Haemato-Biochemical Studies On Parakeratosis And Alopecia In Sheep With a Trial Of Treatment

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ABSTRACT

Fifteen male and female sheep (8-10 month old and 20-25kg .Bwt.) were divided into 3 equal groups (Gps.1-3) to elucidate the possible effect of parakeratosis and alopecia in some haemato-biochemical parameters , Moreover, the concurrently used mineral mixture for treatment of the affected sheep .Gp 1 contained 5 clinically healthy sheep which were free of internal and external parasite, Gp 2 showed different degrees of massive loss of wool (alopecia) without visible skin lesions, beside diarrhea and easily detached wool and Gp 3 showed focal and diffuse thickening of the skin (parakeratosis) with alopecic areas. Skin scrapings were taken from the alopetic areas mixed with 10% potassium hydroxide and examined microscopically for mites. Gp 2 treated with a mineral mixture (2 kgm/ ton ration)and daily zinc oxide ointment on the skin for 20 consecutive day, Gp 3 was treated with a mineral mixture (2 kgm/ton ration) and copper sulphate at a dose of 1gm /10 liter drinking water for 20 consecutive day. Two blood samples were collected from each sheep on the 5^{th} and 15^{th} days post treatment (PT). The first sample was collected on di sodium EDTA as anticoagulant in a tub for hematological examination. The second one was collected in centrifuge tube for obtain clear serum biochemical analysis.

The alopecia and parakeratosis were associated with a significant decrease in the total erythrocytic count, hemoglobin level and packed cell volume beside the level of calcium , inorganic phosphorus, sodium, potassium , copper, iron , zinc, total protein albumin and globulin. The total leukocytic count insignificantly increased when compared with gp 1. The liver function enzymes (AST–ALT-alkaline phosphates) and kidney function parameters (urea and creatinine) were significantly elevated in gps 2 & 3.

The signs, lesion and the abnormal biochemical alterations completely disappeared after treatment.

It could be concluded that the parakeratosis and alopecia are associated with reversible alterations in the blood picture and chemistry as they regained the normal values, 15day PT.

INTRODUCTION

Sheep are of a great economic importance to Egypt as they are a good source of meat, milk, wool and hides *(1)*.

Trace elements had long been known to be of great importance in animal nutrition as they play a major role in metabolism and enzyme functions *(2)*. They play an important role in both nutritional and productive performance of farm animals *(3)*. Alopecia is associated with adverse hematological and biochemical blood changes due to impaired of nutrition which affected the liver function and general health condition of the affects animal *(4)*. Alopecia and parakerarotsis are usually caused by deficiencies of micro and macro-elements *(5)*. Moreover, Some trace elements have an important role in the immunocompetence as they are components of the enzymes which control the immune response *(6)*. Natural deficiency of a single trace element rarely occurs in domestic animals, however a combination of mineral deficiencies is more common *(7)*. Zinc deficiency is usually associated with alopecia and parakeratosiss. Additional signs included growth retardation, swelling of the coronet, of hock and knee joints, rough coat and congested conjunctivitis *(8)*.

The objective of the present study was to study alopecia and parakeratosis in sheep and

elucidate the associated hematological and serum biochemical variations ,Moreover chemotherapeutic trials were evaluated.

MATERIAL AND METHODS

Drug

A-Amcofos: It is a trade name of mineral mixture produced by Amicomed Company for Veterinary Pharmaceutical preparation, Egypt. each 1 liter contains:

Phosphorus 235gm Magnesium diacid phosphate 1.8gm

Sodium diacid phosphate 45.2gm Zinc diacid phosphate 10.2gm.

Copper diacid phosphate 2.5 gm Cobalt diacid phosphate 0.1gm.

Calcium diacid phosphate 10.3 gm Purfied water up to 1 liter.

Animals

The present investigation was carried out on 15 sheep of both sexes (8-10 months old) and about 20-25 kg body weight. They were divided into 3 equal groups(gps1-3). Gp (1) was healthy and free from internal and external parasite, Gp (2) showed different degrees of massive loss of hair (alopecia) easily detached wool and diarrhea, Gp (3) showed focal and diffuse thickening and scalling of skin with alopecic areas. The sheep were obtained from a private farms in Sharkia Province.

Experimental design

Gp (2was daily treated with a mineral mixture 2 kgm/ton ration and 10% zinc oxide ointment on skin for 20 consecutive day, Gp (3) treated with a mineral mixture 2 kgm ton ration and copper sulphate in a dose of 1gm/10 liter drinking water for 2 consuctive day.

