Seasonal Variation and Relationships Between Copper of Serum and Various Tissues in Copper Poisoned Sheep in Kerman Province, Iran

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Abstract:

BACKGROUND: Soil and plant contamination in copper exposure causes chronic copper poisoning (CCP) in animals following the consumption of plants in these soils.

OBJECTIVES: The present study was carried out on 10 clinically affected copper poisoned cases (in each season) from autumn 2015 to the end of summer 2016, in four seasons in Kerman province to evaluate the seasonal effects of CCP in sheep.

METHODS: All samples were taken from clinically affected cases of copper poisoning that were then necropsied and the poisoning was confirmed by pathognomic pathological findings and serum copper concentration. Samples from liver, lung, kidney, heart and spleen were collected immediately after death and their copper concentration was measured.

RESULTS: The highest levels of copper in lung, heart, spleen and kidney were observed in the summer. Liver had the highest amounts of copper in spring and the levels of copper in wool and serum were detected in winter. The copper levels of liver and kidney were positively correlated in autumn, but the copper levels in these tissues were negatively correlated in both spring and winter.

CONCLUSIONS: Severity of the copper toxicity (judged by the liver copper concentration) is season-dependent in sheep and seasonal variations affect this toxicity. Environmental climate and stressors may be the main causes of copper contents of different tissues and the liver is the main organ to reserve copper in poisoned sheep.

Keywords:
Copper poisoning, Serum, Tissue, Season, Sheep
Introduction

Copper, an essential trace metal for organisms, plays an important role as a co-factor of crucial enzymes. It is necessary for energy production, connective tissue formation, and pigmentation (Bost et al., 2017). Sheep are the species most susceptible to chronic copper toxicity (Gupta 2018), because their liver cells have a high affinity for copper and they excrete copper into the bile at a very low rate, leading to a build-up of liver copper stores over time. Copper is a strong oxidizing agent and it binds to proteins in the liver cells which are stored in lysosomes within hepatocytes. As long as the copper remains stored in lysosomes it does not cause tissue damage. (Kumaratilake, 2014; López-Alonsoa et al., 2017). There is a narrow range between required level of copper and the level at which toxicity can occur (Constable et al., 2017). For sheep, 5 to 10 μg of copper/g of diet is recommended together with a normal level of molybdenum (1μg/g dw) (Menzies et al., 2003). The proper copper: molybdenum ratio should be 6:1. Lower molybdenum content in the diet can cause copper toxicity even if the amount of copper is appropriate (Kessler et al., 2012).

Chronic copper poisoning is the most common form of copper toxicity in farm animals. Its occurrence is associated with a long-term intake of copper compounds of different origin (Rosa1 et al., 2016). The characteristic feature of industrial copper intoxication is that the animals are reared close to industrial plants, and ingest copper from industrial deposits through feed or from the air, mostly together with other toxic elements throughout their entire life (Maggini et al., 2007). As a result, animals reared under such conditions adapt to a certain degree to chronic intake of increased doses of copper and clinical and pathological manifestations of intoxication are not always characteristic. Chronic copper poisoning (CCP) is well recognized as a hazard in sheep production (Maggini et al., 2007). It is usually associated with decreasing value of production parameters and decreasing adaptation ability to environmental conditions. During chronic ingestion of copper, its passive accumulation may occur over a period from a few weeks to more than a year. Copper toxicity is considered having two distinct phases: first pre-hemolytic phase without clinical symptoms and second phase called hemolytic crisis which is fatal (Cregar et al., 2012). Copper-induced changes were reported in the blood, liver, kidney and reproduction and central nervous system of the animals (Theil and Calvert, 1978).

