Behavioral and Biochemical Alterations in Sheep With Trace Elements Deficiency: A Trial for Treatment

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Abstract

Introduction: Micro-minerals imbalances have a great impact on animal’s physiology and cause various problems. Trace mineral deficiency is not recognized easily, especially in the sheep and goats as the deficiency exists in the flocks in sub-clinical form and affects the fertility and animal welfare.

Methods: This study was carried out to detect trace elements deficiency through behavioral observation, haematological and biochemical analysis in sheep with a trial of treatment with a trace elements mixture.

Results: A total number of fifteen diseased baladi ewes were used, blood samples were obtained at the beginning and also ewes were behaviorally observed two weeks before treatment for ascertain trace elements deficiency. Ewes were then treated with mineral mixture drug for two weeks during which they were behaviorally observed. Blood samples were obtained again after treatment for haematological and biochemical analysis. The obtained results following treatment showed significant increased drinking and licking frequencies; copper, cobalt, selenium and magnesium levels and also haemoglobin and red blood cells count, while feeding and lateral recumbancy time, movement frequency, zinc and calcium levels and also white blood cells count and packed cell volume percent were non-significant increased. Furthermore, rumination and standing time and iron and phosphorus levels were non-significantly decreased. However, sodium and potassium levels were significantly decreased.

Conclusions: It can be concluded that trace elements deficiency in sheep could cause behavioural, haematological and biochemical changes which respond to treatment but need long time for complete cures so it is recommended to provide trace element supplementation in ration.


INTRODUCTION

Animal welfare is about what animals experience but it has trade and political aspects. Veterinarians and animal scientists use a range of measurements to assess welfare. The criteria for animal welfare as defined by the European Welfare Quality Project including good housing, nutrition, health and opportunities to behave appropriately [1]. What animals experience cannot be measured and animal scientists and veterinarians use a range of parameters to measure animal nutrition, health, physical comfort and animal behaviour [2]. The role of veterinarian on a large sheep farm may be focused on disease prevention and improved productivity and profitability and this might result in a focus on preventive conditions such as trace element deficiencies rather than treating such problems. Production of small ruminants considered the main economic output, contributing into the farmer’s income in many parts of Asia and Africa [3]. Deficiency of trace minerals can affect the production, and the performance of sheep and goat [4, 5]. Trace mineral deficiencies are more common in the small ruminants reared under the traditional system and/or...
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grazing poor quality pastures [6, 7]. Trace mineral deficiency is not recognized easily, especially in the sheep and goats as the deficiency exists in the flocks in sub-clinical form and affects the fertility, the wool yield, and the growth rate [8]. Also, trace mineral deficiency causes abortion, placental retention, death of lambs, ill thrift, reduction in immunity, increased susceptibility to bacterial, parasitic infections, and affects the animal welfare [9]. Deficiencies of trace elements such as cobalt, copper, iron, zinc, and selenium had been reported to occur in all the climatic zones, and the geographic regions of the world [10].

Cobalt is an element constituent of cobalamine or vitamin B12 [11], which has been recognized for its role in the formation of the red blood cells, and the maintenance of the nerve tissue [12]. Cobalt deficiency in the ruminants is associated with listlessness, reduced milk production, tough hair and coat with low breaking strength, anemia resulting in pale skin, and mucous membrane which followed by emaciation of muscle, incoordination, stumbling of gait, and death [13]. Copper is a vital component as cofactor in many enzymatic systems, and is required in the body for the production of the red blood cells as it is essential for absorption, and transport of iron necessary for hemoglobin synthesis [14]. Copper deficiency is associated with anemia, diarrhea, retarded growth, changes in the color of fur, fragility of long bones, and even death [15].

The iron is a major structural component of hemoglobin, and directly required for the erythropoiesis [16]. Iron deficiency anemia could cause low birth weight, pre-term delivery, and increased oxidative damage to erythrocytes in placenta-fetus unites [17]. Zinc is an important element in the animal feeding, since it plays an important role in the synthesis of hormones [18]. Zinc deficiency results in decline in the feed consumption, lack of appetite, and disruptions in synthesis of proteins containing amino acids with sulphur content [19]. Selenium, together with vitamin E, participates in antioxidant protection of organisms, as it is a constituent of glutathione peroxidase enzyme. Selenium also plays an important role in the immune system, and the metabolism of thyroid hormones. It is also crucial for the reproduction [20-22].