Table 1. Experimental design

Groups	Sheep No	Sheep status and treatment	Treatment and dose
Group1	5	healthy sheep free from internal and external parasite	----
Group2	5	sheep suffering alopecia and treated with mineral mixture	mineral mixture 2 kgm ton ration & 10% zinc oxide ointment on skin daily for 20 consuctive day
Group3	5	Sheep suffering parakeratosis and treated with mineral mixture	mineral mixture 2 kgm ton ration & copper sulphate(1gm /10 liter) drinking water for 20 consecutive day

Skin scraping samples

Skin scarping was collected from alopecic and parakeratotic area . It was used for detection of dermatophytes and metazoan parasites *(9)*.

Blood and serum samples

Two blood samples were collected from the jujular vien of the healthy and diseased sheep before treatment and at 5 and 15 day post treatment (PT).

The 1st sample was collected in a tube containing EDTA disodium as anticoagulant for erythrogram and total leukocytic count *(10)*.The 2nd sample was collected in clean, dry centrifuge tube and left at room temperature then centrifuged at 3000 r.p.m. for 5 minutes to separate clear serum for estimation of serum calcium *(11)*, inorganic phosphorus **(12)**, sodium and potassium *(13)*, copper *(14)*, iron *(15)*, zinc *(16)*, transaminases (AST&ALT) *(17)*, alkaline phosphatase *(18)*, total protein *(19)*, albumin *(20)* and globulin (calculated as difference between total protein and albumin). Moreover the serum urea *(21)*, creatinine *(22)* were determined.

Statistical analysis

The obtained data were tabulated and statistically analyzed *(23).*

RESULTS

The affected sheep with alopecia and parakeratosis were emaciated with pale mucous membranes. They showed massive loss of wool, which was easily detached without visible skin lesions. The wool loss was seen on the dorsal aspects of the thoracic and lumber regions beside face, neck and limbs. The parakeratosis was associated with focal and diffuse thickening of skin with alopecic areas.

Table 2 showed a significant decrease in the total erythrocytic count, haemoglobin level and packed cell volume meanwhile total leukocytic count insignificantly increased in alopecic and parakeratotic

The macro (calciam, inorganic phosphorus, sodium and potassium) and micro (copper, iron and zinc) element were significantly decreased in the alopecic and parakeratotic sheep when compared with the control ones (Tables 3 & 4)

Table 5 showed that alopecic and parakeratotic sheep presented a significant decrease in the serum total protein, albumin and globulin.

Table 6 revealed significant increase in liver enzymes (AST-ALT-alkaline phosphatase) in the alopecic and parakeratotic sheep.

Table 2. Erythrogram and total leukocytic count of healthy, alopecic and Parakeratotic sheep.

Parameter	Healthy lambs (n= 5)	Diseased lambs					
		Alopecic lamb			Parakeratotic lamb		
		Pre Treatment (n= 5)	Post treatment (day)		Pre Treatment (n= 5)	Post treatment (day)	
			5	15		5	15
R.B. Cs. (10^6/c.mm)	9.93± 0.37	5.02± 0.39**	7.08± 0.84*	8.9± 0.39	6.07± 0.41**	7.84± 0.62*	9.35± 0.71
H.B (g m %)	13.04± 0.81	10.18± 0.49**	10.94± 0.81*	12.46± 0.96	10.12± 0.82**	11.94± 0.73*	12.72± 0.83
P. C.V. (%)	39.05± 1.42	33.13± 0.98*	36.09± 0.82*	38.02± 0.85	32.28± 1.17**	35.07± 0.72*	38.37± 01.89
WB.CS. (10^3/cm.m)	10.36± 1.71	10.87± 1.07	10.91± 1.29	10.84± 1.59	10.16± 1.93	10.09± 1.37	10.58± 1.38

*significant at $P < 0.05$ * * significant at $P < 0.001$

Table 3. Some serum macro elements of healthy , alopecic and Parakeratotic sheep.