Some stress factors such as changes in environmental climate, pregnancy, parturition, lambing and lactogenesis may affect the animals and sensitize them to show clinical signs of copper poisoning (Sivertsen and Løvberg, 2014). Multiple factors influence the degree of copper intoxication in animals such as the amount and the period of copper intake, the nutritional status of animals and also season. We hypothesized that, season (with high temperature range and varied plant species composition, diversity and availability, lambing time, subsequent lactation, etc.) may affect the degree of copper intoxication in sheep and one of the aims of the current research was to present the copper levels of wool, serum and different organs such as liver, lung, spleen, heart, kidney in chronic copper poisoned sheep.
at different seasons during a year to clarify the effect of season. The relationships among all copper levels of studied samples were also obtained to better understand the proper interactions among different organs and tissues in poisoned sheep. Furthermore, based on the regression formulas between copper serum and tissues in copper poisoned sheep at different seasons, it may estimate the copper levels of different tissues based on the circulating copper contents at different seasons. There is also a hope that the study may contribute to the understanding of the epidemiology of CCP in sheep.

**Materials and Methods**

**Animals and samples:** The present study was carried out from autumn 2015 to the end of summer 2016, in Kerman province, Iran. This study was commenced in autumn and continued seasonally during a period of one year (4 times in a year). Ten live copper poisoned sheep (in each season) were selected and blood samples were taken via jugular vein in plain tubes to obtain sera after centrifugation. Sera were stored at -20 °C until assayed. Wool samples were taken from the neck of these animals and then washed and dried. Samples from liver, lung, kidney, heart and spleen were collected immediately after death. These tissues were put in formalin 10% until analyzed for copper content.

It should be noted that all samples were taken from clinically affected cases of copper poisoning and serum was provided at this stage. The clinical signs of copper poisoned sheep were sudden in onset and affected animals became increasingly weak. Some sheep spent time wandering aimlessly or head-pressing. Jaundice developed in some of them and breathing became shallow and rapid due in part to the development of anemia. Cases were necropsied and the poisoning was confirmed by pathognomonic pathological findings and serum copper concentration. Samples from control cases were obtained from 10 otherwise healthy cases far from copper industries.

**Evaluating copper concentration of serum, tissues and wool:** In order to evaluate the copper contents, 150 mg of tissues, 500 µL of serum and 250 mg of wool samples were processed and digested. The digestion procedure for serum and tissues was done by using nitric acid (HNO3; 65%, P.A., Vetec 191; Sigma-Aldrich Ltd., São Paulo, SP, Brazil) and perchloric acid (HClO4; 70%, P.A., ACS, Vetec 909; Sigma-Aldrich Ltd., Brazil) at a ratio of 70:30. One mL of the digested tissues was then heated at 85 °C until the solution became translucent and a brownish smoke stopped being released, which indicated the complete digestion of the organic matter. The tubes were allowed to cool at room temperature. Then, the digested samples were stored at 4 °C for 24 h and atomic absorption was done on these samples, subsequently.

**Statistical analyzes:** Data are presented as mean±SE. Statistical differences of copper content in different samples, among various seasons were analyzed by One-Way ANOVA and LSD post-hoc test. The relationships among the copper content of se-
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Table 1. Copper concentration of serum, tissues and wool (ppm; mean±SE) of copper poisoned sheep at different seasons during a year (n=10). In each column, different letters indicate significant differences among seasons (P<0.05).

