Relationship of some Trace Elements (Copper and Zinc) in the Blood and Wool in Egyptian Sheep

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Abstract
The present study aimed to determine the levels of zinc (Zn) and copper (Cu) in serum and wool of sheep during different physiological states and the correlation coefficient between them. For this purpose, serum and wool samples were taken from sheep of different ages, sex and different physiological state. The concentration of Zn and Cu in serum and wool samples were measured. The result of the study showed that there was minor fluctuation in the levels of Zn and Cu in wool in the two groups. In present study, when making correlation groups of animals founding that there is a strong linear positive correlation between Cu in serum and Cu in wool of male animals in both groups A and B (significant at 0.05), and there is a strong linear negative correlation in non-pregnant animals of both groups. In present study, when making correlation between Zn levels in serum and Zn in wool in all groups of animals founding that there is a strong linear positive correlation between Zn in serum and Zn in wool in non-pregnant animals of group A (significant at 0.01), there is mild linear positive correlation in dam animals of group B and there is a strong linear negative correlation in lambs (significant at 0.05), male animals and non-pregnant animals of group B (significant at 0.01).

Keywords
Copper, Correlation, Sheep, Wool, Zinc

1. Introduction
Sheep fleece, similar to other animal’s coat, present a special chemical indicator, and a concentration of the elements it reflects both feed and nutrition quality, as well as climate and the environment. Many other factors, such as breed, age, sex, physiological composition of wool and coats can change the chemical composition of the wool and coat. (Ramirez-Perez et al., 2000). As well, Zarski (1988), confirmed significant correlations between Fe, Mg, Zn and Na concentration in the hair and liver of doe deer. However, Hair is known to be a good bio indicator of the state of the environment, air pollution, water, and soil better than blood, urine or milk. Enne et al., (1989) reported high concentrations of toxic metals (Cu, Zn, Cd, pb) in sheep wool from mining region in Italy.

Zinc and copper have been demonstrated to be essential micronutrients for ruminants (Selim et al., 2020). Knowing the concentration of these elements in plasma and wool is very important to manipulate their concentration in the diet. On the other hand, there has been some research concerning effects of season and pasture on blood serum and wool samples of native sheep, but no study was found to determine Zn and Cu level of blood and wool of sheep at different physiological phases, to manipulate their diets. Therefore, this study aimed to determine Zn and Cu levels of serum and wool of Egyptian sheep at different physiological status.

2. Materials and Methods
2.1. Animals
100 animals were used for the current study. The animals are present in two groups from two different farms (each group contains 50 animals); The Farm of faculty of veterinary medicine, Beni-Suef University (group A) and a private farm in Beni-Suef province (group B). Each group of them subdivided into 5 groups (each one of them contain 10 animals):

- **Group I**: lambs (In the first 7 days after lambing).
- **Group II**: adult male sheep (2–4 years).
- **Group III**: adult non-pregnant female ewes (2–4 years).
- **Group IV**: pregnant female ewes (2–4 years).
- **Group V**: dams (lactating sheep) (2–4 years).
2.2. Diets
Group A, Sheep was fed on commercial concentrate mixture and yellow corn. The commercial concentrate mixture contained of 16% crude protein, 15% crude fiber, 12% ash, 12% moisture, 2% ether extract, and 65% total digestible nutrients. In summer, sheep in group B was daily fed one kg commercial concentrate mixture and about 0.5 kg barseem hay. Rice straw was added ad- libitum. In winter, sheep were fed the identical diets, but with replacement of hay with fresh barseem (about 700g/animal/day). As well, before the breeding season, the Diet changed by increasing the commercial concentrate mixture up to (1.50kg/sheep/day) while the amount of other elements was similar as before. The commercial concentrate mixture formed of 14% crude protein, 15% crude fiber, 9% ash, 12% moisture, 2% ether extract, and 65% total digestible nutrients. The diets were allowed to sheep as a percentage of body weight in consonance with NRC (National research council) of sheep (2007).

2.3. Sample Collection
2.3.1. Serum Samples
The samples of blood were obtained from each animal through jugular vein and collected in a sterile dry centrifuge tube for gathering a non-hemolysed serum. The blood samples were centrifuged at 3000rpm for 10min. The clear serum was separated and kept in a sterile dry Ependorf tubes at -20°C for evaluating the levels of Cu and Zn. The serum Zn was estimated using test kits supplied by Biodiagnostic, according to the method described by Sharma and Singh (2009). The serum Cu was estimated using test kits provided by Biodiagnostic, in consonance with the method reported by Abe et al., (1989).

2.3.2. Wool Samples
Wool samples were obtained from the para lumbar fossa using stainless steel sheep clippers. Feed and other foreign particles were removed from the wool and washed by warm water, then they were soaked into ethanol for 5min to remove the organic dusts and over dried at 60°C for at least 24h.

