Experimental study on zinc deficiency in sheep

Ibrahim, S.O., Helal, M.A., Abd El Raof, Y.M., Elattar, H.M.

Department of internal medicine Faculty of veterinary medicine – Benha university

ABSTRACT

This study was conducted for evaluation of some hematological alteration that associated with Zn deficiency. Eight lambs with average age (5-6) months and body weight (28-30) kg were fed on basal high calcium corn soya bean meal diet with crude protein, calcium, phosphorus and zinc 14%, 1.6%, 0.8%, 26ppm, respectively. All lambs were fed on the experimental ration mixture 450-500 gm / Head / day and hay and water ad libitum until the end of the study at 20th weeks. Serum and whole blood sample were collected weekly for monitoring hematological changes control values were determined at zero day before start feeding on the experimental ration. Clinical signs showed alopecia, skin abnormalities, loss of appetite and emaciation. A significant ($p < 0.05$) decrease in bodyweight, ruminal movement at 6th week and temperature at 12th week. A significant ($p < 0.05$) increase in respiratory rate, pulse rate appeared from the 8th week till the end of the study. There was significant ($p < 0.05$) decrease in WBCs, Hb, lymphocyte and RBCs from 4th, 6th, 4th, 10th, weeks, respectively. Serum zinc concentration was significantly decreasing ($p < 0.05$) from the 6th week. From this study we can conclude that induced Zn deficiency has a negative impact on the general health condition as well as on some hematological and immunological parameters in sheep.

1. INTRODUCTION

There is a hypothesis that Zn ions present in cytoplasm at 10-11mol/l and in equilibrium with numerous zinc metalloenzyme and transcription factors, act as a master hormone particularly in relation to cell division and growth (Vallee 1959). Zn is widely distributed in food mainly bound to protein. The bioavailability of dietary Zn is dependent upon the digestion of these proteins to release Zn and allow it to bind to peptides, amino acids, phosphate, and other legends within the intestinal tract (Jacob et al. 1998). Zn is a component of thymulin (a hormone produced by thymic cells that regulates cell-mediated immunity) and also is a component of many metalloenzyme such as Copper-Zn superoxide dismutase (Cu-Zn SOD), carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphates and RNA polymerase, which affect on the metabolism of carbohydrate, proteins, lipids and nucleic acid (NRC, 2001). also Zn has an influence on immune system (Shankar and Prasad 1998). This trace element has profound effect on host defense mechanism relative to infectious disease. The animals deficient in Zn exhibit atrophy of the thymus gland, depressed cell-mediated immunity (Nauss and Newberne 1982). The aim of this study was to evaluate the clinical and hematological alterations in experimental Zn deficient sheep.

2. Material and methods

2.1. Animals

A total number of eight a clinically healthy young female lambs with age average is 5-6 months and average bodyweight 30.45 ±1.2 kg. Experimental study began at October 2015 and continued for 20weeks for experimental induction of zinc deficiency. Fecal examination, liver function, and kidney function tests were carried out for detection of any internal parasite or any liver and kidney affections that occur for animals after s/c injection of Ivermectine + clorsulon 0.2 mg / kg body weight by2 doses with 2 weeks’ intervals before starting the experiment.

2.2. Clinical examination of animals

Examination of the animals was carried out using the methods described by Radostits et al. (2007).

2.3. Drinking water:
Experimental study on zinc deficiency in sheep

Water analysis was carried out for detection the level of (Zn) in three different sources of tap water, ground water, and distilled water and finally ground water was used.

2.4. Diet:

Animals feed on basal high calcium corn soya bean meal diet and the composition and analysis of the experimental ration as shown in Table 1 and Table 2. Roughages: wheat straw and rice straw. All lambs were fed on the experimental ration mixture 450-500 gm / Head / day and hay and water ad libitum until the end of the study at 20th week.

2.5. Blood sampling

Blood samples were collected from jugular vein according to (Pugh 2002) two sets blood samples were obtained from each lamb, the first set of samples were collected on labeled test tube containing 5 mg k2EDTA in concentration of 1 mg/1ml blood (Coles 1986) as an anticoagulant for determination of hematological parameters (total erythrocyte count, hemoglobin content, total leukocytes count and lymphocytes). The second set of blood samples were allowed to flow freely and gently over the inner surface of a clean and dry centrifuge tube. The samples were allowed to clot in slanting position at room temperature for about 2 hours then the samples were centrifuged at 3000 rpm for 10 minutes, the clear sera were aspirated carefully by automatic pipette and transferred into clear dry labeled Eppendorf tubes and stored at -20°C till examination.

