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ALOPECIA IN SHEEP DUE TO NUTRITIONAL DEFICIENCY IN SOUTH SINAI

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ABSTRACT
This study was carried out on fourteen (14) sheep were housed in Raas Seder station in south Sinia. Among them, seven diseased sheep suffered from different degrees of alopecia, emaciation, lameness, and unthriftiness, as well as sharp decrease of body weight, were treated by using mineral mixture contains Copper, Zinc, Manganese, Iron, and Selenium which were added on the ration and drinking water. Multivitamins were also injected and the affected alopecic areas were treated topically by Thyme oil. Blood samples were collected for estimation of blood picture and biochemical analysis before and after treatment for the diseased sheep as well as for healthy sheep as control group. Alopecic sheep showed a significant decrease of haemoglobin and erythrocytic count, while total leucocytic count did not change significantly. Differential leucocytic count indicated a non significant decrease of lymphocytes and a non-significant increase of neutrophils in comparsion to control and treated groups. Biochemical examination revealed that there were significant decrease in copper, zinc, manganese, iron, total protein, albumin and globulin in diseased ewes when compared to control and treated groups. On the other hand, there was a significant increase in serum ALT, AST and urea. It was concluded that alopecia commonly occurs due to multiple trace element deficiencies, particularly copper and zinc. The use of supplementation is recommended to support the maintenance of mineral status in homeostasis and the reproductive performance of sheep. Thyme oil can be used as aromatherapy for treating alopecia by improving scalp blood circulation. Sheep breeder must pay attention to and give balanced ration to prevent alopecia.

KEYWORDS: Alopecia, Sheep, Mineral Deficiency, Thyme Oil, Sinai.

INTRODUCTION
Alopecia is a local or general loss of hair, fur or wool. True alopecia is baldness without other visible skin disease. However, the term alopecia is also associated with many inflammatory skin disorders. Congenital alopecia has been described as hairlessness accompanies goiter. Acquired alopecia is due to a variety of diseases and intoxication, e.g. gastroenteritis, pneumonia, dietary deficiencies, infectious diseases, and poisoning as by mercury, thallium, iodine or formaldehyde. Disorders of the thyroid, pituitary and gonads can cause hair loss. Temporary alopecia is seen in advanced pregnancy or lactation. Localized hair loss may result from repeated local friction, the continued application of chemicals or irritants, and the presence of ectoparasites and X-rays (Otto et al., 1979) or due to nutritional deficiency (Radostits et al., 2000). The animals obtain these elements either as minerals salts or as organic compounds. The need for these elements changes with age, productivity, climates as well as the levels of these elements in soil. The insufficiencies cause lesion throughout the body and specific skin diseases such as alopecia (Underwood, 1977). Trace elements deficiency, in particular copper, zinc and cobalt have been incriminated in the etiology of alopecia and wool eating habit in sheep (Meyer and Lohse, 2002). However, the deficiency symptoms of one mineral may predominate and affect the performance in the ruminant. Trace elements function probablyact as an activator of enzymes, co-factors and a stabilizer of the secondary molecular structures (Aitken, 2008; Anderson & Rings, 2008). In a successful surveillance management, assessment the levels of the trace minerals is beneficial for detecting the main sources of the diseases/problems of the livestock (Anderson & Rings, 2008). The essential element in sheep is copper; it is easily available in diet and rapidly absorbed through the abomasums and the duodenum. When copper incorporate into specific cuproenzymes. It serves as a critical co-factor, catalyzing the facile electron transfer reactions required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation, and connective tissue formation (McDonald et al., 1984, Balamurugan & Schaffner, 2006). Lack or low concentration of copper in animal feed may cause wool abnormalities as the fine wool becomes limp and glossy and losses its crimps (Radostits et al., 2000, Suttle, 2010). Zinc is fundamental for the