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Serum</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Spleen</th>
<th>Wool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>8.37±0.22a</td>
<td>26.78±2.52a,c</td>
<td>744.62±223.47a</td>
<td>34.32±0.72a,c</td>
<td>35.01±0.16a</td>
<td>70.01±1.23a,c</td>
<td>28.35±5.18a,b</td>
</tr>
<tr>
<td>Summer</td>
<td>6.89±0.63b</td>
<td>36.35±1.57b</td>
<td>374.19±83.46b</td>
<td>48.08±4.84b</td>
<td>36.34±0.91a</td>
<td>82.31±2.21a,c</td>
<td>27.40±4.95a,b</td>
</tr>
<tr>
<td>Autumn</td>
<td>8.08±1.89a</td>
<td>33.45±1.27b,c</td>
<td>161.44±30.43b</td>
<td>38.50±3.38a,c</td>
<td>35.18±0.99a</td>
<td>55.47±16.15a</td>
<td>20.44±7.28a</td>
</tr>
<tr>
<td>Winter</td>
<td>8.70±0.26a</td>
<td>29.70±0.62c</td>
<td>95.94±21.58b</td>
<td>37.24±3.67a,c</td>
<td>31.35±1.83b</td>
<td>30.62±0.91b,c</td>
<td>51.51±15.86b</td>
</tr>
<tr>
<td>Control</td>
<td>3.90±0.32c</td>
<td>23.14±0.74d</td>
<td>31.31±1.72c</td>
<td>21.29±0.41d</td>
<td>21.75±0.38d</td>
<td>3.64±0.22c</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The relationships among copper contents of all samples of copper poisoned sheep (n=10) at each season. Stars indicate significant relationships between copper contents of samples (P<0.05).

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Serum</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
<th>Heart</th>
<th>Spleen</th>
<th>Wool</th>
</tr>
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<tbody>
<tr>
<td>Spring</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>-0.078</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Liver</td>
<td>0.237</td>
<td>-0.737*</td>
<td></td>
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</tr>
<tr>
<td>Lung</td>
<td>0.308</td>
<td>0.164</td>
<td>0.303</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.130</td>
<td>0.497</td>
<td>0.193</td>
<td>0.404</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.330</td>
<td>-0.122</td>
<td>0.710*</td>
<td>0.843*</td>
<td>0.602</td>
<td></td>
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</tr>
<tr>
<td>Wool</td>
<td>0.240</td>
<td>0.157</td>
<td>-0.306</td>
<td>-0.178</td>
<td>-0.146</td>
<td>-0.291</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Kidney</td>
<td>0.295</td>
<td></td>
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</tr>
<tr>
<td>Liver</td>
<td>0.261</td>
<td></td>
<td>0.126</td>
<td></td>
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</tr>
<tr>
<td>Lung</td>
<td>-0.127</td>
<td>-0.142</td>
<td>-0.544</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.202</td>
<td>-0.088</td>
<td>0.950*</td>
<td>-0.309</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>-0.279</td>
<td>-0.490</td>
<td>-0.748*</td>
<td>0.880*</td>
<td>-0.505</td>
<td></td>
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</tr>
<tr>
<td>Wool</td>
<td>-0.386</td>
<td>0.575</td>
<td>-0.277</td>
<td>-0.034</td>
<td>-0.431</td>
<td>-0.126</td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>-0.017</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.019</td>
<td></td>
<td>0.791*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lung</td>
<td>0.096</td>
<td>-0.242</td>
<td>-0.048</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>-0.085</td>
<td>-0.364</td>
<td>-0.658*</td>
<td>-0.720*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Spleen</td>
<td>0.086</td>
<td>-0.080</td>
<td>-0.056</td>
<td>0.961*</td>
<td>-0.684*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wool</td>
<td>-0.119</td>
<td>0.332</td>
<td>0.442</td>
<td>-0.017</td>
<td>-0.294</td>
<td>-0.029</td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.211</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>-0.404</td>
<td></td>
<td>-0.797*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.687*</td>
<td>0.511</td>
<td>-0.795*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>0.689*</td>
<td>0.231</td>
<td>-0.652*</td>
<td>0.952*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.706*</td>
<td>0.065</td>
<td>-0.367</td>
<td>0.855*</td>
<td>0.920*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wool</td>
<td>-0.180</td>
<td>-0.478</td>
<td>0.499</td>
<td>-0.202</td>
<td>-0.090</td>
<td>0.116</td>
<td></td>
</tr>
</tbody>
</table>

Copper and different tissues and wool in various seasons of copper poisoned sheep were analyzed by Pearson’s correlation test and regression formula was obtained. The correlations among copper contents of all samples were also assayed by this test. Statistical analyses were performed by using SPSS software (SPSS for Windows, version 11.5, SPSS Inc., Chicago, Illinois). The level of significance was set at P<0.05.