2.6. Induction of Zn deficiency:

Induction of Zn deficiency was carried out by increase the calcium and phosphorus level in the ration as shown in table (2) by addition of ground limestone and steamed bone meal.

2.7. Hematological examination

The total erythrocytes count (TEC), total leukocytes count (TLC), hemoglobin (Hb) and lymphocytic count were determined by using automatic cell counter according to Jain (1993).

2.8. Zn analysis

Serum Zn was estimated by using atomic absorption spectrophotometer as described by Fuwa et al. (1964).

2.9. Analysis of experimental ration:

This method describes the determination of Zn in total diet by using the method according to Murthy et al., (1971)

2.10. Statistical analysis

Statistical analysis was conducted with prism 6 (Graphpad software) quantitative data is presented as the mean with standard error of mean (SEM) and p < 0.05 was considered to be statistical significant.

3. RESULT

3.1. Clinical parameters (Table 3)

Skin abnormalities (rough skin, thickened, cracked, wrinkled with dandruff), paleness of mucus membranes loss of appetite and emaciation Figure (1) and decreased growth rate. There was significant (p < 0.05) decreased in body weight from the 6th Week in zinc deficient sheep than that of the control point until the end of the experiment on the 20th week Figure (2). There was significantly (p < 0.05) decreased in temperature from the 12th Week in zinc deficient sheep than that of the control point until the end of the experiment on the 20th week Figure (3). There was significant (p < 0.05) increased in respiratory rate from the 8th week in zinc deficient sheep than that of the control zero point until the end of the experiment at 20th week Figure (4). There was significant (p < 0.05) increased in pulse rate from the 8th week in zinc deficient sheep than that of the control zero point until the end of the experiment on the 20th week Figure (5). There was significant (p < 0.05) decreased in ruminal movement from the 6th week in zinc deficient sheep than that of the control point until the end of the experiment on the 20th week Figure (6).

3.2. Hematological parameters Table4

There was significant decrease in WBCs from the 4th week in zinc deficient sheep than that of the control zero point until the end of the experiment on the 20th week Figure (7). There was significant decreased in Hb from the 6th week in zinc deficient sheep than that of the control point until the end of the experiment on the 20th week Figure (8). There was significant decreased in lymphocyte from the 4th week in zinc deficient sheep than that of the control point until the end of the experiment on the 20th week Figure (9). There was significant decreased in RBCs from the 10th week in zinc deficient sheep than that of the control point until the end of the experiment on the 20th week Figure (10). Serum zinc concentration were significant decrease in the experimentally induced zinc deficient sheep from the 6th week than that of the
### Table 1 Composition of Basal Diet

<table>
<thead>
<tr>
<th>Element</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn</td>
<td>76.5</td>
</tr>
<tr>
<td>Soybean meal 44% protein</td>
<td>11</td>
</tr>
<tr>
<td>Alfalfa leaf meal</td>
<td>5</td>
</tr>
<tr>
<td>Brewer’s dried yeast</td>
<td>3</td>
</tr>
<tr>
<td>Ground lime stone</td>
<td>2</td>
</tr>
<tr>
<td>Steamed bone meal</td>
<td>2</td>
</tr>
<tr>
<td>Plain salt</td>
<td>0.5</td>
</tr>
<tr>
<td>Supplemental Cu</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Supplemental vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>2.3 μg/kg</td>
</tr>
</tbody>
</table>

### Table 2 Analysis of the Experimental Ration

<table>
<thead>
<tr>
<th>Element</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>14</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.6</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>(26 ppm)</td>
</tr>
</tbody>
</table>