Parameter	Healthy lambs (n= 5)	Diseased lambs					
		Alopecic lamb			Parakeratotic lamb		
		Pre Treatment (n= 5)	Post treatment (day)		Pre Treatment (n= 5)	Post treatment (day)	
			5	15		5	15
Calcium (mg/dl)	7.41± 0.57	4.28± 0.83**	6.48± 0.68*	7.19± 0.89	5.94± 0.28*	6.59± 0.72*	7.11± 0.84
Inorganic Ph.(mg/dl)	5.02± 0.49	3.27± 0.51**	4.16± 0.32**	5.1± 0.59	3.77± 0.35*	4.20± 0.48*	4.93± 0.93
Sodium (mEq/L)	131.13± 3.69	96.26± 5.93**	107.12± 6.19*	126.48± 9.27	130.16± 6.28*	104.51± 5.11*	127.82± 713.41
Potassium (mEq/L)	5.04± 0.32	3.38± 0.18*	3.94± 0.50*	4.85± 0.92	3.15± 0.48*	4.17± 0.46*	4.89± 0.52

*significant at $P < 0.05$ * * significant at $P < 0.001$

Table 4. Some serum micro elements of healthy , alopecic and Parakeratotic sheep

Parameter	Healthy lambs (n= 5)	Diseased lambs					
		Alopecic lamb			Parakeratotic lamb		
		Pre Treatment (n= 5)	Post treatment (day)		Pre Treatment (n= 5)	Post treatment (day)	
			5	15		5	15
Copper Ug/dl	116.03± 3.83	83.69± 5.48	96.48± 3.95***	110.29± 5.93	92.14± 2.51	103.36± 4.58	113.48± 4.89
iron Ug/dl	105.69± 3.98	91.27± 3.52	99.72± 4.02***	103.28± 4.79	88.32± 3.58	97.39± 3.93	104.36± 3.38
Zinc Ug/dl	93.15± 3.26	62.27± 3.19	72.92± 3.82**	88.48± 4.19	67.93± 3.61	79.18± 3.73	88.38± 3.19

*significant at $P < 0.05$ * * significant at $P < 0.001$

Table 5. Protein profile of healthy , alopecic and Parakeratotic sheep.

Parameter	Healthy lambs (n= 5)	Diseased lambs					
		Alopecic lamb			Parakeratotic lamb		
		Pre Treatment (n= 5)	Post treatment (day)		Pre Treatment (n= 5)	Post treatment (day)	
			5	15		5	15
T. protein (gm./dl)	9.09± 0.61	6.82± 0.78**	7.74± 0.31**	8.96± 0.42	7.09± 0.48	8.48± 0.49	8.82± 0.94
Albumin (gm./dl)	4.67± 0.53	3.12± 0.25	3.92± 0.31	4.42± 0.52	3.49± 0.31	4.41± 0.26	4.51± 0.45
Globulin (gm./dl)	4.42± 0.63	3.70 ± 0.21	3.82± 0.35	4.54± 0.47	3.60 ± 0.21	4.07 ± 0.16	4.31 ± 0.34

*significant at $P < 0.05$ * * significant at $P < 0.001$

Table 6. Liver enzymes and kidney function of healthy , alopecic and Parakeratotic sheep.

Parameter	Healthy lambs (n= 5)	Diseased lambs					
		Alopecic lamb			Parakeratotic lamb		
		Pre Treatment (n= 5)	Post treatment (day)		Pre Treatment (n= 5)	Post treatment (day)	
			5	15		5	15
AST (U/L)	53.45± 1.51	69.04± 2.32***	63.27± 1.17	55.35± 1.45	66.73± 1.83***	60.51± 1.23	54.38± 1.93
ALT (U/L)	41.27± 1.37	61.16± 1.82***	54.24± 1.04	45.18± 1.84	64.38± 1.73***	58.39± 1.37	45742± 1.93
ALP (u/L)	79.82± 1.97	89.38 ± 3.26***	82.73± 3.45	80.36± 438	91.68 ± 2.18***	87.93± 2.94	82.83 ± 493

*significant at $P < 0.05$ * * significant at $P < 0.001$

DISCUSSION

The alopecic and parakeratotic sheep were emaciated with pale mucous membranes. The wool loss was seen on the dorsal aspect of the thoracic and lumber regions beside face, neck and limbs. The wool was easily detached and the skin was scally, crusting and alopecic. These results are similar to previous findings *(24)* where zinc deficiency was the cause of similar skin lesions. Depression, wool eating, flexed knees and a markedly stiff gait were attributed to zinc deficiency *(8)*. Wool abnormalities were usually related to deficiency of copper and zinc *(25)* Zinc deficiency in sheep and goats led to alopecia and parakeratosis *(26)*. Zinc deficiency caused alopecia and parakeratosis *(27)* that could be

attributed to interrupted ordinary growth of the epidermal cells. *(28)* The same authors recorded that alopecia, cessation of wool growth and pale mucous membranes were the most common results of zinc deficiency in sheep. Parakeratosis in lambs was attributed to low levels of zinc and cupper *(29 , 30).*