functioning of more than (200) enzymes (Keen et al., 2003), which found in all of the major metabolic pathways involved in proteins, lipids, nucleic acids and carbohydrates metabolism (O'Dell & Sunde, 1997; Keen et al., 2003). Zinc also plays an essential role in maintaining hoof tissues by stimulation of growth of epithelial cells, production of keratin, improved wound healing and improved cellular integrity (Brown et al., 2007) as well as play a key role in immunological responses (Al-Saad et al., 2010). Zinc deficiency is associated with parakeratosis, growth retardation, wrinkled skin, wool loss (Kendall et al., 2000). Mineral deficiencies may cause clinical disorders that have dramatic effects on the health and survival of sheep, or marginal deficiencies that result in subtle and often undetected effects on productivity (Balamurugan et al., 2017). Alopecia and wool abnormalities were observed in sheep suffering from deficiency of copper, zinc, iron, and manganese (Abd El-Roaf and Ghanem, 2006). Hormonal studies revealed a significant increase in prolactin and cortisone hormones in postpartum alopecia (Ramadan, 2012). The serum levels of mineral bio-markers were more sensitive to the reproductive stage than to the level of concentrate supplementation. In general, the use of supplementation is recommended to support the maintenance of mineral status in homeostasis and the reproductive performance of ewes (Wessam Saleh, 2019). Aromatherapy can be used as a supplement to treat alopecia. It uses highly concentrated extracts which are derived from the flowers, leaves, bark and the roots of various plants such as Thyme vulgaris oil. These oils work not only on a cellular level to strengthen the nervous system, but also it stimulates hair follicles by improving scalp blood circulation (Kaushik et al., 2011). The objectives of this study were to investigate the level of some minerals related to alopecia and haematobiochemical alterination accompined by this disease as well as a trial for an effective treatment and to find out the effect of thyme oil on the affected alopecic lesions.

MATERIALS AND METHODS

Animal and experimental design

The present study was carried out on fourteen (14) sheep (3 years old) which were housed in Raas Seder station in south Sinia. These animals were divided to tow groups; Group A composed of seven clinically healthy sheep and were served as control group; group B composed of seven diseased animals which showed different degrees of alopecia, emaciation, lameness, and unthriftiness, as well as sharp decrease of body weight (average weight 29.3 kg).

The seven diseased sheep were treated by using the following medication

- Mineral mixture contains Copper, Zinc, Manganese, Iron, and Selenium which were added on the ration.
- Multivitamins injection contains Vit A, D, E, B1, B6, B2, B12.
- Multimineral solution added on drinking water.
- The affected alopecic areas were treated typically by Thyme oil.

The treated animals were fed on balanced concentrate mixture and roughage and were treated with the above medications for one month.

Haematological analysis

One ml of blood was withdrawn from jugular vein into heparinized test tubes. Haemoglobin concentration (Hb), erythrocytic (RBCs) count, white blood cell (WBCs) count and the differential leucocytic count were estimated according to Coles (1986).

Biochemical analysis

Blood samples were collected from jugular vein and centrifuged for serum separation to examine the biochemical parameters.

The blood samples collection were conducted at zero time and one month after treatment.

Serum Copper, Zinc, Manganese, and Iron were determined using atomic absorption spectrophotometer AAS.

Serum total proteins: Serum total proteins were determined spectrophotometrically by using of special kits according to the method that described by Pagana (2010).

Serum albumin: Serum albumin was determined spectrophotometrically by using of special kits according to the method that described by Fischbach and Dunning (2009).

Serum urea: Serum urea was determined spectrophotometrically by using of special it's according to the method that described by Patton and Crouch (1977).

Alanine amino transferase (ALT) and Aspartate amino transferase (AST) were determined according to method of Frankel (1970).

STATISTICAL ANALYSIS

The data were statically analyzed using one way Analysis of Variance (ANOVA) and independent-samples T test in the experimental study as previously described (Bailey, 2008). All the statical evaluations were done using the computer program SAS (2005) using the linear model. Values were represented as means ± standard error (SE). All differences were considered significantly among groups of the experimental study when $P \leq 0.0$.

