

*Original Paper*

The relationship between deficiency of some trace elements, oxidative stress, immunoglobulin E and vitamin A in sheep affected with skin diseases

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ARTICLE INFO**Keywords**

Nutritional

Oxidative stress

Sheep

Skin diseases

Received 29/02/2020

Accepted 25/04/2020

Available On-Line

08/09/2020

ABSTRACT

This work aimed mainly to study the status of some trace elements, oxidative stress, immunoglobulin E and vitamin A in sheep with skin diseases. In this study a survey was done on 190 of sheep of both sexes from sheep flocks around Menoufia Governorate showing skin diseases of 2 ± 0.5 years of age. All cases were subjected to clinical examination; body temperature was taken also pulse rate, respiratory rate; rumen movements, mucous membrane, wool and body condition score. 58% of these animals suffered from infectious causes. The other 42% of animals were associated with nutritional deficiencies. A significant decrease ($p < 0.01$) was found in the level of zinc, copper and vitamin A than compared to apparently healthy animals. Also, there was significant decrease ($p < 0.05$) in alkaline phosphatase activity. However, a significant increase ($p < 0.05$) was found in catalase activity. A significant decrease ($p < 0.01$) was found in superoxide dismutase activity compared to apparently healthy animals. We found significant increase ($p < 0.05$) in Malondialdehyde level than that taken from apparently healthy animals. A significant increase ($p < 0.05$) was detected in IgE level compared to apparently healthy animals. According to the results of our study we concluded that the prevalence of non-infectious skin affections was (42%) and there was significant relationship between non-infectious skin affections and oxidative stress, some trace elements, vitamins, immunoglobulin levels in sheep under field conditions

1. INTRODUCTION

Skin diseases in sheep severely affect wool production and quality which is the major product of sheep industry. Both malnutrition and trace elements deficiencies are important factors that affect wool production (Scott, 2007). Any factor that affecting the body has a direct effect on wool production such as any systemic disease, stress or nutritional deficiency that leads to weakening of the wool, thinning of the fibers which causing break of the wool at any weak point. (Hindson and Winter, 2002).

The quality of wool is determined by its fiber diameter, yield, crimp, color and strength. Nutrition has an important role in wool production. The most important wool characteristic is fiber diameter that determines the quality and price of wool. It considers mean to determine animal nutrition as the sheep will divert nutrients from wool production to other requirements needed to survive. All infectious agents as external parasites, fungi, bacteria and viruses cause loss of mechanical protection between animal and environment due to skin destroying that facilitates invasion by other diseases affecting the general health of the animal. The leather industry also affected by damage of the skin (Kusiluka and Kamarage, 1996). Skin, being the outermost barrier of the body, is exposed to both endogenous and environmental pro-oxidants (Cross

et al., 1998). In skin diseases, the body has several potent antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), GSH-peroxidase activities, and prooxidants as malondialdehyde (MDA), and the joined interactions between these antioxidants' activities depend on the lysis of peroxides and free radicals (Camkertan et al., 2009). Body fights against excess free radicals via antioxidant defense system that comprises antioxidant enzymes and nutritional antioxidants, such as vitamins, zinc, and copper. Antioxidant vitamins (non-enzymatic antioxidants), such as vitamins E and C, act by quenching singlet oxygen and prevent free radical formation. Their supplementation in diseases associated with oxidative stress hastens clinical recovery (Morita et al., 2006).

Unbalanced using of nitrogenous fertilizers can reduce copper, cobalt, molybdenum and manganese content of the pasture. Also using extra lime reduces plant copper (Cu), cobalt (Co), zinc (Zn) and manganese (Mn) while increase molybdenum contents. According to these deficiencies in plants content, the animal feeding on these plants suffers from trace element deficiency (Constable et al., 2017).

The exact impact of deficiencies can be uncertain, although the vitamins and trace elements are essential for general health and productivity. So, some animals may remain healthy but have sub-normal blood

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levels of these elements and others may show unthriftiness (Shuttle and Jones, 2007).