2.3.3. Microwave Digestion of Wool Samples
For selected mineral analysis, 1ml of wool samples was pitted into a vessel, resistant to temperature and pressure, with 8ml nitric acid and 2ml of hydrogen peroxide for microwave digestion. The digested sample was allowed to cool before quantitively transferred into clean falcon tubes and completed to 25ml. The final volume with deionized water and analyzed in the flame atomic absorption spectrophotometer (Aydin, 2008).

2.4. Statistical Analysis
Zinc and copper contents of serum and wool samples were analyzed statistically using SAS. The data of plasma and wool minerals were subjected to one way ANOVA. Differences between means were considered significant at p<0.01. Also we made a correlation between the Cu and Zn levels in blood and wool.

3. Results and Discussion
Concerning the serum Zn levels (Table 1, Fig. 1), it is significantly lower (p<0.01) in males (96.2±9.9) than other sheep in group A. While the serum level of Zn is significantly higher (p<0.01) in lambs (132.5±1.4) than other sheep in group B and these results were in harmony with Jeffery (2006) who said that, the “normal range” for Zn and Cu in body tissues would be higher in early newborn animals than in an adult animal which may be assigned to accumulation of these elements in feti at different levels during gestation, necessitating adequate aging of the fetus for interpretation. In addition, these elements, for which little amount is secreted in milk, accumulate at higher concentrations during gestation to provide newborn animals with sufficient body reserves for survival until foraging. By comparing between the two groups, we observed that its level was significantly higher (p<0.01) in males and lambs in group B than A and significantly lower (p<0.01) in pregnant ewes and dam in group B than A.

As mentioned in Table (1) and Fig (2), we found that serum Cu level was significantly higher (p<0.01) in male animals (20.3±1) than other sheep in group A. While its level was significantly higher (p<0.01) in non-pregnant ewes (18.1±2.7) than other sheep in group B. These results were agreed with Chhabra (2006) who found that no influence of age on blood Cu concentrations and were not agreed with Jeffery (2006). By comparing between the 2 groups, we observed that serum Cu level was significantly lower (p<0.01) in sheep of group B than group A, and these may be attributed to difference in breed and ration between the 2 groups.

Concerning the concentrations of Zn in wool (Table 2, Fig. 3) there were minor fluctuation throughout the study in the 2 groups and the ranges were (0.069±0.030 – 0.128±0.021 ppm/g) and (0.065±0.001 – 0.087±0.017ppm/g) in group A and B respectively. By comparing between the two groups, found that the concentrations of Zn in wool were significantly higher (p<0.01) in all animals of group A than group B except in males and pregnant ewes, their levels were lower in group A than B. Investigations by Patkowska-Sokola et al., (2009), Zn level ranged from 73.62 to 88.80 mg/kg in the wool of sheep, while Patkowska-Sokola et al., (2003) denoted that Zn concentration in sheep wool covers a wide range from 18.3 to 336.9 mg/ kg. In consonance with Scott (1999), a normal Zn content in sheep wool ranges from 35 to 195mg/kg depending on its concentration in animal diet and the physiological state of sheep.

Concerning the levels of Cu in wool (Table 2, Fig.4) there were minor fluctuation throughout the study in the two groups and the ranges were (0.007±0.003 – 0.09±0.004 ppm/g) and (0.008±0.001 – 0.029±0.008ppm/g) in group A and B respectively. In our study, we found that the highest level of Cu in wool in the 2 groups was in male animals and its level was significantly higher (p<0.01) in males of group A than group B.

A number of authors estimated Cu concentration in the wool of sheep, Patkowska-Sokola (2009) reported that the Cu...
level ranged from 5.30 to 10.30 mg/kg in the wool of sheep, while Patkowska-Sokola et al., (2003) proved that Cu level in sheep wool covers a wide range from 1.70 to 25 mg/kg. Ramirez-Perez et al., (2000) report that to a large degree, it depends on its concentration in animal diet and low Cu level in serum can affect its diminished content in wool.

In the present study, when we make correlation in all groups of sheep (Table, 3) found that there is no significant linear correlation between Zn in serum and Zn in wool in lambs, male animals and dams of group A and pregnant females of both groups, there is strong linear positive correlation between Zn in serum and Zn in wool in non-pregnant animals of group A (significant at 0.01), there is mild linear positive correlation in dams of group B and there is strong linear negative correlation in lambs (significant at 0.05), male animals and non-pregnant ewes of group B (significant at 0.01).

In the present study, when we make correlation in all groups of sheep (Table, 3) found that there is no significant linear correlation between Cu in serum and Cu in wool of lambs, pregnant females and dams of both groups A and B, there is strong linear positive correlation between Cu in serum and Cu in wool of male animals of both groups (significant at 0.05), and there is strong linear negative correlation in non-pregnant animals of both groups A (significant at 0.01) and B (significant at 0.05).

