادت إلى معدلاتها الطبيعية في وقت اقل وكان التحسن اكثر وضوحا وذلك عند استخدام فيتامين هـ والسيلينيوم مع الانروفلوكساسين.

SUMMARY

Subclinical mastitis is one of the most important diseases affecting farm animals causing economic losses. So, the present study was designed to determine the effect of vitamin E and selenium treatment on subclinical mastitis and to find the relationship between clinicobiochemical changes in mastitis in subclinical mastitic buffaloes. The present study was carried out on thirty lactating buffaloes, aged between 4-6 years, belonging to some private dairy farms in Sharkia governorate. The animals were divided into 3 equal groups. Group I: consisting of 10 healthy animals. Group II and III, ten buffaloes in each, were positive for intra mammary mastitis infection. Group II buffaloes treated only with Enrofloxacin for five days, while those of group III received combined treatment with Enrofloxacin and Vitamin E plus selenium for five days by intramuscular route. Milk and blood samples were collected just before treatment, 3, 8 and 15 day post treatment. The prevalence of subclinical mastitis in examined animals was 44.89 % of examined quarter milk samples. The bacteriological examination of milk samples revealed isolation of 4 types of bacteria: *E coli* (36.36%), *Staphylococcus aureus* (20.45%), *Streptococcus dysagalactiae* (11.36%) and *Pseudomonas aerugenosa* (4.54%). The mixed infections were *Staphylococus aureus* with *E. coli* (18.18%), *Streptococcus dysagalactia* with *E. coli* (9.09%). The results of this study showed that phagocytic activity and phagocytic index in milk were significantly lower in mastitic buffaloes before treatment compared with healthy ones. The blood picture revealed that, buffaloes suffering from subclinical mastitis showed anemia associated with significant increase in total leukocytic count with neutrophilia, lymphopenia and eosinopenia in addition to highly significant increase in E.S.R. Concerning the serum mineral profiles, of mastitic buffaloes before treatment, there was no significant difference in serum concentration of sodium between mastitic and control buffaloes. However, there was a significant decrease in calcium and potassium level in mastitic animals. The results also, showed that cortisol, total sialic acid (TSA) and lipid bound sialic acid (LBSA) were

significantly higher in subclinical mastitis group compared with control ($P<0.001$). Protein bound sialic acid did not change in subclinical mastitis in comparison with control group. In the current study, our data indicated reduction of SCC, enhancement cellular defense mechanism of the diseased mammary gland in animals of group III treated with Enrofloxacin and Vitamin E plus selenium as compared to the Enrofloxacin treatment alone. Most of studied hematological, biochemical and inflammatory markers returned to their normal range by the end of the experimental period. However, the cure rates of animals in group III was faster and more pronounced than group II. Hence Vitamin E plus selenium therapy may be added along with the antibiotics for effective amelioration of intramammary infection in buffaloes.

Key words: *Subclinical mastitis, Vitamin E-Selenium, Buffaloes.*

INTRODUCTION

Mastitis is the most important disease of dairy animals, despite of improved management practices and dry cow therapy, mastitis remains worldwide problem of major economic importance to the dairy farmers (National Mastitis Council 2005).

The subclinical mastitis is defined as a diseased characterized by the presence of a significantly increased somatic cell counts in milk from affected glands and it can only be diagnosed with more sensible methods (Philopot, 1986).

Inflammatory reaction accompanying mastitis is generally caused by intra mammary bacterial infection. Severe inflammation damages the mammary secretory epithelium and subsequently reduced milk production. The phagocytic cells release many cytotoxic radicals and pro-inflammatory cytokines during inflammation, these reactive molecules attributes to lipid peroxidation, genotoxicity and inhibition of cellular metabolic pathways (Kannapen *et al.*, 1999).

Antibiotics cannot prevent the inflammatory reaction driven by the host leukocyte, while the antioxidants which are the intracellular defense mechanism against oxidation, can reduce mammary cell damage during acute inflammation. Many workers have studied the protective role of antioxidants in inflammatory conditions. Vitamin E and selenium enhances mammary immunity in dairy cattle and low plasma level of vitamin E and glutathione peroxidase (GSH-Px) activity have been observed in bovine mastitis (Hogan *et al.*, 1993).

