Risk assessment

TOXICOLOGICAL ASPECTS IN SHEEP BRED IN A COPPER-POLLUTED AREA

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Abstract. The main goal of this study was to emphasise different aspects that appear in sheeps after ingestion of fodder with a high copper level; 5-year old ewes farmed all their life near an industrial unit that pollutes with copper were used for this study. Through investigation methods we tried to find clinical and pathological modifications that appear in the animals farmed for a long period in a copper-polluted area, insisting on toxicological investigations; both clinical and laboratory analyses indicated copper chronic poisoning. The studied animals presented the specific clinical signs of chronic copper poisoning, initially being discreet (apathy, anorexia), and then digestive, respiratory and nervous signs and even death. The sheep farmed at a longer distance from the pollution source did not express characteristic clinical signs. Results of the biochemical analyses showed an increase of the serum transaminases activity proportionally to closeness of the pollution source that demonstrates liver function damage in chronic copper poisoning. High concentrations, exceeding the normal level 20 times were found in liver; maximum level was found in animals farmed near the pollution source.

Keywords: copper, sheep, liver, wool, copraemia.

AIMS AND BACKGROUND

The industrial process for extracting and processing non-ferrous ore has a continuous and a negative impact on the environment. The area of Zlatna, where the copper processing industry is developed, is badly polluted with heavy metals, as powders and aerosols, silicon dioxide powders and gases. Heavy metals accumulate and concentrate in the soil, ground water, vegetation and animal tissues, and the presence of sulphur dioxide in the atmosphere enables the appearance of acid rain, affecting human health, vegetation, animals health and buildings. Metallurgy gases resulting from the process of extracting copper from copper-bearing concentrated mixtures produce, in addition to local pollution, a long-range pollution due to corridor conditions and the height of the dispersion stack. Nowadays, when environment pollution and the depreciation of the natural surroundings manifest themselves aggressively in the world, Romania not being

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an exception from these harmful phenomena, it is stringently necessary to take
decisive and effective action to protect the environment.

This study is trying to reveal and underline some of the toxicological aspects
deriving from the ingestion of feed and water with high content of copper by
sheep, and to investigate the clinical signs and pathological anatomical changes
(macroscopic and microscopic), appearing as a consequence of copper pollution
in sheep growing in the area, with practical importance especially as regards
chronic intoxication. The chronic copper poisoning is the most frequent expres-
sion of this metal poisoning in farm animals\textsuperscript{3}. The chronic poisoning with copper
salts is associated with administering of different therapeutic agents containing
copper or either with the industrial copper poisoning induced by respiratory or
digestive exposure, during their growth and productive live\textsuperscript{4,5}. In most cases the
animals are in a continuous adaptative biological challenge, and the clinical and
anatomopathological findings are not always characteristic\textsuperscript{6}.

EXPERIMENTAL

5-year old ewes farmed all their life near a copper polluting industrial unit were
used for the study. There were investigated the following 3 groups of ewes farmed:
near the pollution source (Zlatna area) – batch A; at 6 km distance (the Ampoiului
valley) – batch B; and at 12 km distance from the pollution source – batch C. The
results were compared with those in a control group farmed on a non-polluted area
(Belciugatele – Calarasi) – batch D.

The performed investigations, by using a spectrophotometry methods (with
atomic absorption), in order to determine serum, wool and copper level prelevated
from the studied animals; another goal was to identify the clinical and pathologi-
ical changes that appear in the animals which were farmed for a long period on a
copper-polluted area, insisting on toxicological investigations, and both clinically
and laboratory analyses indicated copper chronic poisoning.

RESULTS AND DISCUSSION

The studied animals – feeded exclusively with local hay (1.5 kg/day) and combined
fodder (0.3 kg/day) presented the specific clinical signs of chronic copper poison-
ing. Initially the clinical signs were discreet (apathy, anorexia), and then digestive,
respiratory and nervous signs (in group A) and even death.

The sheep that were farmed at a higher distance from the pollution source did
not expressed characteristic clinical signs.

Extremely high values of copper, but also of other heavy metals (Zn, Fe,
Mo, Se, As, Cd and Pb) were found in administered provender to the animals
(Table 1).
Table 1. Elements included in food (mg/animal/day)

<table>
<thead>
<tr>
<th>Daily quantity</th>
<th>Cu</th>
<th>Zn</th>
<th>Fe</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland hay (1.5 kg)</td>
<td>16.92</td>
<td>59.55</td>
<td>358.39</td>
<td>1.125</td>
</tr>
<tr>
<td>Concentrates (0.3 kg)</td>
<td>2.11</td>
<td>10.58</td>
<td>22.86</td>
<td>0.372</td>
</tr>
<tr>
<td>Drinking water</td>
<td>0</td>
<td>1.20</td>
<td>0.25</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Daily quantity</th>
<th>Se</th>
<th>As</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland hay (1.5 kg)</td>
<td>0.385</td>
<td>0.280</td>
<td>0.079</td>
<td>0.285</td>
</tr>
<tr>
<td>Concentrates (0.3 kg)</td>
<td>0.063</td>
<td>0.028</td>
<td>0.017</td>
<td>0.033</td>
</tr>
</tbody>
</table>