### Table 3 Clinical Parameters in Experimentally Induction Zinc Deficiency in Sheep

<table>
<thead>
<tr>
<th>Time</th>
<th>Body temperature</th>
<th>Ruminal mov. /2 min.</th>
<th>Heart rate/min</th>
<th>Respiratory rate/min</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0- days</td>
<td>39.43 ± 0.06 a</td>
<td>4.50 ± 0.18 a</td>
<td>66.80 ± 1.23 a</td>
<td>23.38 ± 0.71 a</td>
<td>30.45 ± 1.29 a</td>
</tr>
<tr>
<td>2 weeks</td>
<td>39.30 ± 0.05 a</td>
<td>4.50 ± 0.18 a</td>
<td>68.13 ± 1.23 a</td>
<td>23.75 ± 0.88 a</td>
<td>32.73 ± 1.17 a</td>
</tr>
<tr>
<td>4 weeks</td>
<td>39.08 ± 0.07 a</td>
<td>4.00 ± 0.25 a</td>
<td>71.25 ± 1.47 a</td>
<td>24.38 ± 0.92 a</td>
<td>35.15 ± 1.14 a</td>
</tr>
<tr>
<td>6 weeks</td>
<td>39.09 ± 0.04 a</td>
<td>3.63 ± 0.17 b</td>
<td>71.63 ± 1.79 a</td>
<td>27.75 ± 1.00 a</td>
<td>37.13 ± 1.13 b</td>
</tr>
<tr>
<td>8 weeks</td>
<td>38.84 ± 0.05 a</td>
<td>3.63 ± 0.17 b</td>
<td>77.50 ± 1.53 b</td>
<td>29.88 ± 0.99 b</td>
<td>39.19 ± 1.09 b</td>
</tr>
<tr>
<td>10 weeks</td>
<td>39.13 ± 0.07 a</td>
<td>3.13 ± 0.28 c</td>
<td>80.88 ± 1.55 b</td>
<td>32.38 ± 1.10 b</td>
<td>40.79 ± 1.12 b</td>
</tr>
<tr>
<td>12 weeks</td>
<td>38.39 ± 0.09 b</td>
<td>2.88 ± 0.28 c</td>
<td>93.13 ± 3.05 c</td>
<td>33.63 ± 1.10 b</td>
<td>42.13 ± 1.25 b</td>
</tr>
<tr>
<td>14 weeks</td>
<td>38.25 ± 0.08 b</td>
<td>2.50 ± 0.18 c</td>
<td>96.00 ± 3.23 c</td>
<td>35.38 ± 0.98 c</td>
<td>43.48 ± 1.31 c</td>
</tr>
<tr>
<td>16 weeks</td>
<td>38.03 ± 0.06 c</td>
<td>2.25 ± 0.15 d</td>
<td>100.63 ± 2.72 c</td>
<td>36.88 ± 0.78 c</td>
<td>44.54 ± 1.35 c</td>
</tr>
<tr>
<td>18 weeks</td>
<td>37.49 ± 0.03 d</td>
<td>2.25 ± 0.15 d</td>
<td>102.50 ± 2.17 d</td>
<td>37.50 ± 0.64 d</td>
<td>45.73 ± 1.45 d</td>
</tr>
<tr>
<td>20 weeks</td>
<td>37.49 ± 0.03 d</td>
<td>2.25 ± 0.15 d</td>
<td>105.00 ± 1.98 d</td>
<td>38.17 ± 0.54 d</td>
<td>46.94 ± 1.55 d</td>
</tr>
</tbody>
</table>