The erythrogram in the parakeratotic and alopecic sheep revealed microcytic hypochromic anemia represented by a significant decrease in the total erythrocytic count,hemoglobin level and , packed cell volume, Meanwhile total leukocytic count was insignificantly increased on the 5th day post treatment. These results are inaccordance with previous findings in goats suffering parakeratosis *(31, 32)* .Our results attributed the change in erythrogram to the failure of bone marrow to produce enough erythrocyte, as a result of deficiency of the raw materials needed for RBCs production as copper, cobalt and iron,The reduced total erythrocytic count was justified the impairmed of nutrition of the alopecic goats *(4).* Other investigators *(33)* suggested that the copper deficiency alters the mechanical properties of the erythrocytes and decreases the erythrocytic survivability. Copper deficiency induced significant decrease in the total erythrocytic count and hemoglobin content *(34)*. The hematological parameters in diseased sheep were improved towards the normal level 15 day post treatment with mineral mixture. These results are in agreement with others (35) who reported that the treatment alopecic calves with mineral mixture improved the erythrogram. The insignificant increase in the total leukocytic count,observed in sheep suffering from alopecia and parakeratotosis gets along with the previous studies *(36 , 37).*

The present investigation revealed a significant reduction in the serum calcium, inorganic phosphorus, sodium and potassium in sheep suffering from alopecia and parakeratotosis.Similar finiding were previously decribed in buffaloes *(38, 39)* where siginficant low levels of calcium, inorganic phosphorus, sodium and potassium were found, Moreover a significant decrease in level of serum calcium, inorganic phosphorus,sodium and potassium were recorded in alopecic animals *(40)*.The serum sodium and potassium level were low in calves suffering alopecia and parakeratosis *(41).* Low levels of inorganic phosphorus, calcium, potassium, sodium and chloride in blood serum was recorded lambs *(42).*

The current study pointed out a significant decrease in the copper, zinc and iron levels of the alopecic and parakeratotic sheep.Similar findings were perivously obtained *(43)* where significant decrease of the values of serum copper, iron, zinc, cobalt and manganese was eluciated in alopicic sheep. Furthermore other authors found significant decrease in copper zinc, manganese and iron levels in alopecic and parakeratotic sheep. A significant decrease in the serum iron in alopecic and parakeratotic was cited in lambs and ewes *(30,44)*. Moreover other finiding revealed low serum copper levels in alopicic sheep *(45)*.The reduction of the serum iron and copper could be triggered by diatatry deficiency diet or due to cupper deficiency which decrease the absorption with subsequnce release from the body store for hemoglobin synthesis *(32).*

The serum total protein, albumin and globulin level were significantly decreased in currently studied sheep suffering alopecia and parakeratosis. Similar results were previously reported *(3, 46)* .The decreased level of serum albumin was attributed anorexia *(46)*.The decreased total protein and albumin levels in the present study may be explained by inappetence and albumin loss as a result of increased capillary permeability in copper deficient and alopecic animals *(47).* Zinc is a constituent of numerous metalenzymes and required for normal protein synthesis and metabolism *(25).* Zinc deficiency may be primary or secondary due to inadequate levels in the ration. Secondary it may result from the presence of a substance which interfers with its absorption or metabolism, in spite of the normal diet concentration *(48).* The total protein and globulin values in alopecic sheep were low *(44)* where parakeratotic skin lesions

of sheep having a marked reduction in serum albumin levels.

The serum liver enzyme (AST,ALT and alkaline phosphatase) activity beside the urea and creatinine levels were significantly increased in currently investigated sheep, suffering alopecia and parakeratosis. Elevated liver enzymes (AST ,ALT and alkaline phosphatase) was recorded in alopecic sheep *(1)* .This rise in the hepatic enzymes might be attributed to the leakage of these enzymes through damaged hepatocyte-membrane by oxidative process *(3).* The zinc deficiency, for the free radicals. It is antioxidant thus its the increased in the serum AST *(49).*

The treatment of alopecia (with mineral mixture and zinc oxide ointment) and parakeratosis (with mineral mixture plus cupper sulphate) reduced in the clinical signs and improved the health status, Moreover the blood picture and biochemical parameters .Similar results were previously documented *(50)* where alopecia and parakeratosis were successful treated with zinc supplemented diet which led to healthy of the skin lesions and wool regrowth .The oral administration of zinc oxide efficiently in treatment of parakeratosis in sheep with disappearance the clinical signs.