The hypothesis was that trace elements deficiency could affect animal health and welfare without being detected. The aim of the present study was to detect trace elements deficiency through behavioral observation, haematological and biochemical analysis in Egyptian sheep. In addition, the efficacy of the supplementary treatment to correct the deficiency was another target.

METHODS

Ethical Approval

Experimental procedures were conducted in accordance with the Alexandria University Animal Ethics Committee guidelines. This research was conducted with strict rules to preserve and safeguard the animal welfare without subjecting them to any degree of suffering or stress.

Animals and Treatment

The study was done in September, 2013 on a total number of fifteen diseased Baladi Ewes (four to five years old) randomly selected from a flock of sheep at a governmental farm in Abes, Alexandria city, Egypt, marked by paint spray on their back. Clinical examination of affected ewes showed that body condition was mild to moderately debilitated, body temperature ranged from 38.5-39.5 º C, visible mucous membranes were pale and anemic, anorexia, wool affections including loss of wool crimp, steely appearance, loss of wool and depigmentation and enlargement of knee and hock joints which was diagnosed as mineral deficiency as shown in Figs 1a and 1b. The sheep were housed in concrete floor building with asbestos ceiling under natural light cycle and grazing on pasture with a minimal amount of concentrates after getting indoor ad libitum with free access to water. The diseased sheep were treated with a drug FOS (Ragab Pharm Industries, Egypt) containing copper sulphate, cobalt carbonate, zinc oxide, iron sulphate, sodium selenite, manganese sulphate and phosphoric acid and was given orally to ewes by a therapeutic dose of 5 ml/head daily for two successive weeks.

Figure 1: Ewes Showing Loss of Wool Crimp and Steely Appearance
Behavioral Observation

Focal sample observation was carried out according to Martin et al. [23] for ingestive, body care, resting and locomotor behavior. These were done for two days per week during two weeks before treatment and also for another two weeks during treatment.

Haematological and Biochemical Measures

Blood samples were collected by jugular vein puncture at the beginning of the study to ascertain mineral deficiency and after the two weeks of treatment, two tubes were taken, one tube containing EDTA for haematological evaluation and another plain vacationer tube for serum biochemical analysis of major and trace-elements. Haemoglobin content (Hb), packed cell volume (PCV), red blood cell count (RBCS) and white blood cell count (WBSCS) were determined according to Stockham et al. [24]. Biochemical analysis were done for determination of serum levels of copper, cobalt, zinc, iron and selenium by atomic absorption spectrophotometer (AAS) as described by Meret and Henkin [25]. Also, the level of magnesium, calcium, phosphorus, sodium and potassium determined by biochemical analyzer (ERMA, Japan) using commercially available kits (Biomedical Systems, Barcelona, Spain). The analyses were carried out according to the manufacturer’s recommendations.

Fecal Examination and Skin Scraping

Fecal examination and skin scraping were carried out according to the method described by Kelly [26] for detection of any internal or external parasites.

Statistical Analysis

Behavioral, haematological and biochemical data were analyzed by student t-test by SAS (Statistical Analysis system, version 6, 4th Edition, SAS Institute, Cary, NC. USA.), proc T. Data was expressed as means ± S.E.M., n = 15 and P-values < 0.05 were considered significant in all tests, unless stated otherwise.

RESULTS

Behavioral Observation

The obtained results from the behavioral observation showed in Table 1 revealed a highly significant increase in drinking, and licking frequencies, while there was a non-significant increase in feeding, and lateral recumbancy time, and movement frequency after treatment. However, rumination and standing time were decreased non-significantly after treatment.

Haematological and Biochemical Parameters

The data obtained from haematological examination shown in Table 2 revealed that there was a highly significant increase in hemoglobin level and red blood cells count after treatment with non-significant increment in packed cell volume percent and white blood cells count. However, the results obtained from biochemical analysis showed a highly significant increase in the levels of copper, magnesium, selenium and cobalt after treatment of the affected ewes. Moreover, a non-significant increase in the levels of zinc and calcium were reported after treatment of the affected animals, while there was a non-significant decrease in the levels of iron and phosphorus and a significant decrease in serum sodium and potassium levels (Table 3).