RESULTS AND DISCUSSION

Clinical examination of diseased sheep showed the presence of alopecia at the neck, legs, and abdomen, (Fig. 1a, Fig.1b and Fig.1c). Additional signs included

inappetance, pale mucous membrane, lameness, staggering gait and diarrhea. A sharp decrease of body weight was a marked sign science the average weight of the diseased sheep was about 29 Kg in comparison to about 40 Kg of the healthy sheep (about 25% weight loss). These signs were nearly similar to those recorded by Fahmy et al., 1980, Taha et al., 1993, Metwalli et al., 1997, Mobarak, 1998, Radostits et al., 2000, Abd El-Roaf and Ghanem, 2006, and Wessam Salah, 2019.

After one month of treatment, there was an improvement of general health conditions include increase in appetite and weight (average weight 39 Kg). Improvement of animal movement and growth of strength hair follicles were also observed as shown in Fig. 2a, b, c.

Thyme oil was used to enhance the growth of new healthy hair follicles through its effect on the scalp blood circulation, Kaushik et al., 2011. As shown in fig. 2, new strength hair follicles replaced the old alopecic wool. The using of such Aromatherapy has additional advantages such as being natural drugs of less side-effects, easy availability, and low-cost for treatment of Alopecia.

Wool abnormalities were usually related to deficiency of copper and zinc (Church and Pond, 1988). The alopecia and the loss of crimp (steely wool) might be attributed to defective keratinization (Davis and Mertiz, 1987 and Linder, 1991). The polypeptide chain of keratin fibers are cross-linked by disulphide bonds which are formed by oxidation of the –SH group of the cysteinyl residue present in the polypeptide chain. This process is copper-dependent and affects the chemical and physical properties of wool and hair (Linder, 1991 and Frank et al., 2000). Locomotor disturbance and lameness with enlargement of hock and knee joints might be regarded to deficiency of manganese, zinc and copper (Mobarak, 1998). It has been found that copper deficiency interferes with osteoblast activity in bone because of the inactivation of lysyl oxidase activity, which is a copper-dependent enzyme (Goonratne et al., 1989). Unthriftiness and anemia (manifested by paleness of ocular and oral mucous membranes) could be attributed to deficiency of iron, zinc and copper (Radostits et al., 2000).

RESULTS OF HEMATOLOGY

Table 1 Showed a significant decrease of the values of haemoglobin (Hb) and erythrocytic count (RBCs) in comparison to control group, while there is no significant difference between treated and control group. These results agreed with Abd-El Raof and Ghanem, 2006, Ramdan, 2012, and Wessam Salah 2019 who reported anemia in cases of alopecia in sheep. This anemia may be due to disturbance in iron metabolism as result of copper deficiency which decreases the absorption of iron, releasing of iron from body stores and utilization in hemoglobin synthesis (Church and Pond, 1988). Also these results agreed with Al-Saad et al., 2010 who reported normocytic normochromic anemia in sheep suffering zinc deficiency. Regarding to leukocytic examination, there were no significant differences between alopecic and control groups. These results mean that the alopecic sheep didn't suffer from microbial infections. This finding indicates that the reason of alopecia is a result of nutritional deficiency and this is agreed with the results of Mobarak, 1998. Differential leucocytic count indicated a non-significant decrease of lymphocytes and a non-significant increase of neutrophils. This result was nearly similar to those mentioned by Arthington et al., 1996. On the other hand this result disagreed with (Ramdan, 2012) who recorded significant leukocytosis in diseased group.

RESULTS OF BIOCHEMISTRY

Biochemical examinations (Table 2) revealed that there were significant decrease in total protein, albumin and globulin in diseased ewes when compared with control one. These results were agreed with Abd-El-Raof and Ghanem, 2006 and Al-Saad et al, (2010). This may be due to loss of appetites and loss of albumin as result of increased capillary permeability in copper deficient in alopecic ewes (Rucker and Tinker, 1977). Also these results agree with Nelson et al., 1984 who reported that, there were significant decrease in total protein and globulin values in sheep with alopecia. Also agreed with (Fouda et al., 2011) and (Ramdan, 2012) who reported reduction in the level of total protein, albumin and globulins in case of diseased sheep. This reduction is attributed to the increased level of blood cortisol in diseased animals. Increased cortisol cause catabolism of protein leading to negative nitrogen balance and increased urinary elimination of nitrogen (McDonald 1980).

