Clinical and Haemato-Biochemical Studies on Cases of Alopecia in Sheep Due to Deficiency of Some Trace Elements

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ABSTRACT
This study was carried out on twenty Balady sheep collected from two flocks of sheep at Kalubia Governorate. Ten sheep of them (from one flock) suffered from different degrees of alopecia, unthriftiness, lameness and diarrhea. The other ten sheep (from other flock) were clinically healthy and considered as control. Skin scrapings were taken from alopecic sheep for microscopic examination after addition of KOH 10%, but there were no mites. Two blood samples were obtained from each animal. The first sample was collected with anticoagulant for estimation of blood picture, while the other was collected without anticoagulant for separation of serum on which biochemical analysis was performed. Alopecic sheep showed a significant decrease ($P \leq 0.05$) 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. Biochemical analysis of serum showed a highly significant decrease ($P \leq 0.01$) of copper and zinc and a significant decrease ($P \leq 0.05$) of manganese, iron, total proteins, albumin and A/G ratio. On the other hand, there was a significant increase ($P \leq 0.05$) in serum ALT, AST and urea. It was concluded that alopecia syndrome commonly occurs due to multiple trace element deficiencies, particularly copper and zinc and deficiency of a single element seldom occurs under field condition.

INTRODUCTION
Deficiency of hair or wool in comparison to normal pilosity of the skin area is referred to as alopecia. There are two kinds of alopecia; one is caused by follicle dysfunction and the other by injury to the fiber as ringworm and trauma. The capacity of the follicular epithelium to produce a fiber may be congenitally defective or may temporarily be reduced because of nutritional deficiency (Radostits et al., 2000). Bacteria, virus, parasites, toxic agents, metabolic disorders and nutritional insufficiencies are important factors in the aetiology of alopecia in sheep. Among the important nutritional factors causing alopecia are deficiencies of micro and macro-elements (Akgul et al., 2000). Alopecia and deficient fiber growth are consistent outcomes of deficiencies of biotin, riboflavin, pyridoxine, folate and pantothenic acid. Copper has direct effects on the activity of an unidentified enzyme on oxidation of thiol groups to form disulphide linkage, so wool in copper-deficient sheep becomes weak, lustrous and lack crimps. Moreover, zinc, similar to copper, is required for normal keratinization of fibers (Hynd, 2000). Deficiency of a single trace element rarely occurs in domestic...
animals in the field, however a combination of mineral deficiencies is more common \cite{Hidiroglou1980}.

Zinc and Copper are very important for sheep health and production. Zinc is a constituent of numerous metalloenzymes and required for normal protein synthesis and metabolism \cite{Church1988}. Zinc deficiency may be primary due to inadequate levels in the ration or secondary as a result of the presence of a substance interfering with its absorption or metabolism, in spite of the normal diet concentration \cite{Wikse1992}. These antagonistic minerals include molybdenum, sulfur and iron \cite{Phillipo1987}.

Clinical signs of zinc deficiency include alopecia and thickening or keratinization of epithelial cells. Additional signs included growth retardation, swelling of the coronet, hock and knee joints, rough coat and congestion of the eye mucous membrane \cite{El-Attar1979,Church1988,Radostits2000}. In zinc deficiency, there was an elevation in haematocrit value. However, leukopenia and lymphocytopenia were recorded in albino rats \cite{Macapinlac1966,Miller1968}. Moreover, in zinc deficiency, there was a reduction in serum glucose, while, there was an increase in serum lipids, AST and bilirubin values \cite{Garcia-Partida1985}.

Copper is required for the activity of enzymes associated with ferrous metabolism, elastin and collagen formation, melanin production and integrity of central nervous system \cite{McDonald1984}. Copper is a cofactor of several metalloenzymes and other metalloproteins (such as ceruloplasmin, superoxide dismutase, cytochrome oxidase, lysyl oxidase, and metallothionein) \cite{Minatel2002}. Primary copper deficiency developed when the copper content of the ration is less than animal requirements. Secondary deficiency produced when copper of the ration is marginal but absorption and utilization of ingested copper is impaired by other minerals \cite{Wikse1992}. The symptoms of copper deficiency in sheep include wool abnormalities as the fine wool becomes limp and glossy and losses its crimps. Moreover, the black wool showed depigmentation. Additional signs include anaemia, scouring, swayback and bone deformities \cite{Radostits2000}. Copper deficiency affects various physiological characteristics that may be important in immunological defense against pathogenic challenge \cite{Stable1993}. Copper deficiency induces hypochromic macrocytic anemia \cite{Church1988}. Haemoglobin level and erythrocytic count are depressed in advanced cases of primary copper deficiency \cite{Radostits2000}.