The clinical signs were discreetly maintained, until the 14th day when the lamb No 9 was totally apathy. The animal remained more down and gave signs of total wickness. It refused food and starting with the 26th day of the study, full lack of appetite was observed; the water that it drink decreased as volume. Since the day 28th the nervous symptoms become numerous and they were characterised by racking, trismus and continuous mastication. In this period the animals were placed in lateral positions and did not respond to outside stimuli. The conjunctiva, mouth and vaginal mucous membranes as well as the skin had a pale colour. It was as well observed the brown chocolate-like colour of blood obtained by puncture and a low haemoglobinuria. The fasciae were full of water and coloured in brown-green. The animal died after a total tiredness in the 36th day of the study. There have been seen similar clinical signs and death in the 37th day of observation at sheep No 7 and No 3. Food accepting decreased in the 14th day of study. The animals maintained their down position and had different degrees of position. Since then, all animals had an easy diarrhea and 5 of them had a profound diarrhea between the 21st and 28th day. The diarrhea symptoms persisted during all period. Next period was characterised by an engrowing lack of wool, total weakness and shepiness in all sheep observed. The symptoms stayed still in this form until the 45th day of study where a big part of animals had a strongly diminished status with strong modifications which could not be repaired, so the cutting animals were imputed and the necropsy exam was made that showed anatomic-pathological changes. Samples of organs were examined by spectrophotometric atomic analysis to find out the copper level in the main affected organs: liver, kidney and spleen. We have tried all over the study to have a good monitoring of corporal weight of the isolated animals and we have made encounters in a few days of study.

The weight of sheep during the study increased compared to first value during the all investigation period. This result was not significant and owed to a strictly care and to a low density of animals, because they were isolated and had better care all over the observation.

An obvious elevation of the serum transaminase activity in sheep can be observed, with clinical signs (batch A) as an expression of the hepatic injuries, because the first target of the copper in excess is the liver (Table 2).
Table 2. Average values/batch of the investigated biochemical parameters

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>GOT (AST) (U/l), 37°C</th>
<th>GPT (ALT) (U/l), 37°C</th>
<th>GGT (U/l), 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>reference value</td>
<td>60–110</td>
<td>&lt; 22</td>
<td>30–95</td>
</tr>
<tr>
<td>1</td>
<td>batch A</td>
<td>160.42</td>
<td>63.99</td>
<td>108.35</td>
</tr>
<tr>
<td>2</td>
<td>batch B</td>
<td>129.92</td>
<td>42.04</td>
<td>104.5</td>
</tr>
<tr>
<td>3</td>
<td>batch C</td>
<td>99.92</td>
<td>22.04</td>
<td>75.78</td>
</tr>
<tr>
<td>4</td>
<td>batch D</td>
<td>57.78</td>
<td>14.28</td>
<td>43.14</td>
</tr>
</tbody>
</table>

These results are similar to those regarding the copper levels from blood serum and wool, as indicators of the chronic exposure to this element in accordance with the distance to the pollution sources (Table 3).

Table 3. Average values of copper in liver samples from the ewes in investigated batches

<table>
<thead>
<tr>
<th>No</th>
<th>Batch</th>
<th>Distance to the pollutant source</th>
<th>Average values of copper (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>close to the pollutant source</td>
<td>60.02</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>6 km to the pollutant source</td>
<td>47.65</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>12 km to the pollutant source</td>
<td>20.12</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>witness</td>
<td>5.44</td>
</tr>
</tbody>
</table>

Note: The allowable limit conformable with Healthy Minister Order No 975/1998 is 3 ppm.

Recorded values in kidney samples in dead animals as a result of poisoning or sacrificed had shown increased values that exceed copper allowable limit, proportionally reversed with the distance from the pollution source (Table 4). So, the values were about 18 times higher than allowable limit near pollution source and 14 times higher at a 6 km distance from the pollution source.

Table 4. Average values of copper in kidney samples from the ewes in investigated batches

<table>
<thead>
<tr>
<th>No</th>
<th>Batch</th>
<th>Distance to the pollutant source</th>
<th>Average values of copper (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>close to the pollutant source</td>
<td>53.17</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>6 km to the pollutant source</td>
<td>43.12</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>12 km to the pollutant source</td>
<td>19.12</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>witness</td>
<td>3.76</td>
</tr>
</tbody>
</table>

Note: The allowable limit conformable with Healthy Minister Order No 975/1998 is 3 ppm.

In the spleen samples from sheep of Zlatna area, copper values exceed with 6–17 times, depending on the distance from the pollution source (Table 5).