Liver enzymes examination (Table 2) revealed that there were significant increase in alanine aminotransferase, aspartate aminotransferase activities in diseased sheep compared to control group while there were no significant difference between treated group and control one. These result conceded with Abd-El- Raof Ghanem, 2006, Al-Saad et al., 2010 and Ramdan, 2012 who reported significant increase in ALT and AST in alopecic sheep. The higher level of the enzyme occur in disorders or conditions that involve the hepatocyte or muscles damage (Stockham and Scott, 2002) . It has been found that absorbed copper appears first in plasma as cupric ion loosely bound to albumin. During hepatic synthesis of ceruloplasmin, copper is bound to this metaloprotein, which is then released to general circulation (Scheinberg and Sternlieb, 1960). Ultimately cuproprotein is present in brain, erythrocyte and liver as cerebrocuprein, erythrocuprein and hepatocuprein, respectively (Scheinberg and Sternlieb, 1960). Therefore, it is possible that copper deficiency reduces the hepatocuprein in liver andadversely affects liver function. In addition, zinc is essential for maintaining the activity of superoxide dismutase, a copper-dependant enzyme, in the liver that function as antioxidant (Sidhu et al., 2005). Therefore, copper and zinc deficiency results in reduction in the activity of superoxide dismutase that

enhances the oxidative stress within hepatic cells. Urea examination revealed that there was significant increase in urea level in diseased group while non significant change in creatinine level when compared with controls one. While there were no significant diference between treated groups and control ones. These results agree with Abd-El- Raof and Ghanem, 2006 and Ramdan, 2012 who reported increase urea level in alopecic sheep which may be due to trace elements deficiency particularly cupper., Mineral analysis showed significant decrease of copper, zinc, Mangnes and iron levels in diseased group compared to control ones on the other hand there were no significant difference between treated and control ones. Other previous related studies (Abd El-Raof & Ghanem, 2006, Aitken, 2008, Constable et al., 2016, and Wessam Saleh, 2019) have been documented similar findings. Primarily, rough hair coat condition and/or wool abnormalities were usually related to deficiency of copper, zinc and cobalt (Pond et al., 2004). Therefore Alopecia and steely wool might be attributed to defective keratinization. The pathway of alopecia in zinc deficient animal may attribute to the reduction of the follicular epithelium capacity to produce fiber (Radostits et al., 2006). Zinc and copper deficiency cause loss of hair and unthriftiness (Abd-El-Raof and Ghanem, 2006, Wessam Saleh, 2019).

It was concluded that nutritional deficiency causes mineral deficiency especially copper, zinc, Mangnes and iron and play an important role in alopecia in sheep. Thyme oil can be used as aromatherapy for treating alopecia by improving scalp blood circulation. The use of supplementation is recommended to support the maintenance of mineral status in homeostasis and the reproductive performance of sheep. Sheep breeder must pay attention to give balanced ration to prevent alopecia.

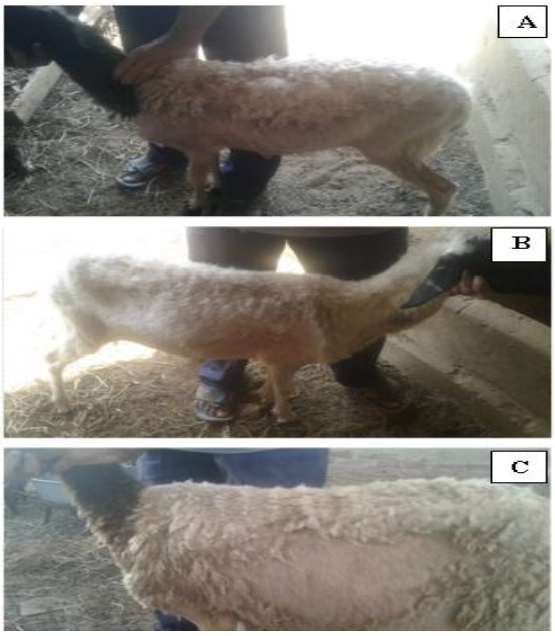

Fig. 1: Diseased sheep showed emaciation and the presence of alopecia at the neck, legs, and abdomen.