### Table 4 Hematological Parameters and Serum Zn in Experimentally Induction Zinc Deficiency in Sheep

<table>
<thead>
<tr>
<th>Time</th>
<th>WBCs</th>
<th>L%</th>
<th>RBCs</th>
<th>Hb</th>
<th>Serum Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0- days</td>
<td>30.00 ± 2.14 a</td>
<td>93.13 ± 1.71 a</td>
<td>10.58 ± 0.39 a</td>
<td>11.39 ± 0.41 a</td>
<td>2.67 ± 0.14a</td>
</tr>
<tr>
<td>2 weeks</td>
<td>37.69 ± 1.05 a</td>
<td>58.40 ± 0.58 a</td>
<td>10.35 ± 0.29 a</td>
<td>11.58 ± 0.13 a</td>
<td>2.2 ± 0.07 a</td>
</tr>
<tr>
<td>4 weeks</td>
<td>38.05 ±1.41 b</td>
<td>67.89 ± 1.85 b</td>
<td>10.11 ± 0.28 a</td>
<td>10.81 ± 0.10 a</td>
<td>1.73 ± 0.06 a</td>
</tr>
<tr>
<td>6 weeks</td>
<td>40.36 ± 1.22 b</td>
<td>70.43 ± 1.29 b</td>
<td>9.73 ± 0.26 a</td>
<td>10.26 ± 0.09 b</td>
<td>1.61 ± 0.03 b</td>
</tr>
<tr>
<td>8 weeks</td>
<td>33.99 ± 1.25 b</td>
<td>69.55 ± 2.11 b</td>
<td>9.31 ± 0.22 a</td>
<td>10.04 ± 0.15 b</td>
<td>1.41 ± 0.11 b</td>
</tr>
<tr>
<td>10 weeks</td>
<td>33.45 ± 1.27 b</td>
<td>67.29 ± 1.69 b</td>
<td>7.73 ± 0.33 b</td>
<td>10.34 ± 0.38 b</td>
<td>1.25 ± 0.08 b</td>
</tr>
<tr>
<td>12 weeks</td>
<td>26.38 ± 0.54 c</td>
<td>61.28 ± 2.53 c</td>
<td>5.81 ± 0.40 c</td>
<td>6.49 ± 0.14 c</td>
<td>1.09 ± 0.07 b</td>
</tr>
<tr>
<td>14 weeks</td>
<td>17.73 ± 0.93 c</td>
<td>53.89 ± 1.66 c</td>
<td>3.69 ± 0.09 d</td>
<td>5.78 ± 0.11 c</td>
<td>0.74 ± 0.04 c</td>
</tr>
<tr>
<td>16 weeks</td>
<td>12.23 ± 0.48 c</td>
<td>50.63 ± 1.58 c</td>
<td>3.83 ± 0.09 d</td>
<td>5.79 ± 0.20 c</td>
<td>0.40 ± 0.04 c</td>
</tr>
<tr>
<td>18 weeks</td>
<td>9.05 ± 0.42 d</td>
<td>39.75 ± 0.98 d</td>
<td>3.81 ± 0.06 d</td>
<td>5.90 ± 0.16 c</td>
<td>0.25 ± 0.02 d</td>
</tr>
<tr>
<td>20 weeks</td>
<td>6.10 ± 0.40 d</td>
<td>29.75 ± 0.96 d</td>
<td>3.36 ± 0.08 d</td>
<td>6.39 ± 0.17 c</td>
<td>0.11 ± 0.02 d</td>
</tr>
</tbody>
</table>

a, b, c, d referred to significant differences
Experimental study on zinc deficiency in sheep

Figure (1) Showing lambs on the 20th week (end of the study) with wool loss and emaciation

Figure (2) Body weight (kg) in experimentally Zn deficient sheep

Figure (3) Body temperature in experimentally Zn deficient sheep

Figure (4) Respiratory rate in experimentally Zn deficient sheep

Figure (5) Heart rate in experimentally Zn deficient sheep
Figure (6) Ruminal Movements in experimentally Zn deficient sheep

Figure (7) WBCs in experimentally induced zinc deficient sheep

Figure (8) Hb concentration in experimentally induced zinc deficient sheep

Figure (9) Lymphocytes in experimentally induced zinc deficient sheep

Figure (10) RBCs experimentally induced zinc deficient sheep

Figure (11) Serum Zinc levels in experimentally induced zinc deficient sheep.
control point then there was no significantly decrease ($p < 0.05$) from 6, 8, 10 weeks than that 6 week then there was significant decrease ($p < 0.05$) until the end of the experiment on the 20th week Figure (11).

4. DISCUSSION

Zinc is an essential trace element that is required by all cells in animals as well it plays a clear and effective roles in numerous enzymatic reactions, nevertheless deficiency of Zn are associated with reduced growth rate, poor immune function, decrease reproductive performance, as well as affecting skin in severe cases (Chan et al. (1998); Gooneratne et al. (1989); Mozaffari and Derakhshanfar (2007).

Decrease of appetite in Zn deficient sheep which represented by significant decrease in the ruminal movement may due to reduced ability to taste and smell foods (Droke et al. 1993a) whereby changes in appetite are associated with changes in the concentration of amino acid derived neurotransmitters in the brain, thus some trace elements deficiency as Zn may reduce the appetite by impairing the taste because it postulated that the sense of taste is mediated through the salivary zinc dependent therefore low salivary zinc concentration leads to a reduction of taste (Berger 2002) and reduced appetite. (Failla 2003).

Reduced appetite has been also reported in buffalo calves affected with Zn deficiency (Al-Saad et al. 2006). The reduced appetite and ruminal movement induced reduction in the body weight (Van Wouwe 1989) and body weight gain in experimentally Zn deficient sheep in this study. Alopecia was the second most frequent sign in sheep with Zn deficiency. This finding is in accordance with those of others in calves (Machen et al. 1996); (Radostitis et al. 2000); (Sharma and Joshi 2005). Buffalo calves (Al-Saad et al. 2006). Of all tissues, the skin has the third highest abundance of zinc in the body. In the skin, the zinc concentration is higher in the epidermis than in the dermis, owing to a zinc requirement for the active proliferation and differentiation of epidermal keratinocytes (Ogawa et al. 2016) that may explain the dermatological changes observed in this study.