Table 1. Serum Zn and Cu concentrations of sheep at different physiological phases in two farms in group A, B (means ± S.D).

<table>
<thead>
<tr>
<th>Group</th>
<th>Lambs</th>
<th>Male</th>
<th>None pregnant female</th>
<th>Pregnant female</th>
<th>Dam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (Ug/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>98.8±7.0*</td>
<td>96.2±9.9*</td>
<td>108.0±15.3*</td>
<td>105.2±7.4**</td>
<td>104.6±9.7**</td>
</tr>
<tr>
<td>B</td>
<td>132.5±14*</td>
<td>106.2±11.8*</td>
<td>105.4±8.6*</td>
<td>98.2±5.9*</td>
<td>88.9±1.7*</td>
</tr>
<tr>
<td>Cu (Mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>13.7±1.3*</td>
<td>20.3±1.0*</td>
<td>18.9±2.8**</td>
<td>18.8±0.9*</td>
<td>11.9±0.7*</td>
</tr>
<tr>
<td>B</td>
<td>12.8±0.9*</td>
<td>14.7±3.0*</td>
<td>18.1±2.7*</td>
<td>11.7±1.2*</td>
<td>10.6±0.7*</td>
</tr>
</tbody>
</table>

The means with different letters are significant at 0.01, Group A: sheep in farm of Faculty of Veterinary Medicine, Beni-Suef University, and Group B: sheep in private farm in Beni-Suef province.

Table 2. Wool Zn and Cu concentrations of sheep at different physiological phases in two farms in group A, B (means ± S.D).

<table>
<thead>
<tr>
<th>Group</th>
<th>Lambs</th>
<th>Male</th>
<th>None pregnant female</th>
<th>Pregnant female</th>
<th>Dam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (Hair Ppm/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.102±0.003*</td>
<td>0.069±0.030*</td>
<td>0.105±0.037*</td>
<td>0.079±0.005*</td>
<td>0.128±0.021*</td>
</tr>
<tr>
<td>B</td>
<td>0.065±0.001*</td>
<td>0.087±0.017*</td>
<td>0.071±0.010*</td>
<td>0.089±0.002*</td>
<td>0.075±0.001*</td>
</tr>
<tr>
<td>Cu (Hair Ppm/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.011±0.001*</td>
<td>0.090±0.004*</td>
<td>0.008±0.003*</td>
<td>0.011±0.003*</td>
<td>0.007±0.002*</td>
</tr>
<tr>
<td>B</td>
<td>0.012±0.001*</td>
<td>0.029±0.008*</td>
<td>0.008±0.001*</td>
<td>0.013±0.003*</td>
<td>0.013±0.002*</td>
</tr>
</tbody>
</table>

The means with different letters are significant at 0.01, Group A: sheep in farm of Faculty of Veterinary Medicine, Beni-Suef University, and Group B: sheep in private farm in Beni-Suef province.

Table 3. Correlation coefficient among Zn and Cu in serum and wool samples at different physiological phases.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zn (r (correlation coefficient))</th>
<th>Cu (r (correlation coefficient))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb (Group A)</td>
<td>0.338</td>
<td>0.460</td>
</tr>
<tr>
<td>Lamb (Group B)</td>
<td>-0.661*</td>
<td>-0.009</td>
</tr>
<tr>
<td>Male (Group A)</td>
<td>-0.614</td>
<td>0.734*</td>
</tr>
<tr>
<td>Male (Group B)</td>
<td>-0.945**</td>
<td>0.686*</td>
</tr>
<tr>
<td>Non-pregnant (Group A)</td>
<td>0.803**</td>
<td>-0.859**</td>
</tr>
<tr>
<td>Non-pregnant (Group B)</td>
<td>-0.885**</td>
<td>-0.762*</td>
</tr>
<tr>
<td>Pregnant ewe (Group A)</td>
<td>0.572</td>
<td>0.223</td>
</tr>
<tr>
<td>Pregnant ewe (Group B)</td>
<td>0.479</td>
<td>0.539</td>
</tr>
<tr>
<td>Dam (Group A)</td>
<td>-0.538</td>
<td>-0.442</td>
</tr>
<tr>
<td>Dam (Group B)</td>
<td>0.581</td>
<td>0.491</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).
4. Conclusion
The results found in this research are difficult to interpret undeniably except when there was only detailed analysis of the mineral composition of feed. Soil and water could be proved the answer, but there is some possibility of using wool to accelerate the selection programs and improve some of the productivity trail of local Arabian sheep.

5. Conflict of Interest
The authors declare no conflict of interest.

6. References

Fig. 3. Zinc levels in wool of sheep in group A and B.

Fig. 4. Copper levels in wool of sheep in group A and B.


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