Inflammatory mediators released by the inflammatory cells also reduce the GSH-Px activity, which is a regulator in controlling free radicals. Dietary supplementation of higher levels of vitamin E and selenium enhances the GSH-Px activity and reduces the severity and duration of mastitis (Hemmingway, 1999).

So, the present study was designed to determined the effect of vitamin E and selenium supplementation on subclinical mastitis treatment and to find the relationship between clinicopathological and biochemical changes and mastitis in subclinical mastitic buffaloes.

MATERIALS and METHODS

1- Animals:

49 lactating buffaloes, aged between 4-6 years, belonging to three private dairy farms at Sharkia governorate, were monitored and sampled for subclinical mastitis. Only 98 milk samples were collected from these animals at a rate of one sample from rear quarters and another one from front quarters from each animal. On the basis of somatic cell count (SCC) < 0.5 million/ml of milk with milk sample negative for pathogenic microorganism, 10 healthy animals were selected and served as control (Group I). While Groups II and III, ten buffaloes in each, were positive for subclinical mastitis, screened on the basis of SCC > 2 million cells/ ml milk and positive for intra mammary infection. Group II buffaloes received only antibiotic treatment (Enrofloxacine 5 mg/kg B.w)* for five days by intramuscular route., while those of group III received combined treatment with antibiotic and vitamin E plus selenium at rate of (vitamin E 1.5 mg/kg B.w – Selenium 0.022 mg/kg B.w)** for five days by intramuscular route.

2- Samples:

(A) Milk samples:

The samples were collected (200ml) from each examined buffalo in sterile vials after cleaning the teat orifice with 70% ethanol and after discarding few streams of milk. The milk samples were collected on day 0, 3, 8 and 15 from the beginning of treatment from all groups.

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*Enrofloxacin solution (Baytril) ®: from Bayer Company containing 10% of the active substance in aqueous solution for parenteral administration.

**Viteselens 15®: manufactured by Egypt Company for Chemical and Pharmatheutical (ADWIA).

1- Determination of somatic cell count of milk samples: it was done as the method of Dang *et al.* (2007). SCC of each milk sample was determined within 2 hours post-collection. The milk was heated to 40^{o}C in a water bath held for 15 minutes at that temperature before being cooled to 20^{o}C with careful stirring. 0.01ml of milk was spread on a 1cm^{2} (0.5 x 2cm) area of a degreased microscopic slide and was dried in a horizontal position, then staining milk smear with Giemsa stain. The SCC were measured under a magnification of 400 X in 50 fields and average number of cells per field was multiplied by the microscopic factor (0.882).

2- Determination of phagocytic activity and phagocytic index of isolated milk PMNs: by the method described by Fox *et al.* (1987). Standard strain of *Staphylococcus aureus* was used, eighteen hours culture was oposonised with pooled bovine serum in incubator for one hour. Equal volume (500µl) of PMNs, which isolated as the method of Daley *et al.* (1991), and 500µl opsonised bacterial suspension was incubated at 37oC for half an hour, keeping PMNs and bacteria in 1: 5 ratio. Thereafter, 500µl of Acridine orange stain was added in it, and centrifuged 13000rpm to get a cell pellet. Finally, 500µl crystal violet was added and cetntrifuged as above and pellet was resuspended in cold sterile phosphate buffer saline (500µl) and wet mount seen under ultraviolet source with excitation filter of 530nm. Phagocytic activity expressed by the percentage of neutrophil that had phagocytosed (1–6) bacteria and phagocytic index expressed as average number of the bacteria per leukocyte counted in 100 cells.

3- Isolation and identification of pathogens: was carried out as described by Cruickshank *et al.* (1975). Milk samples were activated by incubation aerobically at 37^{o}C for 24 hrs, then centrifuged at 3000rpm for 20 minutes. The cream and supernatant fluid were discarded. A loop full from the sediment was streaked onto the surface of blood agar, nutrient agar, MacConkey's agar and incubated at 37^{o}C for 24-48 hours and examined for bacteriological growth. Purified isolates were identified morphologically and biochemically.

4- Antibiotic sensitivity test: using several types of antibiotics by disc diffusion method described by Cruickshank *et al.* (1975).