Results

Copper contents of serum, various tissues and wool (ppm; mean±SE) of poisoned sheep in different seasons during a year are presented in Table 1. The highest levels of copper in lung, heart, spleen and kidney were observed in the summer. Liv-
er had the highest amounts of copper in spring and highest concentrations of copper in wool and serum were detected in winter. The lowest amounts of copper in kidney, liver, heart and spleen were detected in winter. However, the lowest levels of copper in serum, lung and wool were observed in summer, spring and autumn, respectively. It is notable that the severity of poisoning was evaluated on the basis of the concentration of copper, mainly in liver as compared to the liver copper concentration in control cases. The relationships between course and severity of clinical findings with seasons were not investigated. The relationships among copper contents of all samples of copper poisoned sheep in each season are presented in Table 2. The copper levels of liver and kidney were positively correlated in autumn ($P<0.05$), but the copper levels in these tissues were negatively correlated in both spring and winter ($P<0.05$). Copper contents of lung and spleen had significant and positive relationships in all studied seasons. In winter serum copper levels were positively correlated with copper level of lung, heart and spleen in copper poisoned sheep ($P<0.05$).

The linear regression formulas between copper levels of serum and different tissues in different seasons are presented in Figs. 1 to 3.

**Discussion**

Environmental contamination of soil and plant material occurs naturally in cupriferous soil outcrops. The production of copper has risen over the last decades due to which, copper quantities in the environment have
increased (Elshkaki et al., 2016). World’s copper production is still rising which primarily means that more and more copper ends up in the environment. Pollution has also been caused by mine tailings and corrosion of metallic copper. This contamination is the result of a high concentration of copper in the superficial soil layers as copper is not a mobile element (Merry et al., 1986). Copper does not break down in the environment and because of that it can accumulate in plants and animals when it is found in soils. On copper-rich soils only a limited number of plants have a chance of survival. That is why there is not much plant diversity near copper-disposing factories. Due to the effects upon plants copper is a serious threat to the productions of farmlands. When the soils of farmland are polluted with copper, animals will absorb concentrations that are damaging to their health. Mainly sheep suffer a great deal from copper poisoning, because the effects of copper are manifested at fairly low concentrations (Bhavani and Sujatha, 2014). Sheep have also been poisoned from ingesting pasture contaminated by copper which has been deposited from industrial pollution such as mine tailings (Merry et al., 1986).