This work aimed mainly to study the status of some trace elements, vitamin A, immunoglobulin E, and its relation to some skin affections in sheep. In addition, evaluation of the oxidative stress and its relation to some skin affections in sheep was performed.

2. MATERIAL AND METHODS

2.1. Animals:

In this study a survey was conducted on 190 sheep showed skin diseases of both sexes of 2 ± 0.5 years of age. All 190 cases were subjected to clinical examination including body temperature also pulse rate, respiratory rate, ruminal movements, mucous membrane and wool. 110 (58%) of these animals suffering from ring worm, mange, internal and external parasites (fleas, ticks, lice). The other 80 (42%) animals were suspected to have nutritional deficiencies. 35 apparently healthy sheep were used as control. All samples were collected from sheep flocks around Menoufia Governorate in the period from May to September 2017.

2.2. Samples:

All serum samples were collected from diseased cases and apparently healthy animals. Skin scraping samples were collected from skin lesions of all animals. Faecal samples were collected from all animals for faecal examination. Blood samples were collected from jugular vein according to Pugh (2002). One blood sample was collected of 5 ml that allowed to flow freely and gently over the inner surface of a clean and dry centrifuge tube. The samples were allowed to clot in a slanting position at room temperature for about 2 hours then the samples were centrifuged at 3000 rpm for 10 minutes. The clear serum was aspirated using automatic pipette and put into clean dry labeled Eppendorf tubes and freeze until biochemical analysis. The clear non-hemolyzed sera were used for biochemical determination into three sets; The 1st for measurement of zinc, copper, cobalt. The 2nd for determination of vitamin A. The 3rd sample for evaluation of immunoglobulin E. The 4th sample for evaluation of antioxidant capacity (SOD, CAT, Alkaline Phosphatase and pro-oxidants MDA level).

2.3. Clinical examination of the animals:

Body temperature, respiratory rates, pulse rates, rumen movements were examined and recorded as mentioned by Constable et al. (2017).

2.4. Serum biochemical analysis:

Serum zinc and copper were determined by using an atomic absorption spectrophotometer using 0.5 ml of serum added to 5 ml of 6% butanol as recorded by Maret and Henkin (1971). Serum vitamin A was determined using colorimetric determination of serum retinol and carotene level by semi-micro method of Neeld and Pearson (1963). Serum CAT activity was determined by method described by Luck (1974). Serum alkaline phosphatase (ALP) was determined by spectrophotometer using special kits according to method described by REC. GSCC (DGKC) (1977).

Serum L-malondialdehyde (L-MDA) was determined calorimetrically according to the method recorded by Satoh (1978) and Ohkawa et al. (1979). Serum superoxide dismutase (SOD) concentration was determined calorimetrically according to the method mentioned by Nishikimi et al. (1972). Quantitative determination of IgE concentration serum using microplate enzyme immunoassay determined according to method of Plebani et al. (1998).

2.5. Skin scraping specimens for mite infestations:

Specimens of skin scraping were collected in clean dry tubes from the lesion margins by deep scraping and diluted with ethyl alcohol 70% and kept in refrigerator and that according to Baker (2010) and Danbirni et al. (2011).

2.6. Diagnosis of dermatophytosis:

The samples are collected from skin scrapings, these scrapings soaked in 25% KOH or NaOH with 5% glycerol then heated gently to emulsify lipids then examined under microscope with 3400 magnification (Thomas 2003)

2.7. Faecal parasitological examination:

In clean plastic containers fecal samples were collected individually from the rectum or immediately after defecation to be examined as soon as possible. Each sample was examined macroscopically and microscopically for any gross parasites as well as for cestodes eggs, nematodes or Eimeria oocysts using concentration floatation technique. Using egg counting to determine the degree of infestation by modified McMaster technique according to Solusby (1982).

2.8. Statistical analysis

The obtained results from the experiments were expressed as mean \pm SEM and were analyzed using *t*-test Statistics for Windows, version 23.0. Armonk, NY: IBM Corp). Differences were declared significant when $P < 0.05$.