(B) Blood samples:

Two blood samples were collected from the jugular vein of each animal just before treatment and after 3, 8 and 15 day post treatment. The first one was taken on heparin for hematological examination. The second blood sample was left to clot at room temperature for about 2 hours, stored overnight in a refrigerator at $4^{o}C$ and centrifuged at 3000 rpm for 15 min. Serum samples were drawn in dry clean capped tubes and kept in deep freeze at $-20^{o}C$ for the biochemical analysis.

1- Hematological studies:

The erythrocytic count (RBCs) hemoglobin concentration (Hb %) and packed cell volume (PCV) were determined. The erythrocytic indices {mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)} were calculated. Moreover, the total leukocytic and differential counts were conducted in addition to determination of erythrocytic sedimentation rate E.S.R (Jain, 1986).

2- Biochemical analysis:

*Serum total protein and albumin were measured spectrophotometrically according to Peters, (1968) and Webster (1974) respectively. Serum calcium was estimated according to Tietz (1970) while sodium and potassium were measured by flame photometer according to Bauer (1982).

*Serum cortisol was determined by radio-immunoassay technique according to the method of Kowalaski and Paul (1976) using IMMULITE 2000.

*Serum total sialic acid concentration (TSA) was determined by method described by Sydow (1985). Lipid-bound sialic acid (LBSA) concentration was determined by the method described by Katopodis and Stock (1980). Protein bound sialic acid (PBSA) concentration was measured by subtracting serum total sialic acid from lipid bound sialic acid.

3- Statistical analysis:

The data obtained from this investigation wee statistically analyzed by Student's t-test according to Snedecor and Cochran (1994).

RESULTS

Table 1: Prevalence of subclinical mastitis in examined quarter milk samples.

Types of samples	Number of samples	Positive samples for bacteriology	
		Number	Percentage
Quarter buffaloes milk samples	98	44	44.89

Table 2: The distribution of isolated bacteria from examined quarter milk samples

Single infection			Mixed infection		
Bacterial isolates	Number	%	Bacterial isolates	Number	%
E. coli	16	36.36	*Staphylococcus aureus* and *E. coli*	8	18.18
Staphylococcus aureus	9	20.45			
Streptococcus dysagalactiae	5	11.36	*Streptococcus dysagalactae and E. coli*	4	9.09
Pseudomoneus aerugenosa	2	4.54			

Table 3: Results of antibiotic sensitivity tests for isolated bacteria

Antibiotic discs	*Staph. aureus*	*E. coli*	*Strept. dysagal.*	*Pseudo. aeurog.*
Enerofloxacine 10µg	+ + +	+ +	+ + +	+ + +
Gentamicin 10µg	+ + +	+ + +	+ + +	+ +
Ceftifure 30µg	+ +	+ +	+ +	-
Danofloxacin 5µg	+ + +	+ +	+ +	+ +
Amoxicillin 30µg	+	+	+ +	+
Spectinomycin 100µg	+ +	+ +	+	+ + +
Cloxacillin 25µg	+	+	+ +	+

(-) Resistant, (+) less sensitive, (++) moderate sensitivity, (+++) highly sensitive.

Table 4: Somatic cell count (1 X 10^5 cell / ml), Phagocytic activity and phagocytic index (Mean values ± S.E) of healthy control and subclinical mastitic buffaloes before and after treatment with either Enrofloxacin alone (group II) or Enrofloxacin + Vitamin E & selenium (group III).

Groups / Parameters	Healthy control buffaloes Group I	Subclinical mastitic buffaloes before treatment	Subclinical mastitic buffaloes on 3rd day post treatment		Subclinical mastitic buffaloes on 8th day post treatment		Subclinical mastitic buffaloes on 15th day post treatment	
			Group II	Group III	Group II	Group III	Group II	Group III
SCC 1 X 10^5 cell/ml	3.82± 0.85	29.61***±1.41	16.44***±1.21	13.45**±1.94	9.11**±1.07	6.55±1.05	7.4*±1.14	4.29±0.97
Phagocytic activity	26.71±1.6	17.25**±1.55	19.22*±2.13	22.14±1.3	20.16* ± 1.5	22.9 ± 1.18	21.7± 1.6	23.2 ±1.13
Phagocytic index	3.21±0.35	1.96*±0.26	2.07*±0.29	2.22±0.41	2.12* ± 0.23	2.51 ± 0.32	2.31±0.24	2.86 ± 0.33

* Significant at P < 0.05 ** Significant at P < 0.01

Table 5: Erythrogram (Mean values ± S.E) of healthy control and subclinical mastitic buffaloes before and after treatment with either Enrofloxacin alone (group II) or Enrofloxacin + Vitamin E & selenium (group III).