Copper is essential for life since it is needed for normal iron metabolism, synthesis of elastin and collagen, melatonin production and integrity of the central nervous system (Bhattacharya et al. 2016). It is essential in keratin (wool) production. More recently, it has been demonstrated that copper is one of the key trace minerals necessary for an effective immune response (Maggini et al., 2007). Sheep are the species most susceptible to chronic copper toxicity, because their liver cells have a high affinity for copper and they excrete copper into the bile at a very low rate, leading to a buildup of liver copper stores over time. Copper is a strong oxidizing agent. It binds to proteins in the liver cells and is stored in lysosomes within hepatocytes. As long as the copper remains stored in lysosomes it does not cause tissue damage (Kumaratilake, 2014). Sheep require about 5 ppm of copper in their total diet. Toxicity can develop at levels above 25 ppm (Constable et al., 2017). Copper toxicity in sheep usually results from the accumulation of excess copper in the liver over a period of a few weeks to more than a year with no clinical signs, followed by a sudden release of liver copper stores to cause toxicity (rapid breakdown of red blood cells). In these situations, CCP may result from excessive copper intakes (Tchounwou et al., 2008). Mature ewes of British breed origin appear to be the most vulnerable and there is evidence to suggest that Finn Sheep and Texels also have a tendency to accumulate more copper in the liver than other breeds (Soli et al., 1977). Stresses, such as weather, environment, poor nutrition, transportation and handling, can also cause the liver cells to die and liberate the stored copper into the bloodstream (Gaetke and Chow, 2003). Nowadays, both clinical and subclinical episodes of CCP represent the main toxicological problem in this animal species, being responsible for significant economic losses over the world (Lopez-Alonso, 2012). The first remarkable finding in this study was a characteristic seasonal variation in the hepatic copper concentrations in the studied sheep (Table 2). This pattern was evident not only in average values, but also for each individual sheep, as illustrated by Figs. 1 and 2. In the studied sheep, the hepatic copper levels fell only moderately from November/December till March.
representative for the situation in the flock over time, one might have expected some of the cases in this flock to occur also in mid-winter. It may be due to the combined effect of high hepatic copper levels and stress factors related to return from pasture, change of feed, lambing, etc. (Gaetke and Chow, 2003). In ruminants, the tissues showing a high concentration of copper, in decreasing order, are the liver, kidney, gastrointestinal tract, adrenals, thymus, gallbladder and bile; those of medium concentration include the pancreas, red bone marrow, intestinal lymph, blood, spleen, heart, lung, and reproductive organs; very low concentrations of copper are found in the white bone marrow, muscle, bladder, ligament, cartilage, bone, eye and nerve tissues. It is obvious that the liver, which shows the highest concentration, serves as the chief storage organ (Comar et al., 1948). In our study, copper poisoned sheep were confirmed by their serum copper levels, clinical and postmortem evaluations (Constable et al., 2017). Assay of copper in different tissues including liver was carried out on samples taken during necropsy. Liver biopsies have been used for many years in the study of copper metabolism in sheep (Bickhardt et al., 1997). But it has been stated that the relationship between chronic hepatic copper accumulation and the outbreak of clinical disease in individual animals is not straightforward. Experimentally, typical CCP may be induced reproducibly in 2–3 months, by oral copper dosing (Soli et al., 1977). Under practical conditions, however, a large number of sheep may be heavily affected by hepatic copper overload, without showing any signs of disease (Constable et al., 2017). This is why we extended our study on copper content of serum, wool and other tissues in otherwise healthy control sheep. Additional stress factors are important for the induction of clinical disease (Moeller et al., 2004). The sensitivity to these stress factors may vary. It has been shown that copper sensitive breeds differ from copper tolerant ones not only in their tendency to accumulate copper, but also in their sensitivity to the effects of this accumulation (Soli et al., 1977). On the other hand, hepatic copper accumulation is generally acknowledged as a prerequisite for the development of clinical disease. The critical level reported by different authors set between 150 and 250 g/g ww (Moeller et al., 2004; Constable et al., 2017). Some stress factors such as changes in environmental climate, pregnancy, parturition, lambing and lactogenesis may affect the animals and sensitize them to show clinical signs of copper poisoning (Sivertsen and Løvberg, 2014). Hence, in the present study, the higher values of copper content in different tissues were detected in spring and summer. The concentrations of different elements in an organism can vary with the season, independently of environmental concentrations (Villares et al., 2002). Some researchers have reported the highest heavy metal contents of plants, such as copper, during autumn and relatively low concentrations during spring (Kim and Fergusson, 1994; Brekken and Steinnes, 2004). Liver had the highest amounts of copper in spring and the highest concentration of copper in wool and serum were detected in winter. The highest levels of copper in lung, heart, spleen and kidney were observed in the summer. It should be noted that the concentration of copper in these tissues was higher than normal in summer and it was concurrent with lower liver copper level that was still within higher ranges. It is possible that
unknown factors may cause release of some copper to the body, especially to these tissues when signs of copper poisoning are unremarkable or absent. Although the lowest amount of copper in liver was detected in the winter, its concentration was still in the toxicity range and possible occurrence of toxicity still exists. The highest levels of copper in wool and serum were detected in winter. This phenomenon may show that certain unknown conditions may help the release of copper from liver to sustain higher than normal copper concentration in these sources. It is also possible that the higher availability of copper in dried forage and subsequent higher absorption may be the cause of this observation (Constable et al., 2017). The higher concentration of copper in lung, heart, spleen and kidney in summer and higher copper level of liver in spring may be partly due to higher vitamin D status in these seasons. Schwalfenberg and Genuis (2015) reported that vitamin D intake can facilitate the absorption and assimilation of essential inorganic elements such as copper. The copper levels of liver and kidney were positively correlated in autumn ($P<0.05$). This shows the same and the degree of changes in copper concentration of these tissues in this season. The copper levels in these tissues were negatively correlated in both spring and winter ($P<0.05$). The cause of which may be related to various, still unknown causes. We found a negative correlation between liver copper and kidney at times of higher copper concentration in liver. It is possible that at these times, higher copper concentration causes the release of more copper in the blood stream with subsequent more binding to ceruloplasmin (the copper containing enzyme in blood). This phenomenon at a certain level of blood copper result in the more unavailability of ceruloplasmin which results in the excretion of the copper in urine. The phenomenon has been also reported in Wilson’s disease in man (Reed et al. 2018). The positive correlation between liver and kidney reported in autumn is occurred at time of lower copper concentration in liver. This is when the ceruloplasmin may not be completely saturated and copper is maintained in blood stream and the copper is not excreted in the urine. This may lead to a corresponding increase in kidney copper level when lower concentration of copper was observed in liver. It is notable that liver rather than kidney tissue is the target tissue to accumulate and metabolize copper and this causes more copper burden to be observed in liver tissue. With respect to the fact that liver has the highest capacity for copper but kidney has limited capacity, when a certain level of liver copper is reached, the corresponding increase in kidney copper does not occur.