### Table 1: Least Square Means and Their Standard Error to the Effect of Trace Element Deficiency Before and After Treatment on Behavior of Sheep

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>After</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingestive Behavior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding, min/h</td>
<td>29.08 ± 1.31</td>
<td>30.46 ± 1.64</td>
<td>1.38 ± 1.74</td>
</tr>
<tr>
<td>Drinking, Freq/h</td>
<td>0.63 ± 0.07</td>
<td>0.89 ± 0.08</td>
<td>0.26 ± 0.09 *</td>
</tr>
<tr>
<td>Rummation, min/h</td>
<td>9.96 ± 1.36</td>
<td>8.08 ± 1.44</td>
<td>1.88 ± 2.11</td>
</tr>
<tr>
<td>Body Care Behavior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Licking, Freq/h</td>
<td>1.18 ± 0.16</td>
<td>2.51 ± 0.27</td>
<td>1.33 ± 0.29 *</td>
</tr>
<tr>
<td>Scratching, Freq/h</td>
<td>0.22 ± 0.06</td>
<td>0.22 ± 0.05</td>
<td>0.00 ± 0.07</td>
</tr>
<tr>
<td>Resting Behavior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral Recumbancy, min/h</td>
<td>22.79 ± 1.97</td>
<td>24.21 ± 1.79</td>
<td>1.42 ± 1.96</td>
</tr>
<tr>
<td>Standing Ideal, min/h</td>
<td>15.25 ± 2.06</td>
<td>11.75 ± 1.54</td>
<td>3.50 ± 2.04</td>
</tr>
<tr>
<td>Locomotor behavior, Freq/h</td>
<td>5.63 ± 0.34</td>
<td>6.03 ± 0.37</td>
<td>0.40 ± 0.40</td>
</tr>
</tbody>
</table>

Means significant (P < 0.05).
* Means highly significant (P < 0.01).

### Table 2: Least Square Means and Their Standard Error to the Effect of Trace Element Deficiency Before and After Treatment on Haematological Parameters of Sheep

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>After</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin, g/L</td>
<td>8.33 ± 0.09</td>
<td>9.03 ± 0.18</td>
<td>0.71 ± 0.16 *</td>
</tr>
<tr>
<td>RBCS, 10⁶/C mm</td>
<td>8.43 ± 0.12</td>
<td>9.23 ± 0.28</td>
<td>0.80 ± 0.23 *</td>
</tr>
<tr>
<td>WBCS, 10³/C mm</td>
<td>12.37 ± 0.55</td>
<td>12.49 ± 0.50</td>
<td>0.12 ± 0.47</td>
</tr>
<tr>
<td>Packed Cell Volume, %</td>
<td>33.63 ± 1.22</td>
<td>35.83 ± 1.07</td>
<td>2.21 ± 1.22</td>
</tr>
</tbody>
</table>

Abbreviations: RBCs, red blood cell count; WBCs, white blood cells count.
Means significant (P < 0.05)
* Means highly significant (P < 0.01).
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Table 3: Least Square Means and Their Standard Error to the Effect of trace Element Deficiency Before and After Treatment on Biochemical Parameters of Sheep

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>After</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, µg/dL</td>
<td>101.65 ± 2.42</td>
<td>142.89 ± 4.45</td>
<td>41.25 ± 3.38 *</td>
</tr>
<tr>
<td>Zinc, µg/dL</td>
<td>58.43 ± 1.83</td>
<td>61.71 ± 2.54</td>
<td>3.27 ± 1.85</td>
</tr>
<tr>
<td>Selenium, µg/dL</td>
<td>3.36 ± 0.16</td>
<td>4.78 ± 0.33</td>
<td>1.42 ± 0.37 *</td>
</tr>
<tr>
<td>Cobalt, µg/dL</td>
<td>1.44 ± 0.11</td>
<td>3.75 ± 0.32</td>
<td>2.31 ± 0.33 *</td>
</tr>
<tr>
<td>Iron, µg/dL</td>
<td>96.43 ± 0.96</td>
<td>93.10 ± 1.70</td>
<td>3.33 ± 2.17</td>
</tr>
<tr>
<td>Magnesium, mg/dL</td>
<td>2.03 ± 0.05</td>
<td>2.58 ± 0.07</td>
<td>0.55 ± 0.09 *</td>
</tr>
<tr>
<td>Potassium, mg/dL</td>
<td>4.05 ± 0.08</td>
<td>3.36 ± 0.06</td>
<td>0.69 ± 0.09 *</td>
</tr>
<tr>
<td>Sodium, mg/dL</td>
<td>109.49 ± 0.99</td>
<td>90.16 ± 1.69</td>
<td>19.33 ± 1.44 *</td>
</tr>
<tr>
<td>Phosphorous, mg/dL</td>
<td>4.75 ± 0.08</td>
<td>4.62 ± 0.07</td>
<td>0.13 ± 0.08</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>6.23 ± 0.09</td>
<td>6.49 ± 0.14</td>
<td>0.26 ± 0.14</td>
</tr>
</tbody>
</table>