Groups / Parameters	Healthy control buffaloes Group I	Subclinical mastitic buffaloes before treatment	Subclinical mastitic buffaloes on 3rd day post treatment		Subclinical mastitic buffaloes on 8th day post treatment		Subclinical mastitic buffaloes on 15th day post treatment	
			Group II	Group III	Group II	Group III	Group II	Group III
RBC_S 10^6/UL	8.1 ± 0.4	6.33**± 0.35	6.48* ± 0.5	6.78 ± 0.6	6.62 ± 0.6	7.12 ± 0.4	7.23 ± 0.4	7.54± 0.6
Hb gm/dl	9.2 ± 0.33	7.3**± 0.43	7.46*± 0.5	7.58*± 0.4	7.55* ± 0.6	7.96 ± 0.6	7.88 ± 0.7	8.3 ± 0.51
PCV %	31.0 ± 1.22	26.0* ± 0.62	28.0 ± 1.13	28.5 ± 1.14	29.0 ± 1.16	29.0 ± 1.24	29.5 ± 1.43	30.0 ± 1.42
MCV FL	38.3 ± 3.8	41.0 ± 3.9	43.2 ± 3.6	42.0 ± 2.9	43.8 ± 4.1	40.7 ± 2.9	40.8 ± 2.5	39.8 ± 2.4
MCH Pg%	11.4 ± 0.85	11.53 ± 0.68	11.51 ± 0.9	11.18 ± 0.76	11.4 ± 0.9	11.18 ± 0.8	10.9 ± 0.6	11.01 ± 0.7
MCHC %	29.7 ± 1.7	28.08 ± 2.12	26.6 ± 2.16	26.59 ± 1.9	26.03 ± 1.86	27.45 ± 1.5	26.7 ± 2.4	27.7 ± 1.75

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table 6: Leukogram and E.S.R (Mean values ± S.E) of healthy control and subclinical mastitic buffaloes before and after treatment with either Enrofloxacin alone (group II) or Enrofloxacin + Vitamin E & selenium (group III)

Groups / Parameters	Healthy control buffaloes Group I	Subclinical mastitic buffaloes before treatment	Subclinical mastitic buffaloes on 3rd day post treatment		Subclinical mastitic buffaloes on 8th day post treatment		Subclinical mastitic buffaloes on 15th day post treatment	
			Group II	Group III	Group II	Group III	Group II	Group III
TLC 10^3/UL	10.24± 0.52	13.2**± 0.43	12.6*± 0.7	12.0* ± 0.58	11.86 ± 0.84	11.48 ± 0.82	11.23 ± 0.62	10.88 ± 0.53
Neutrophil 10^3/UL	6.2 ± 0.41	9.91**± 0.62	9.3* ± 0.72	8.43* ± 0.66	8.47* ± 0.61	7.67 ± 0.58	7.79 ± 0.61	6.97 ± 0.54
Lymphocyte 10^3/UL	2.9 ± 0.22	2.2* ± 0.18	2.3* ± 0.16	2.5 ± 0.19	2.38 ± 0.18	2.7 ± 0.19	2.41 ± 0.17	2.8 ± 0.18
Eosinophil 10^3/UL	0.6 ± 0.03	0.4**± 0.04	0.44* ± 0.05	0.51 ± 0.05	0.46 ± 0.06	0.54 ± 0.04	0.47 ± 0.05	0.56 ± 0.03
Monocytes 10^3/UL	0.54 ± 0.06	0.69 ± 0.07	0.56 ± 0.05	0.56 ± 0.04	0.55 ± 0.06	0.57 ± 0.05	0.56 ± 0.03	0.55 ± 0.04
E.S.R mm/2hrs	1.02 ± 0.063	1.76***± 0.05	1.33** ± 0.041	1.26** ± 0.03	1.31**± 0.024	1.19* ± 0.04	1.28** ± 0.035	1.11 ± 0.036

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table7: Some biochemical parameters (Mean values ± S.E) of healthy control and subclinically mastitic buffaloes before and after treatment with either Enrofloxacin alone (group II) or Enrofloxacin + Vitamin E & selenium (group III)