Copper contents of lung and spleen had significant and positive relationships in all studied seasons. This fact shows that these tissues may have relatively limited capacity to store copper in face of higher concentration of copper in other tissues. In winter serum copper levels were positively correlated with copper level of lung, heart and spleen in chronic copper poisoned sheep ($p<0.05$). It should be reminded that serum samples were taken from live animals in the same season and it is interesting that the level of serum concentration was well correlated with tissue samples (lung, heart). This shows the fact that serum copper in live animals and copper in lung, heart and spleen samples rise accordingly in chronic copper poisoned sheep in winter.

In conclusion, the results of the current
research showed that severity of the copper toxicity is season-dependent in sheep and seasonal variations affect the level of toxicity. Environment, climate and stressors may be the main causes of copper contents of different tissues and the liver remains the main organ to reserve copper in poisoned sheep. Furthermore, the pattern of the relationships among tissue and circulating copper were various in different seasons. Furthermore, evaluating the copper content in each tissue based on other ones may contribute to assessing the level of toxicity in different seasons.

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Conflicts of interest

The author declared no conflict of interest.

References


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PMID: 22871931


تغییرات فصلی و ارتباط بین مس سرم و بافت‌های مختلف در گوسفندان مسموم با مس در استان کرمان، ایران

سید جواد افصح هجری، غلامرضا مصباح، علی اصغر چالمه، بهنام کشاورزی، احمد عریان، مهرداد پورجعفر، خلیل بدیعی، سید جواد افصح هجری 
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چکیده
زمینه مطالعه: آلودگی خاک و مواد گیاهی در مواجهه با مس باعث مسمومیت مزمن با مس در حیوانات می‌شود.
هدف: مطالعه در مورد مسمومیت با مس در گوسفند در فصول مختلف و ارتباط بافت‌ها و مس در همان منطقه.
روش‌کار: انتخاب‌نامه‌ها در فصل‌های مختلف از پاییز تا بهار انجام شد.
نتایج: در بیشتر فصول مس در سرم و پشم بالاترین مقدار را داشت.

کلیدواژه‌ها: مسمومیت با مس، سرم، پشم، فصل، گوسفند

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