3. RESULTS

From 190 examined sheep showing skin diseases of both sexes of 2 ± 0.5 years of age we found 110 (58%) of these animals suffered from ring worm, mange, internal and external parasites (fleas, ticks, lice). The other 80 (42%) animals were suspected to nutritional deficiencies. 35 apparently healthy sheep were used as control.

3.1. Clinical examination of sheep affected with skin diseases associated with nutritional deficiencies:

Sheep affected with skin diseases associated with nutritional deficiencies included in this study were 2 ± 0.5 years of age, 64 (80%) were non-pregnant ewes, 16 (20%) were males. The clinical examination of the 80 (42%) cases of sheep affected with skin diseases associated with nutritional deficiencies revealed that 14 cases (18 %) of cases show anorexia, 16 cases (20%) show emaciation with 24 cases (30 %) suffered of easily detached wool and areas of alopecia, also diarrhea detected in 16 cases (20%) with 8 cases (10%) pasty

feces, nervous symptoms were shown in 2 case (2%) (Table 1).

3.2. Laboratory diagnosis of sheep affected with skin diseases associated with infectious causes :

Of all 110 cases (58%) suffered from infectious causes we found that 55 (50%) of cases were positive to external parasites (fleas, ticks and lice), 44 (40 %) of cases were positive to *Sarcoptes scabii* Var *Ovis* and 11(10%) of cases were positive to ring worm infestations.

Table 1 Frequency of clinical signs in skin affected sheep

Signs	No. of cases	%
Anorexia	7	9
Emaciation	8	10
Easley detached wool	24	30
Diarrhea	16	20
Pasty feces	8	10
Nervous symptoms	2	2
Alopecia	8	10
Parakeratosis	7	9
Total	80	100

3.3. Biochemical determination of serum markers of sheep affected with skin diseases associated with nutritional deficiencies :

Zinc level in serum of sheep with skin diseases was significant decrease ($p<0.01$) than apparently healthy animals (Table 2). Copper level in serum from sheep with skin diseases decrease ($p<0.01$) than apparently healthy animals (Table 2). Vitamin A level in serum sheep with skin diseases was significant decrease ($p<0.05$) apparently healthy animals (Table 2).

Table 2 Levels of copper, zinc and vitamin A of sheep affected with skin diseases associated with nutritional deficiency

	Copper ($\mu\text{mol/l}$)	Zinc ($\mu\text{g/l}$)	Vit. A (IU/dl)
Skin affected animals	15.7 \pm 0.7**	7.1 \pm 0.1**	52.2 \pm 7.7*
Apparently healthy animals	20.9 \pm 1.2	12.3 \pm 0.5	70.5 \pm 13.2

* $p<0.05$, ** $p<0.01$

Alkaline phosphatase level in serum from sheep with skin diseases decrease ($p<0.05$) than apparently healthy animals (table 3). CAT activity in serum from sheep with skin diseases increase ($p<0.05$) than apparently healthy animals (Table 3). SOD activity in serum from sheep with skin diseases decrease ($p<0.01$) than from apparently healthy animals (Table 3). MDA level in serum from sheep with skin diseases increase ($p<0.05$) than —apparently healthy animals (Table 3). IgE level in serum from sheep with skin diseases was significantly increase ($p<0.05$) than apparently healthy animals (Table 3).

4. DISCUSSION

In our study, a survey was done on 190 of sheep showing skin diseases of both sexes of 2 ± 0.5 y of age. 110 (58%) of these animals suffering from ring worm, mange, internal and external parasites (fleas, ticks, lice). The other 80 (42%) animals were associated with nutritional deficiencies. 35 of apparently healthy sheep were used as control. We also found that the

prevalence of skin diseases was of all 110 cases (58%) suffered from infectious causes. We found that 55 (50%) of such cases were positive to external parasites (fleas, ticks and lice), 44 (40 %) of cases were positive to mite infestations and 11(10%) of cases were positive to ring worm infestations. Although the prevalence of skin diseases in sheep and goat in a study of Teshome (2016) was as followed: out of 631 sheep and 152 goats were examined, found 268 sheep (42.47%) and 58 goats (38.16%) exhibited skin diseases. The skin disease was more frequently observed in young animals than in adults and in poor body condition than in good and medium body condition animals.