Groups / Parameters	Healthy control buffaloes Group I	Subclinical mastitic buffaloes before treatment	Subclinical mastitic buffaloes on 3rd day post treatment		Subclinical mastitic buffaloes on 8th day post treatment		Subclinical mastitic buffaloes on 15th day post treatment	
			Group II	Group III	Group II	Group III	Group II	Group III
Total protein (gm/dl)	7.93 ± 0.66	7.34 ± 0.4	7.43 ± 0.42	7.48 ± 0.37	7.41 ± 0.39	7.6 ± 0.72	7.37 ± 0.63	7.77 ± 0.41
Albumin (gm/dl)	4.7 ± 0.21	3.62**± 0.26	3.79* ± 0.24	3.82* ± 0.30	3.8* ± 0.33	3.93 ± 0.41	3.83 ± 0.41	4.11 ± 0.33
Total globulin (gm/dl)	3.23 ± 0.26	3.72 ± 0.24	3.64 ± 0.31	3.66 ± 0.28	3.61 ± 0.29	3.67 ± 0.32	3.54 ± 0.31	3.66 ± 0.41
Calcium (mg/dl)	8.8 ± 0.6	7.1*± 0.4	7.3 ± 0.6	7.32 ± 0.8	7.4± 0.8	7.5 ± 0.6	7.7 ± 0.8	8.1 ± 0.5
Sodium (mEq/l)	118.9 ± 4.1	126 ± 5.6	127 ± 4.6	124 ± 3.5	125 ± 3.9	122 ± 2.4	124 ± 5.1	121 ± 3.6
Potassium (mEq/l)	6.84± 0.32	5.25* ± 0.5	5.61* ± 0.4	5.63 ± 0.6	5.6* ± 0.5	5.81 ± 0.4	5.8 ± 0. 3	6.1 ± 0.3

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table 8: Cortisol and sialic acid levels (Mean values ± S.E) of healthy control and subclinical mastitic buffaloes before and after treatment with either Enrofloxacin alone (group II) or Enrofloxacin + Vitamin E & selenium (group III).

Groups / Parameters	Healthy control buffaloes Group I	Subclinical mastitic buffaloes before treatment	Subclinical mastitic buffaloes on 3rd day post treatment		Subclinical mastitic buffaloes on 8th day post treatment		Subclinical mastitic buffaloes on 15th day post treatment	
			Group II	Group III	Group II	Group III	Group II	Group III
Cortisol mg/dl	0.86± 0.12	1.33*± 0.11	1.29*± 0.09	1.27* ± 0.11	1.02 ± 0.08	0.98 ± 0.09	0.99± 0.08	0.91 ± 0.04
TSA Mmol/L	2.46 ± 0.03	2.65*± 0.05	2.62* ± 0.05	2.60* ± 0.04	2.6* ± 0.05	2.57 ± 0.06	2.56 ± 0.04	2.53 ± 0.05
LBSA Mmol/L	1.16± 0.02	1.33** ± 0.03	1.30** ± 0.03	1.29* ± 0.04	1.28* ± 0.03	1.26 ± 0.04	1.25 ± 0.04	1.22 ± 0.03
PBSA Mmol/L	1.30 ± 0.02	1.32± 0.01	1.32 ± 0.02	1.31 ± 0.03	1.32 ± 0.03	1.31 ± 0.02	1.31 ± 0.02	1.31 ± 0.03

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

DISSCUSION

Mastitis is an inflammatory disease which if not prevented or treated in the early phase, leads to permanent damage to mammary tissue. The importance of subclinical mastitis is well documented. In bovine herds, the prevalence of subclinical mastitis is 25-30 times higher than clinical mastitis (Dodd, 1983).

Table 1 revealed the prevalence of subclinical mastitis in examined animals which was 44.89 % of examined quarter milk samples. This finding is almost identical to that reported by Selim and Alganzoury (2006).

The bacteriological examination of milk samples Table 2, revealed the isolation of 4 types of bacteria: *E coli* (36.36 %), Staphylococcus aureus (20.45%), Streptococcus dysagalactiae (11.36%) and Pseudomonas aerugenosa (4.54 %) each as a single infection. These results were nearly similar to that recorded by Abd-El-Khalek and El-Sherbini (2005). In this study, the mixed infections were *Staphylococus aureus* with *E. coli* (18.18 %), *Streptococcus dysagalactia* with *E. coli* (9.09%). These results agreed with that obtained by Afifi and Moustafa (1991).