It could be concluded that parakeratosis and alopecia in sheep are nutironally induced disease they were associated with many reversible alterations in blood picture and some biochemical parameters such deviations returned to normal values 15day post treatment.

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الملخص العربى

دراسات هيمتوبيوكيميائية على تشوهات الجلد وتساقط الصوف في الأغنام مع محاولة العلاج

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قسم الكيمياء الحيوية والسموم والنقص الغذاء معهد بحوث صحة الحيوان بالزقازيق
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أجريت هذه الدراسة على الاغنام بهدف دراسة تأثير تشوهات الجلد و تساقط الصوف على صورة الدم وبعض الوظائف البيوكيمائية مع محاولة العلاج، وقد تم اجراء البحث على عدد ١٥ من الاغنام من الجنسين(٥ بصحة جيدة و٥ تعانى من تشوهات في الجلد و٥ تعانى من تساقط الصوف) في مزرعة خاصة بمحافظة الشرقية أعمارها تتراوح من ٨ -١٠ شهر وتتراوح اوزانها من ٢٠-٢٥ كجم . تم كحت الجلد فى المناطق المصابة بتساقط الصوف على محلول هيدروكسيد الصوديوم بتركيز ١٠% وذلك لفحصها تحت الميكروسكوب لاستبعاد الإصابة بالجرب. قسمت الى ثلاثا مجموعات متساوية ٥ أغنام بكل مجموعة) المجموعة الأولى أغنام تتمتع بصحة جيدة خالية من الطفيليات الداخلية والخارجية (مجموعة ضابطة)، المجموعة الثانية تعانى من تساقط الصوف ويتم علاجها باستخدام مرهم أكسيد الزنك على المكان المصاب يوميا مع إضافة٢كجم مخلوط الأملاح المعدنية/طن عليقة يوميا لمدة ٢٠يوم متتالية والمجموعة الثالثة تعانى من وجود تشوهات فى الجلد ويتم علاجها باستخدام كبريتات النحاس في مياة الشرب بمعدل ١ جم لكل ١٠ لتر يوميا وإضافة ٢٣كجم مخلوط الأملاح المعدنية / طن عليقة يوميا لمدة ٢٠يوم متتالية. تم أخذ عينتى دم من كل حيوان قبل العلاج وعند اليوم ٥ ,١٥ يوم من نهاية العلاج الأولى على EDTA وذلك لدراسة التأثيرات على صورة الدم والأخرى لفصل مصل الدم وذلك لقياس بعض المؤشرات البيوكيميائية .
بفحص القشور الماخودة من الاماكن المصابة بتساقط الصوف ميكروسكوب وجد إنها خالية من الإصابة بطفيل الجرب والقراع
وقد أشارت النتائج الى حدوث نقص معنوى فى العدد الكلي لكرات الدم الحمراء، تركيز الهيموجلوبين، حجم خلايا الدم المرصوصة مصحوبة بزيادة غير معنوية في العدد الكلي لكرات الدم البيضاء فى الاغنام المصابة بتشوهات الجلد و تساقط الصوف.
تشير نتائج الدراسة أن تشوهات الجلد و تساقط الصوف كانتا مصحوبين بوجود نقص معنوى فى النحاس,الزنك ,الحديد , الكالسيوم ,الفوسفور, الصوديوم ,البوتاسيوم, البروتين الكلى ،الزلال, وزيادة معنوية فى معدل كلا من الترانس امينيز (ALT-AST), والفوسفاتيز القاعدى ,اليوريا والكرياتين والجلوبيولين في مصل الدم .
نستخلص من هذه الدراسة أن تشوهات الجلد و تساقط الصوف كانتا مصحوبين بتغيرات فى صورة الدم وبعض القياسات البيوكيميائية فى الاغنام.وتلك التغيرات استعادت وضعها الطبيعي بعد ١٥ يوم من نهاية العلاج لذلك ينصح باستخدام علائق متوازنة فى التركيبات الغذائية خاصة المعادن النادرة فى تغذية قطعان الاغنام.