The respiratory and heart rates were significantly higher ($p<0.05$) in zinc deficient sheep than in zero point. These could be due to the fact that zinc is a component of the enzyme carbonic anhydrase, which is located in the red blood cells and parietal cells of the stomach and is related to the transport of respiratory carbon dioxide and the secretion of hydrochloric acid by the gastric mucosa (Radostitis et al. 2000) as well as carbonic anhydrases are metalloenzymes that catalyze the reversible interconversion of CO$_2$ and HCO$_3^-$ (Aggarwal et al. 2015), so Zn deficiency causing disturbances in carbonic anhydrases which can no longer perform the CO$_2$ to HCO$_3^-$ (Kimber and Pai 2000) resulting in accumulation of carbonic acid and carbon dioxide which manifested clinically in the form of increased respiratory and heart rate.

Significant decrease in RBC and Hb in Zn deficient sheep reflected anemia. Similar results were reported by Al-Saad et al. (2006); Ali and Al-Amery (2013); Alsaad et al. (2011); Lukaski (2005). This may be attributed to impairment of cell replications and protein synthesis and thus the generations of blood cells which commence with condition of Zn deficiency, and this results were supported by the results obtained by Eze et al. (2015) who found that Zn supplementation can be useful in the management of anemia in rats.

Significant decrease in lymphocyte in Zn deficient sheep may indicate that Zn deficiency had affect on cell mediated immunity (Bires et al. (1992); (Droke and Spears (1993)). Zn status caused significant changes in endocrine function were important in understanding of the secondary physiological changes created by Zn deficiency and of their impact in the immune system specially lymphopoiesis (DePasquale-Jardieu and Fraker 1979). Zn is one of the most relevant nutritional factors for the whole life of an organism because it affects the inflammatory/immune response, metabolic harmony and antioxidant activity (Mocchegiani et al. 2013), a significant decrease in lymphocytes and WBCs in Zn deficient sheep in this study may be attributed to Zinc deficiency decreases the activity of serum thymulin (a thymic hormone), which is required for maturation of T-helper cells results in cell-mediated immune dysfunction (Prasad, 2007). It has been known for many years that zinc deficiency in experimental animal's results in atrophy of thymic and lymphoid tissue (Prasad and Oberleas, 1971). Later studies in young adult zinc-deficient mice showed thymic atrophy, reductions in the absolute number of splenocytes, and depressed responses to both T-cell-dependent and T-cell-independent antigens (Fernandes et al. (1979); Fraker et al. (1977); Prasad (2007). The immune system function is impaired even in cases of moderate Zn deficiency. (Shahraz and Ghaziani,2005) Severe Zn deficiency reduces immune system function. Zn is necessary for the development and activity of T lymphocytes...
and its deficiency causes a reduction in cellular immunity (Yousefichajjan et al. 2016).

Plasma Zn which represents, 0.2% of total body zinc content, was the most frequently measured biomarker of Zn status, thus enabling the most comprehensive analysis of this biomarker (Lowe et al. 2009). Although plasma Zn concentration responds to altered intake over short periods, the homeostatic mechanisms that act to maintain plasma Zn concentration within the physiologic range (namely, adaptive changes in efficiency of absorption and levels of endogenous excretion) may prevent high plasma concentrations from being sustained over a prolonged period. (Lowe et al. 2009). Plasma zinc concentration can fall in response to factors unrelated to Zn status or dietary Zn intake, including infection, inflammation, stress, or trauma. Conversely, tissue catabolism during starvation can release Zn into the circulation, causing a transient increase in circulating Zn levels (Hambidge et al. 1989).

5. CONCLUSION

From the obtained results of this study we can conclude that Zn deficiency cause some clinical and hematological alteration as alopecia, decrease body weight, skin abnormalities, decrease WBCs and lymphocyte

6. REFERENCES


Failla, M.L. 2003 .,Trace elements and host defense: recent advances and continuing challenges. The Journal of nutrition 133, 1443s-1447s.


Experimental study on zinc deficiency in sheep


Pugh, D.G., 20. Text Book of Sheep and Goat medicine, A.W. Saundes, USA, 0-7216-9052-7211.


Sharma, M.C., Joshi, C., 2005. Therapeutic efficacy of zinc sulphate used in clustered model treatment in alleviating zinc deficiency in cattle and its effect on hormones, vitamins and production parameters. Veterinary research communications 29, 609-628.