Means significant (P < 0.05).
* Means highly significant (P < 0.01).

Fecal Examination and Skin Scraping

The results of fecal examination and skin scraping deduced the absence of internal parasites and mite infestation.

DISCUSSION

Abnormal behavior may be the first indicator for a problem with an individual sheep or the whole flock. It is a more sensitive measure than other factors indicating poor welfare such as injury, disease, and reduced intakes and is often used to assess welfare. Such abnormal behaviors observed in the sheep include lethargy, becoming uninterested in feeding, increased vocalization, isolation of individuals from the flock, pica, restlessness and an increased respiration rate [27]. The clinical signs recorded in the present study aroused suspicion of trace elements deficiency in the diseased animals including unthriftness, alopecia, inappetance to anorexia, debilitation, anaemia, and locomotor disturbances, which was agreed with Radostits et al. [28]. Sheep are social animals; they flock, walk, run, graze, and bed down together which are usually initiated, and led by the oldest ewe [29]. They are also grazing animals which would normally spend most of their time seeking and selecting food, eating, and ruminating. The stressed sheep will stop ruminating, and observation of the time spent occupied in this behavior is an indicator of good welfare which could explain the decrease in ruminating time of sheep. Moreover, the decreased feeding time could be attributed to the impaired propionate metabolism resulting from cobalt deficiency leading to higher blood propionate concentrations which inhibit appetite until their elimination from the body, they could be nitrogenous metabolites of protein [36]. This finding was agreed with Wallwork and Sandstead [37] who found after three to four days of zinc deprivation, high food consumption for one to two days followed by low food intake for one to two days. However, Apgar and Fitzgerald [38] stated that the zinc deficient ewes that carried their pregnancies to term increased feed consumption immediately after delivery, therefore there was no increase in intake by these ewes with zinc supplementation. On the other hand, the increment in licking frequency after treatment may be attributed to the improvement in the metabolism especially energy metabolism which was disturbed by cobalt deficiency and lead to a decrease in the body care behavior of animal. The brain tightly regulates metals trafficking as an important part of its normal activity and disruption of this delicate equilibrium may have detrimental effects for the whole brain functioning [39]. Moreover, Sandstead et al. [40] showed that zinc deprivation resulted in anorexia and less activity which explained the inappetance and decreased locomotor. On the other hand, locomotor disturbances with enlargement of hock and knee joints might be regarded to deficiency of zinc, copper and manganese [41]. Moreover, the less improvement in the feeding, locomotor and resting behavior may be due to no significant increase in zinc level after supplementation although there was increment in copper and cobalt level after treatment.