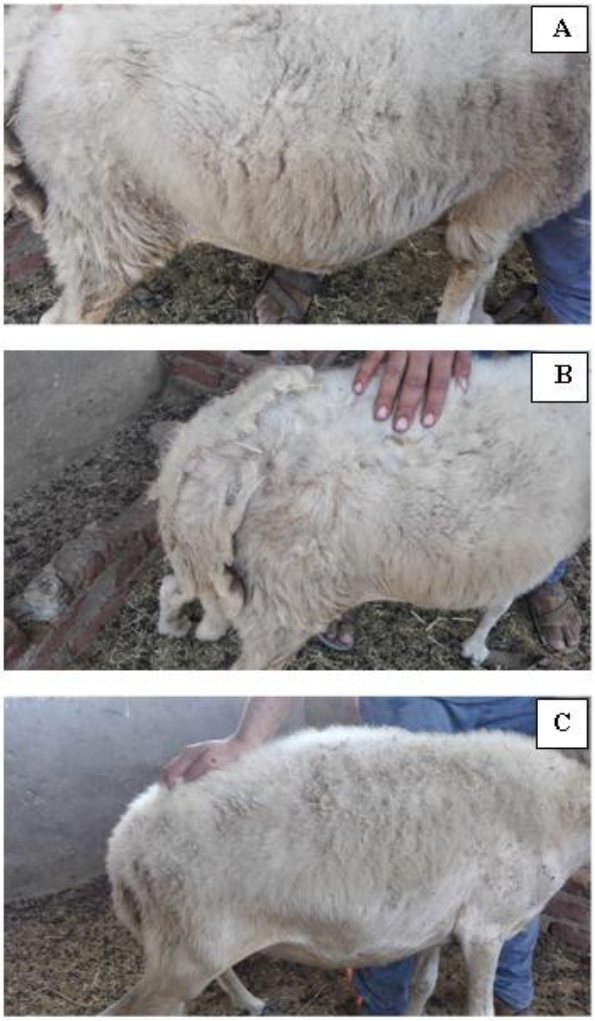

Fig. 2: Treated sheep show growth of healthy strength wool and improvement of weight.

Table. 1: Hematology analysis of control, diseased, and treated sheep.

Parameters	Control Group A	Diseased Group B	Treated sheep	SE
Hb, gm/dl	13.2a	8.2b	12.3a	1.15
RBCs, 10^6/Cmm	11.3a	8.4a	11.6a	1.27
WBCs, , 10^3/Cmm	9.4	8.5	9.0	1.96
Lymphocytes %	56.0	47.0	55.2	2.89
Monocytes, %	2.3	2.6	2.2	0.87
Neutrophil %	41.2b	46a	40.3b	0.69
Eosinophil %	1.7	1.9	1.8	0.40

Table. 2: Biochemical analysis of control, diseased, and treated sheep.

Parameter	Control Group A	Diseased Group B	Treated sheep	SE
Copper (mg/l)	1.26a	0.53b	1.58a	0.115
Zinc (mg/l)	1.12a	0.35b	1.13a	0.069
Manganese (mg/l)	0.058a	0.028b	0.05a	0.012
Iron (mg/l)	4.46a	0.99b	3.8a	0.231
Total protein gm/dl	7.2a	5.3b	6.8a	0.29
Albumin, gm/dl	4.3a	3.2b	4.0a	0.17
AlT, mL	37b	58a	38.2b	2.89
AST, mL	62.7b	87.2a	60b	1.56
Urea (mg/l)	31.2b	49a	33.4b	0.69

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