The results of antibiotic sensitivity tests of the isolated bacteria were shown in Table 3, where Enrofloxacin, Gentamycine and Danofloxacine were the most effective antimicrobial agents against the most isolated strains, while, Spectinomycin in the second degree of efficiency. Similar finding were reported by Abd-El-Khalek and El-Sherbini (2005).

The somatic cell count is an important marker of intra mammary infection, where heavy influx of leukocyte into the infected mammary gland was observed during mastitis (Paape *et al.* (1979). The changes in SCC in the three groups are shown in Table 4. The SCC of the milk isolated from healthy control buffaloes was < 0.4 million cells/ml. Before treatment, the SCC was significantly higher in milk of sunclinical mastitic buffaloes than in milk from healthy ones ($P > 0.01$). The SCC decreased significantly in group II which received Enrofloxacin treatment on day 3,8 and15 post treatment but still higher than that of healthy control buffaloes, group (I), while the SCC in group III which treated with combined therapy of Enrofloxacin and vitamin E and selenium, improved on day 8 and 15 and revealed non significant change as compared with control ones. This result is in agreement with that of Heinrichs *et al.* (2009) who found that dietary supplementation

of selenium and vitamin E in great amount than are required for nutritional adequacy can have complementary functions in reducing somatic cell counts and both the severity and duration of clinical mastitis

In this study, both phagocytic activity and index were studied Table 4, where they were significantly lower in subclinical mastitic buffaloes compared with healthy control ones ($P < 0.05$). The phagocytic activity and index were significantly increased in group III which treated with combined therapy of Enrofloxacin and vitamin E & selenium. This result is similar with that of Smith *et al.* (1997) who reported that, the polymorphonuclear neutrophil (PMNs) is a major defense mechanism against infection in bovine mammary gland and vitamin E with selenium play vital roles in protecting leukocytes and macrophages during phagocytosis, where they help these cells to survive the toxic product that are produced when ingested bacteria are killed. This result is also in agreement to that of Bourne *et al.* (2008) who reported that, dietary supplementation with vitamin E and selenium resulted in a more rapid neutrophil influx into milk following intra mammary bacterial challenge and increased intracellular killing of ingested bactera by neutrophil.

Vitamin E functions as a chain-breaking antioxidant, neutralizing free radicals and preventing oxidation of lipids within membranes. Vitamin E serves as the first line of defense against peroxidation of phospholipids. Selenium as part of glutathione peroxidase is the second line of defense as the enzyme destroys peroxides and hydroperoxides. It has been observed that vitamin E-selenium supplementation during the lactation period, reduced the intra-mammary infection by 42.2% and the obtained milk had lower SCC by 25% than corresponding values of control ones (Kruze *et al.*, 2007). Also, Ndiveni and Finch, (1996) observed increased phagocytosis and production of superoxide after treatment with vitamin E and selenium.

Prognostic diagnosis of subclinical mastitis have commonly been done on the hematological and biochemical examinations of the blood. Biochemical analysis of subclinical mastitic animals may help in diagnosis of subchemical abnormalities and become a helpful means for practice under field conditions (Rose, 1987).

The present study revealed that, buffaloes suffering from subclinical mastitis showed significant decrease in total erythrocytic counts, hemoglobin concentration, packed cell volume Table 5 associated with significant increase in total leukocytic count (TLC) with

neutrophilia, lymphopenia and eosinopenia in addition to highly significant increase in E.S.R Table 6. These hematological changes were attributed to stress condition. Similar findings were reported by Soaad, (2007) and Mona *et al.* (2010).

The occurring of anemia in sub clinically mastitic buffalos before treatment may be attributed to the increased reactive oxygen species especiallyH_2O_2 which resulted in accumulation of hydrogen peroxide and caused oxidation of the sulfhydryl groups of the globulin chains. The erythrocyte cell membrane may be damaged resulting in the removal of the erythrocyte from circulation (Robbins and Kumar, 1994).