Lozoff [42] showed that iron deficient animals are hypoactive and decreased physical activity and impaired skeletal motor activity are thought to be due to altered dopaminergic function and these agreed with Kim et al. [43] who re...
ported that motor impairment in iron deficient rats. 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, these explains the bone disorder present in the study and defects present in locomotion and resting behaviour [44]. There were also changes in behavior in response to zinc-iron deprivation characterized by lower activity levels and the less change after supplementation might be due to that plasma iron concentrations did not recover in response to supplementation and complete recovery of iron status would occur after a longer period of supplementation [45]. Furthermore, the highly significant increment in drinking frequency might be attributed to several factors including the increase in feeding, feed on concentrates and the high temperature during experiment. Copper has a multiple function as iron absorption, hae mopoeis, and various enzyme activities and in the oxidation-reduction process [46]. Furthermore, unthriftiness and anemia; manifested by paleness of visible mucous membranes, could be attributed to iron, zinc and copper deficiency [28]. The decreased level of hemoglobin and red blood cells count in diseased ewes could be based on the role of copper in production of hemoglobin through the re-utilization of iron liberated from normal breakdown of haemoglobin [28]. Moreover, these results were agreed with Church and Pond [47] who reported that copper deficiency decrease the absorption of iron, releasing iron from body stores, and utilization in hemoglobin synthesis. Thus the increment in hemoglobin level, and red blood cells count after treatment could be explained by the finding of Lominadze et al. [48] who stated that copper administration can cause significant increase in hemoglobin and serum copper level. Abou-Zeina et al. [32] suggested that the deceased hemoglobin level and red blood cells count might be a prominent feature of cobalt deficiency which improved after treatment of lambs with cobalt for four weeks. This confirms the essential role of vit.B12 and folate for the production of heme. Reduced activity of heme-enzyme; catalase may possibly result from a decreased formation of succinyl-Co A, necessary for heme synthesis via the co-balalmine-dependent methyl-Co A mutase [49]. Moreover, vitamin B12 co-operates with folate in the synthesis of deoxyribonucleic acid so, a deficiency of either compounds led to disturbed production of DNA and impaired production of red blood cells and causes anemia. This explanation was agreed with the obtained results in which the improvement in the level of hemoglobin and red blood cells count after treatment with drug containing cobalt [50] reported that the decreased number of white blood cells may be attributed to the stress of cobalt deficiency which caused nutritional and metabolic disturbances and stimulate the adrenal gland to secrete corticosteroid hormone resulting in sustained change in leukocyte numbers, and theses decrease was improved after treatment with cobalt. On the other hand, Golub et al. [45] found that marginal iron and zinc deprivation can lead to behavioral and hematologic dysfunction.

The lower serum selenium level in affected ewes was in agreement with Maas et al. [51] who reported that not all selenium-deficient animals develop the white muscle disease and many selenium-deficient animals never show clinical signs. Also, Lopez et al. [52] found that serum selenium concentration was below normal in pregnant ewes from selenium deficient region in Spain but no pathology was associated with selenium deficiency and lambs had no symptoms of nutritional myopathy. Furthermore, the non-significant decrease in iron level could be attributed to that iron is required for the synthesis of hemoglobin and myoglobin as well as many enzymes and cytochrome enzymes of electron transport chain also iron functions in transport of oxygen to tissues, maintenance of oxidative enzyme system and is concerned with ferritin formation so it is used in the correction of anemia caused by copper deficiency [53]. Serum magnesium level was significantly increased after treatment of affected ewes which agreed with Eades and Bounous [54] who mentioned that magnesium concentration are diet dependent and its balance is primarily affected by intestinal absorption and renal excretion. Moreover, the non-significant increase in zinc level after treatment agreed with the finding of Mandour [55] and disagreed with Schwarz et al. [56] who stated that there was inverse relationship between zinc and copper in copper deficiency. One possibility accounting for these differences may be the absorption and bioavailability of zinc in various supplements at the site of small intestine for animals as it has been accepted that zinc sulfate provided more available zinc than other forms like zinc oxide [57]. However, the mean value of serum calcium and inorganic phosphorus revealed a non-significant changes pre and post treatment of affected ewes that agree with earlier studies [58-60]. While, wool abnormalities were usually related to deficiency of copper and zinc [47]. As copper has direct effects on the activity of an unidentified enzyme on oxidation of thiol groups to form disulfide linkage, so wool in copper-deficient sheep becomes weak, lustrous and lack crimps [61]. However, the mechanism of poor wool growth observed in zinc deficiency involved impaired protein synthesis [35]. Moreover, the wool eating may be for receiving additional zinc due the difference in the availability of zinc [38]. It can be concluded that trace elements deficiency in sheep could cause behavioural, haematological and biochemical changes which respond to treatment but need long time for complete curns so it is recommended to provide trace element supplementation in ration to avoid deficiency of such element particularly in trace elements deficient soil.

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CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

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