This study was planned to reach a correct diagnosis of alopecia syndrome among sheep depending on the clinical and laboratory examinations, which included skin scraping and biochemical analysis of serum to estimate the levels of some blood parameters and some liver and kidney function parameters.
MATERIALS AND METHODS

Animals and experimental design
The present investigation was carried out on ten diseased Balady sheep (3-6 years old) of both sexes from a flock of sheep at Kalubia Governorate. Diseased individuals showed different degrees of alopecia, unthriftiness, lameness and diarrhea. Another ten clinically healthy Balady sheep related to another flock at the same locality fed on balanced concentrate mixture and roughage were served as control group.

Skin scrapings
Skin scrapings were taken from alopecic areas. The scraped material was mixed with 10% potassium hydroxide and then examined microscopically for detection of mites (Kelly, 1984).

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
Five ml of blood were withdrawn by puncturing the jugular vein into a test tube without anticoagulant, left to clot and centrifuged for serum separation. Commercially available diagnostic kits were used for colorimetric determination of serum calcium (Glinder and King, 1972), inorganic phosphorus (El-Merzabani et al., 1977), total protein (Doumas et al., 1981), ALT and AST (Reitman and Frankel, 1957) and urea (Patton and Crouch, 1977). Serum iron, zinc, copper and manganese were estimated by atomic absorption spectrophotometer AAS, N as described by Meret and Henkin (1971).

Electrophroesis of sera
The sera were freshly used for electrophoresis of serum proteins by Agarose Gel electrophoresis according to Alper (1974).

Statistical Analysis
Statistical analysis was performed according to Petrie and Waston (1999). A student t test was performed to compare values in the diseased group to the control group. Data were represented as means ± standard error of the mean. The difference between the means was considered significant if $P \leq 0.05$.

RESULTS AND DISCUSSION
Careful clinical examination of diseased sheep revealed the presence of alopecia at the neck, shoulder and abdomen, easily detached wool, rough wool (Fig. 1, Fig. 2 and Fig.4), steely wool Fig. (3) and achromotrichia (wool discoloration). Also, there was enlargement of knee joint. Additional signs included inappetance, poor growth, pale mucous membrane, lameness, staggering gait and diarrhea. These signs were nearly similar to those recorded by Fahmy et al. (1980), Taha et al. (1993), Metwalli et al. (1997), Mobarak (1998) and Radostitis et al. (2000). 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 Mertz,
The poly-peptide 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).

Result of skin scraping demonstrated no mite infestation and therefore hematological and biochemical analysis were subsequently conducted for further investigation to reach accurate diagnosis.

Results of blood picture (Table 1) indicated a significant decrease of the values of haemoglobin (Hb) and erythrocytic count (RBCs). This decrease might be due to disturbance in the regular metabolism of iron as copper deficiency decreases the absorption of iron, releasing of iron from body stores and utilization in haemoglobin synthesis (McDonald et al., 1984 and Church and Pond, 1988). The mean values of total leucocytic count showed a non-significant change. This result was coincided with those 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).

Biochemical analysis of serum samples of alopecic animals (Table 2) revealed a highly significant decrease in copper and zinc levels, while a significant decrease of manganese and iron was recorded. Similar results were obtained by Fahmy et al. (1980) who recorded a significant decrease in the levels of copper, zinc, manganese and iron in sheep with alopecia and wool eating. Moreover, Ali (2000) reported a significant decrease of the values of serum copper, iron, zinc, cobalt and manganese in sheep showing alopecia. On the other hand, serum calcium and inorganic phosphorus showed a non-significant change. These results coincided with those of Farahat (1994).

The values of serum total protein and albumin (Table 2) recorded a significant decrease, which was followed by a compensatory increase in the globulin levels. This result was nearly similar to those of Mobarak (1998), Akgul et al. (2000) and Radostits et al. (2000) and. This decrease of total proteins and albumin might be attributed to inappetence and albumin loss as a result of increased capillary permeability in copper-deficient and alopecic animals [O’Dell (1976) and Rucker and Tinker (1977)]. The activities of serum alanine aminotransferase and aspartate aminotransferase (Table 2) are increased significantly in
alopecic sheep. The increased activity of liver enzymes indicates liver damage. This could be explained by the results of Randhawa and Brar (1998) who demonstrated fatty changes around the central vein in the liver of hypocupraemic sheep. 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 and adversely 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.

Serum urea concentration (Table 2) shows a significant increase in alopecic sheep. This increase in urea concentration suggests that trace elements deficiency, particularly copper is associated with kidney dysfunction in sheep. The exact mechanism by which copper alters kidney function is not fully understood. However, Randhawa and Brar (1998) observed vacuolar degeneration and coagulative necrosis in the kidneys of sheep with hypocupraemia. Consequently, changes in kidney function could be attributed to these histopathological changes.

In summary, alopecia and wool abnormalities observed in sheep were attributed to copper and zinc deficiency. In addition, deficiency of iron and manganese occurs concurrently suggesting that nutritional deficiency problem is mostly caused by deficiency of more than one element and deficiency of a single element seldom occurs under field condition.

Fig. (1): A sheep showing scattered areas of alopecia at neck, shoulder and abdomen (black arrows).
Fig. (2): A sheep showing alopecia (black arrow) and rough wool.