Table 3 Activities of alkaline phosphatase, Catalase, Superoxide dismutase, MDA and IgE levels of sheep affected with skin diseases associated with nutritional deficiency

	Skin affected animals	Apparently healthy animals
ALP (U/l)	240.2 \pm 43.4*	442.8 \pm 45.4
CAT (U/l)	41.7 \pm 5.4*	15.2 \pm 0.2
SOD (mmol/ml)	16.1 \pm 1.4**	37.7 \pm 0.5
MDA ($\mu\text{mol/l}$)	59.6 \pm 4.7*	32.4 \pm 1.4
IgE (IU/ml)	180.6 \pm 19	87.3 \pm 7.6

* $p<0.05$, ** $p<0.01$

The skin of affected sheep associated with nutritional deficiencies included in our study was 2 ± 0.5 y of age, 64 (80%) were non pregnant ewes, 16 (20%) were males. Zinc deficiency found in sheep in the present study caused some hazard effects such as hair loss, parakeratosis, growth retardation, loss of appetite and delayed wound healing and that also reported by Harold et al. (2011) that because zinc as a trace element plays an important roles in numerous enzymatic reactions so zinc deficiency is associated with reduced growth rate, decrease reproductive performance, poor immune responses as well as skin affections also mentioned by El-Attar (1979), Chan et al. (1998), Gooneratne et al. (1989), Mozaffari and Derakhshanfar (2007).

Alopecia is associated with zinc deficiency that was found. Moreover, the skin has high quantity of zinc especially in the epidermis than in the dermis which needed for proliferation and differentiation of epidermal cells as recorded by Hefnawy et al. (2018). Zinc is involved in immune system and metabolism of vitamin A as mentioned by Watson (1998) that may lead to lower vitamin A serum level with zinc deficiency as recorded in our study results. In addition, we found significant decrease in serum zinc level as well as significant decrease in serum vitamin A level in cases showing noninfectious skin affections. The link between vit. A metabolism and zinc metalloenzymes is necessary in conversion of retinol to vit. A aldehyde (underwood and shuttle, 2000). The symptoms we noticed in our survey were matched with what recorded by Kendall et al. (2000) as parakeratosis, wrinkled skin, wool loss, alopecia.

Zinc is involved in antioxidant mechanism as Cu-Zn SOD and protect from iron –induced lipid peroxidation (Zago and Oteiza, 2001) and that explain the link between zinc deficiency and lower serum level of SOD reported in our results that we found significant decrease in serum SOD level as well as significant decrease in serum zinc level in cases showing

noninfectious skin affections. Berger (2002) cited that about 300 enzymes have zinc in their components. Zinc deficiency causing alopecia, crusting, scaling (McGavin and Zachary, 2007). Zinc improves cellular integrity, growth of epidermal cells, production of keratin and wound healing. A proliferation and differentiation of epidermal keratinocytes (Ogawa et al., 2016). Those symptoms were similar to those we reported in our study on cases suspected for nutritional deficiency and associated with significant decrease in serum zinc level. Our results showed significant decrease in ALP activity and zinc levels in noninfectious skin affected sheep these results agreed with El-Sayad et al. (1999) about biochemical results which found that significant decrease in serum zinc level and alkaline phosphatase activity in affected calves. Our results showed significant decrease in serum zinc and copper levels of non-infectious skin affected sheep than that of apparently healthy sheep these results were similar to those recorded by Fahmy et al. (1980), who reported a significant decrease in copper and zinc in sheep with alopecia. Skin lesions associated with zinc deficiency as alopecia, parakeratosis we found in our cases agreed with Hensel (2010), who found that in sheep and goats as well as dogs which associated with skin lesions that included hyperpigmentation and hyperkeratosis suffered from zinc deficiency.