The animals which shown specific clinical signs of chronic copper poison already presented alteration of habits in clinical evaluation moment at the beginning and after isolation (the entire period of 45 days). Feeding status of such animals varied, but in general was good. Lack of appetite, apathy and loss of hair, occasionally occur. Similar, weight of A batch (28.6+/–5.64 kg) measured at the beginning
of evaluation was the results of influence of over 5 years of copper and other risk microelements presented in emission of copper producing plant which produced whole metabolism disturbance in exposed animals, because of liver functional ability disturbances. Much more, the length of time of cumulative stage in copper-poisoned sheep depends of copper dose, length of action, nutritional status of individual, breed and presence of competitive elements in emission.

Table 5. Average values of copper in spleen samples

<table>
<thead>
<tr>
<th>No</th>
<th>Batch</th>
<th>Distance to the pollutant source</th>
<th>Average values of copper (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>close to the pollutant source</td>
<td>50.98</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>6 km to the pollutant source</td>
<td>42.87</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>12 km to the pollutant source</td>
<td>18.01</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>witness</td>
<td>3.05</td>
</tr>
</tbody>
</table>

Note: The allowable limit conformable with Healthy Minister Order No 975/1998 is 3 ppm.

Generally, cumulative stage of copper toxic ingestion can last from weeks to months. Bires (1992) reported that copper poisoning cumulative stage in sheep reared in copper-polluted areas is, usual, about few years¹. Before the chronic effect of released copper by plant to which the studied sheep were exposed, cumulative stage lasts few years with a delayed expression of clinical signs. Because of the long lasting cumulative stage and also in one studied batch of sheep that could not reach the toxic stage of copper poisoning, the clinical status can be explained also through an adaptation process of the evaluated sheep to the ingested copper under these chronic conditions, even before starting the experiment in correlation with the presence of the other risk elements present in the emission material.

Other factors, which contributed to clinical manifestation of industrial poisoning, included individual predisposition of studied animals because similar copper doses had as a result toxic stage with consecutive death only of 3 evaluated sheep. Clinical signs over toxic stage of 3 sheep from A batch were the same with industrial copper poisoning. Such a manifestation can be explained by chronic ingestion of copper by sheep, because of industrial contribution to the period of breeding near the copper-producing plant, time in which individual tissues alteration and functional disturbances of that reach a very elevated level in evaluated sheep.

Biochemical analyses results showed an increase of the serum transaminases activity proportionally to closeness of the pollution source that demonstrate liver function damage in chronic copper poisoning.

It were observed high concentrations that exceeded normal level 20 times in liver, and maximum find level was in animals farmed near the pollution source.

The hematology examination (MGG) reveals an intensive hemolysis with irregular shape of the RBC (Figs 1 and 2).
The most frequent lesional aspects were the congestive and the hemorrhagic, identified in liver, kidneys, gastrointestinal tractus.

The cyto-pathologic changes induced by copper in sheep were dominated by intensive hemolysis, hepatic cell destructions and granulo-vacuolar dystrophy, and obvious destructions of the renal epithelium. The morphopathological changes induced by copper in sheep were: congestions, hemorrhages and toxico-dystrophic changes (Fig. 3).

In liver, from a histopathological point of view (hematoxilin–eosin–methilene blue) the most frequent aspects were the evident mesenchymal reactions in portobiliar space with obvious congestions, hemorrhages and toxico-dystrophic vacuolisation.
In kidney more relevant were the congestions in renal parenchyma and the tubular cell destructions with the remaining of the basal membrane, intraglomerular hypercellularity (Figs 4 and 5).

Constant myocardic lesions were registered (Fig. 6) – the congestions and the mesenchymal reactions were correlated with microvacuolisations in miocardyc
cells (toluidine blue and TEM × 11 000). In sheep is well known the chronic cumulative action, in correlation with the responsible doses and/or exposure time and amount of the copper salts or products.

![Image](image.png)

**Fig. 6.** Congestive, mesenchymal changes and microvacuolisations in miocard

In general, the cumulative stage of copper intoxication (with acute evolution) can be observed after weeks or months after exposure or/and ingestion (in some cases – years).

**CONCLUSIONS**

1. This study was performed for achieving the aspects regarding the clinical and para-clinical changes on sheep bred near to copper-polluted areas.

2. The animals raised in such copper polluted areas manifested clinical signs characteristic for copper intoxication, initially discrete and later specific digestive, nervous and respiratory signs (even death).

3. The animals raised on longer distances from polluted areas did not manifest clinical signs.

4. The results of the biochemical investigations performed in sheep batches reveal an obvious raising of the hepatic transaminase activity, according to the distance to the pollutant sources, as an early and specific indicator of the direct aggression on this organ.

5. After determining the copper level by atomic spectrophotometric method, in blood serum, wool and internal organs samples – from the studied ewes, the results reveal higher concentrations, especially in liver (even 20 times higher in comparison with the reference values).
REFERENCES


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