TLC appeared to be more sensitive indicator of inflammatory conditions of buffaloes. An increase in neutrophil numbers usually occurs during inflammatory conditions and is generally responsible for the increased TLC. Lymphocyte numbers did not increase in the majority of the conditions in sick buffaloes as corticosteroids released during stress cause lysis of lymphocytes (Jain, 1986).

The ESR of the buffaloes appeared to be the most sensitive indicator of an inflammatory reaction. ESR is a non-specific parameter indicating the presence of an abnormal process in the body and is interpreted in a similar way to an abnormal TLC (Jain, 1986).

The results obtained from Table 7 indicated that serum albumin values revealed significant decrease in subclinical mastitic buffaloes compared with the healthy control group. This decrease in serum albumin may be attributed to its infiltration of albumin from the blood to milk due to increase permeability of blood vessels as a consequence of inflammation (Jain, 1986).

Concerning the serum mineral profiles, there is no significant difference in serum concentration of sodium between subclinical mastitic and control buffaloes. However, there is a significant decrease in calcium and potassium level in subclinical mastitic animals Table 7. These results are agreed with that previously recorded by Soaad (2007) and Mona *et al.* (2010).

However, animals that experience subclinical hypocalcemia have lower than the normal range of calcium in the blood stream, but usually do not exhibit any observable clinical signs of hypocalcemia. More recent research has revealed that calcium is also an integral part of the immune system and is involved in intracellular signaling factors of white blood cells (Kimura *et al.,* 2006). It has been called the "second messenger" of the immune system, and the white blood cells of animals

with hypocalcemia have a reduced ability to respond to invading pathogens.

Cortisol is a highly immunosuppressive agent, released from the animals' adrenal glands whenever they undergo stress. In the present study, the subclinical mastitic buffaloes before treatment revealed higher levels of cortisol compared to the control Table 8.

It is interesting to note that animals with subclinical hypocalcemia have blood cortisol levels higher than animals that are normal (Horst and Jorgensen, 1982). These higher levels of blood cortisol result in a more severe level of immune-suppression, making the animal much more susceptible to infectious diseases including mastitis.

Sialic acids are one of the most important molecules of life, since they occupy the terminal position on macromolecules and cell membranes and are involved in many biological and pathological phenomena. The majority of sialic acids are found in either protein (PBSA) or lipid-bounded (LBSA) forms, while little amount is in the free forms. In addition, sialic acid is localized at the end chain of many acute phase proteins. The detection of sialic acid may be a valuable indicator for diagnosis and prognosis of inflammatory diseases (Haq *et al.* 1993).

The results showed that TSA and LBSA were significantly higher in subclinical mastitis group compared with control ($P<0.001$). Protein bound sialic acid did not change in subclinical mastitis in comparison with control group Table 8. Acute phase reactants influence total sialic acid concentrations due to their glycoprotein structure. The increase level of sialic acid may alter receptor-ligand interactions, which are known to play an important role in inflammation and immune response (Karagenc *et al.,* 2005). On the other hand, increase of TSA and LBSA during inflammation and tissue damage is attributed to liberation of sialic acid from cell membrane into circulation as sialic acid is abundantly present in all biological membranes (Nazifi *et al.*, 2011).

The parenteral administration of vitamin E and Selenium in sub-clinically mastitic buffaloes appears to have a positive effect on all studied hematological and biochemical parameters of blood samples. The combination of vitamin E and selenium with the antibiotic treatment was superior to the treatment with antibiotic alone.

Different studies carried out in dairy herds have shown that vitamin E and Selenium supplementation can reduce both the incidence of clinical mastitis and the duration of symptoms of this disease in addition to Improving the ability of the animal to mount an effective

immune response against an invading pathogen (Hemmingway, 1999; Moyo *et al.,* 2005). They reported that vitamin E supplementation was on average associated with a 14% reduction in the risk of intra-mammary infection and a 30% decrease in the risk of occurrence of clinical mastitis.

In the current study, our data indicated reduction of SCC, enhancement cellular defense mechanism of the diseased mammary gland in animals of group III which receive combined treatment of antibiotic with vitamin E and selenium. Moreover, most of studied hematological, biochemical and inflammatory markers returned to their normal range by the end of the experimental period. However, the cure rates of animals in group III was faster and more pronounced than group II.

Hence, we recommended the administration of Vitamin E plus Selenium with the antibiotic treatment regime of subclinical mastitis in buffaloes for better clinical response.

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