Copper has physiological roles related to several functions in the body include bone formation, cellular respiration, connective tissue development and catalytic cofactor of some metalloenzymes (Vazquez- Armijo et al., 2011) copper is needed for ferrous metabolism by enhancing activity of enzymes required for its metabolism, also needed for elastin and collagen formation, melanin production and keep integrity of central nervous system also cited that copper has a direct effect on oxidation of thiol group to form disulphide bond, so in our study we recorded that wool was weak, lack crimps and lustrous and that associated with significant decrease in serum copper and this came along with Abd El-Raof and Ghanem (2006). Also, we recorded in our study that the black wool showed depigmentation, the animal become anemic and diarrheic and that also noticed by Sharma et al., George and fisher (2008) and Hansen et al. (2009). Ceruloplasmin and SOD are two copper dependent enzymes having anti-inflammatory action prevents oxidative tissue damage resulting from inflammation as mentioned by Cerone et al. (2000). Also, Lai et al. (1994) recorded that decrease activity of Cu-Zn SOD as mentioned in our study significant decrease in serum copper level came along with significant decrease in SOD so oxidative stress is expected in copper deficient sheep. That matches with Rikaby (2013) who reported that copper plays an important role in decrease and preventing oxidative stress as antioxidant. Copper has a role in cellular respiration, bone formation, connective tissue development cited by McDowell (2003). The change of hair pigmentation associated with copper deficiency in cattle for reduction in activity of copper dependent enzyme tyrosinase required for melanin synthesis (Underwood et al. 2000) as we noticed in our study there was a depigmentation of black wool in some cases suspected for nutritional deficiency. This also came along with Spears et al. (2008), who recorded that the early sign of dark wool depigmentation may also be seen in case of copper deficiency. It was recorded in our study that copper

deficiency associated with alopecia as well as decreased wool production and this agreed with Niekerk et al. (1989) The result agreed with White (2004), Ali et al. (2012) and Mohammed et al. (2013), who reported that loss of wool appearance of alopecia in sheep with copper deficiency.

Vitamin A is required for normal cellular differentiation and development, which it is essential for the transduction of light to the neural signals required for vision, so they found that vitamin A deficiency leads to epithelial differentiation, metaplasia, xerophthalmia, anorexia, and compromised function of immunity (Green et al., 2012). In our study we found that the cases suffered from alopecia and wool loss were accompanied with decrease of vitamin A and was agreed with Everts et al. (2013). Our results stated that hyper-keratinization associated with significant decrease in serum vitamin A level that agreed with Girard et al. (2006), who found that vitamin A deficiency resulted in hyperkeratosis in human. Deficiency of vitamin A in our study affects normal keratinization as mentioned by Chung et al. (2010) and Everts (2012). Low level of vitamin A reported in our study was the same result recorded by Thomas et al. (2012), who found that hyperkeratosis and dermatitis associated with vitamin A deficiency in juvenile cattle. Hensel (2010) found that poor coat quality, crusting, alopecia and poor wound healing in dogs, horses, caged birds and reptiles that suffered from vitamin A deficiency and that results came along with ours where we found hyperkeratosis, alopecia and poor coat quality in our cases of sheep suffered from vitamin A deficiency.

IgE mediated hypersensitivity is often referred to allergy as noticed by Gershwin (2001), who mentioned that IgE mediated responses includes rhinitis, asthma, atopic skin diseases and anaphylaxis these noticed in humans that many of these conditions are noticed in domestic animals. We found that the cases in our study having noninfectious skin lesions came together with increased level of IgE these results matched with Sujan et al. (2005), who recorded that there is increase in IgE level in serum of patients with allergic dermatitis and psoriasis. Increased levels of IgE in our study also agreed with Kasumagić et al. (2006), who noticed that patients of alopecia areata had elevated serum concentrations of total IgE. Sutton et al. (1993), who found that serum IgE concentrations were high in atopy and parasitosis. Kasperkiewicz et al. (2018) were found that in cases of atopic dermatitis (AD) there was an elevated level of total serum IgE and that came along with our results which showed also increase in level of IgE accompanied with wool loss and wool crimp.