Fig. (3): (A) showing steely wool, and (B) demonstrating wool with normal crimps from apparently healthy ram.

Fig. (4): A lamb showing loss of hair at the knee joints (arrows).

Table (1): Haemoglobin, erythrocytic count, leucocytic count and differential leucocytic count in alopecic and control sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control sheep</th>
<th>Alopecic sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Hb) gm/dl</td>
<td>12.60 ± 0.24</td>
<td>8.40 ± 0.47*</td>
</tr>
<tr>
<td>Erythrocytic count (RBCs) 10^6/C mm</td>
<td>11.97 ± 0.25</td>
<td>8.16 ± 0.19*</td>
</tr>
<tr>
<td>White blood cells count (WBCs) 10^3/C mm</td>
<td>9.30 ± 0.25</td>
<td>8.87 ± 0.17</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>55.60 ± 2.30</td>
<td>48.30 ± 1.90</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>2.40 ± 0.28</td>
<td>2.70 ± 0.33</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>40.20 ± 2.10</td>
<td>47.10 ± 2.10</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>1.80 ± 0.35</td>
<td>1.90 ± 0.29</td>
</tr>
<tr>
<td>Basophils %</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The statistical test is the student $t$ test. Data are presented as means ± SE. * denotes significant different from control at $P \leq 0.05$
Table (2): Some biochemical parameters in the sera of alopecic and control sheep.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control sheep</th>
<th>Alopecic sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (µg/dl)</td>
<td>95.66 ± 3.1</td>
<td>38.30** ± 6.32</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>95.7 ± 2.85</td>
<td>60.63** ± 5.27</td>
</tr>
<tr>
<td>Manganese (µg/dl)</td>
<td>3.80 ± 0.37</td>
<td>2.96* ± 0.15</td>
</tr>
<tr>
<td>Iron (µg/dl)</td>
<td>105.75 ± 5.60</td>
<td>81.08* ± 4.55</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.62 ± 0.58</td>
<td>9.80 ± 0.51</td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/dl)</td>
<td>5.91 ± 0.21</td>
<td>6.14 ± 0.31</td>
</tr>
<tr>
<td>Total protein (gm/dl)</td>
<td>6.38 ± 0.33</td>
<td>5.04* ± 0.28</td>
</tr>
<tr>
<td>Alanine aminotransferase (µ/L)</td>
<td>52.71 ± 1.13</td>
<td>88.10* ± 2.59</td>
</tr>
<tr>
<td>Aspartate aminotransferase (µ/L)</td>
<td>38.96 ± 2.22</td>
<td>59.89* ± 3.00</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>32.45 ± 2.15</td>
<td>48.58* ± 3.16</td>
</tr>
</tbody>
</table>

The statistical test is the student t test. Data are presented as means ± SE
** denotes highly significant different from control at P ≤ 0.01
* denotes significant different from control at P ≤ 0.05

Table (3): Serum electrophoretic pattern in the sera of alopecic and control sheep.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control sheep</th>
<th>Alopecic sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (%)</td>
<td>37.28 ± 1.23</td>
<td>33.26* ± 0.92</td>
</tr>
<tr>
<td>Alpha globulins (%)</td>
<td>18.62 ± 0.90</td>
<td>20.12 ± 1.16</td>
</tr>
<tr>
<td>α1-globulin (%)</td>
<td>8.10 ± 0.65</td>
<td>9.41 ± 0.42</td>
</tr>
<tr>
<td>α2-globulin (%)</td>
<td>10.52 ± 0.67</td>
<td>10.71 ± 0.83</td>
</tr>
<tr>
<td>Beta globulins (%)</td>
<td>11.64 ± 0.78</td>
<td>12.15 ± 0.83</td>
</tr>
<tr>
<td>β1-globulin (%)</td>
<td>5.76 ± 0.54</td>
<td>6.83 ± 0.66</td>
</tr>
<tr>
<td>β2-globulin (%)</td>
<td>5.88 ± 0.36</td>
<td>5.32 ± 0.41</td>
</tr>
<tr>
<td>Gamma globulins (%)</td>
<td>32.45 ± 1.38</td>
<td>34.35 ± 1.18</td>
</tr>
<tr>
<td>χ1-globulin (%)</td>
<td>24.28 ± 1.04</td>
<td>26.35 ± 0.85</td>
</tr>
<tr>
<td>χ2-globulin (%)</td>
<td>8.17 ± 0.56</td>
<td>8.00 ± 0.44</td>
</tr>
<tr>
<td>Total globulins (%)</td>
<td>62.72 ± 1.23</td>
<td>66.62* ± 0.92</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.59 ± 0.03</td>
<td>0.50* ± 0.02</td>
</tr>
</tbody>
</table>

The statistical test is the student t test. Data are presented as means ± SE
** denotes highly significant different from control at P ≤ 0.01
* denotes significant different from control at P ≤ 0.05
REFERENCES


Randhawa, S.S. and Brar, R.S. (1998): Pathological studies in molybdenum induced hypocuprosis in
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