In our study, we found significant increase in serum levels of MDA, CAT activity and we recorded significant decrease in serum activities of SOD, ALP. And that associated with decrease serum levels of zinc and copper. Since Yoshika and Naito (2002). Reported that oxidative stress can expressed as balance disturbance between antioxidant system and free radicals. We found in our study there was a relationship between decrease serum levels of copper and zinc and disturbance in antioxidant mechanism that because zinc and copper were involved in antioxidant enzymatic mechanism as Cu-Zn SOD activity. These results matches with Beattie and Kwun (2004), who cited that zinc deficiency induced endothelial cells susceptibility to oxidant stress. Also Lai et al. (1994) recorded that decrease activity of Cu-Zn SOD as mentioned in our study may be due to significant decrease in serum copper level came along with

significant decrease in SOD so oxidative stress is expected in copper deficient sheep. This result matched with Rikaby (2013), who reported that copper plays an important role in decrease and preventing oxidative stress as antioxidant. MDA is used as oxidative stress indicator in tissues and cells of animals and plants as recorded by Matovic et al. (2010). Many disorders involved with free radical reaction as skin disease and immunodepression as mentioned by Chakraborty (2009). The increase of oxidative stress accompanied with alopecia also seen by Gaetke et al. (2003) as we found alopecia, easily detached wool, crimps and crusts in the current study. These results also matched with Bakry et al. (2014) and Magro et al. (2011), who found that several literature reports can link androgenic alopecia to increased oxidative stress. Increased level of MDA and decreased level of SOD in cases having androgenic alopecia as shown in our result found matched with result shown by Stojan et al. (2016). Also, they found decrease in Cu Zn SOD in patients with androgenic alopecia which acts as strong antioxidant defense mechanism, transforms peroxides into less toxic products.

Increasing activity of CAT and MDA level in our study came similar to Ozturk et al. (2016). Increase levels of MDA in our study were similar to results of Naziroglu et al. (2000), Abdel Fattah et al. (2011), Koca et al. (2005) and Bekpinar et al. (2005). Our results showed decreased serum activity of SOD in contrary to Abdel Fattah et al. (2011) and Koca et al. (2005). But the study of Akar et al. (2002) matched with our results in decrease serum activity of SOD which associated with noninfectious skin affections. The level of MDA was increased in our study which agreed with Cwynar et al. (2017) that reported the increase in level of MDA was shown in cases of alopecia areata as an indicator of the effect of oxidative stress in patients with alopecia areata. Increasing serum level of MDA and decreasing levels of serum zinc and copper as recorded in our study came matched with Najji (2017), who reported these results accompanied with several clinical disorders as loss of appetite, pica, wool loss, alopecia, parakeratosis, weight loss and diarrhea. But in contrary of our results which mentioned significant increase in serum CAT activity, the study of Najji (2017) recorded significant decrease in serum CAT activity. Our recorded clinical symptoms on suspected cases of nutritional deficiency seems referred to zinc and copper deficiency including anorexia and loss and emaciation and came along with Dittmer et al. (2011), who explained that abnormal mineralization caused by zinc deficiency which is responsible for bone mineralization. In our study, increased serum level of MDA represented by increase oxidative stress leading to release of free radicals affecting liver and kidney leading to hypoproteinemia resulting in loss of body weight, wool loss and easily detached wool and this attributed to zinc and copper deficiency this matches with Najji and Zenad (2015) and Jemai et al. (2007). In our study, we recorded increase in activity of serum CAT but in contrast Najji (2017), Nagamani et al (2015) and Al- Derawi (2010) reported that decrease in serum activity of CAT. They studied imbalance between oxidant and antioxidant system, and they connected that to copper and zinc which they act as a part of antioxidant defense mechanism this also matched with Constable (2017) and Najji (2016). Cam et al. (2009) recorded that low cellular resistance against oxidative stress indicated low serum activity of CAT while the high CAT activity indicated to defense against free

radicals. According to the results of our study we can conclude that the prevalence of non-infectious skin affections was (42%) and there was significant relationship between non-infectious skin affections and oxidative stress, some trace elements, vitamins, immunoglobulin in sheep under field conditions.

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