INFLUENCE OF MINERAL NUTRITION ON SUPEROXIDE DISMUTASE ACTIVITY IN BLOOD OF COWS

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The influence of Cu, Zn, Mn, Se and Mo concentrations in feed on the activity of superoxide dismutase in the blood of cows was investigated. It was demonstrated that the activity and values of some blood parameters such as erythrocyte count, haemoglobin and haematocrit were lower in cows with decreased concentrations of the elements. It was thought that nutritional factors can have important influence on increased sensitivity to cellular oxidative damage in cows.

Key words: cows, superoxide dismutase, copper, zinc, manganese.

It was demonstrated that there is a close metabolic interaction between Cu, Zn, Mo, Se and Mn in cattle (12). Its general effect depends on the mineral content in the fodder (1, 2). It is known that copper influences the defence mechanisms against free radicals and erythropoiesis in the bone marrow (4). Besides, copper can also implicate the pathogenic mechanisms of familial amyotrophic lateral sclerosis (Lou Gehrig’s disease) by the CuZnSOD mutant. Moreover, superoxide dismutase (SOD) activity influences the oxidative signals which can cause apoptosis (13). Furthermore, Cu is indispensable for biosynthesis of heme in haemoglobin and for mobilisation and transport of Fe. Considerations of particular haematological indices are also important because liganding of O₂ to haemoglobin is connected with displacement of an electron from the Fe atom orbital to O₂; molecular orbital.

SOD activity depends on Cu-Zn and plays an important role in the cell defence mechanisms against oxidative stress (5, 9, 16). An antioxidative role of SOD is based on dismutation of O₂. The mentioned above reaction is especially interesting due to participation of Cu⁺ and Cu²⁺ complexes, which are involved in reduction and oxidation at the active sites of SOD. However, almost the same catalytic activity like SOD has the aqua complex, some amino acids, and salicylates as copper ligands. The turnover of these ligands is six times smaller than that of SOD. CuZnSOD uses metal ions and is responsible for catalyzing the potentially toxic superoxide radical anion to
$\text{H}_2\text{O}_2$ (6). Then, catalase can eliminate $\text{H}_2\text{O}_2$ (7). Under influence of such minerals as lead and chromium an increase can be observed in the activity of both enzymes during chronic intoxication with cadmium. However, a decreased activity of these enzymes was found in acute or chronic intoxication with cadmium. The reason of this phenomenon is substituting zinc for cadmium to form CuCdSOD. In the brain, prion protein can also decrease CuZnSOD activity (3). The activity of enzymes described above is similar in adaptive processes associated with the intake of metals. Presumably, CuZnSOD plays the principle role in the defence mechanisms against superoxide radical anion. Unfortunately, this enzyme may be inactivated by $\text{H}_2\text{O}_2$ to generate OH (19). Activity of CuZnSOD in the particular cells and body fluids is dependent on period of observation and influence of different endogenous and exogenous factors including oxidative damage and cellular dysfunctions. All of it can play a key role in the development of animal diseases including neurodegenerative processes in animals.

In the Biebrza National Park we can observe low concentration of Cu, Zn, Mo, Se, and Mn in the soil, herbage, fodder, internal organs and body fluids of ruminants (10). That is why the effect of the concentration of minerals in the fodder on SOD activity in the blood and on erythrocytes, haemoglobin, and haematocrit in cows is the object of this paper.

**Material and Methods**

The experiment was performed on 38 clinically healthy, Black and White breed cows, aged 4-8 years, with the average annual milk yield of 4950 liters. Animals were chosen from 6 farms and 2 different regions (A and B). Group A (experimental - 19 cows) was situated in the preserved area of the Biebrza National Park (Region A) and group B (control - 19 cows) in the typical region for the North-East of Poland (Region B). Average concentrations of Zn, Cu, Mo, Se, and Mn in the diet for cows shows Table 1. During the grazing season the animals were kept on pasture while in winter their diet consisted of hay, protein food, potatoes, and straw. The experiment lasted 3 months.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fodder</th>
<th>Se</th>
<th>Zn</th>
<th>Cu</th>
<th>Mo</th>
<th>Mn</th>
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<tr>
<td></td>
<td></td>
<td>mg/kg d.m.</td>
<td></td>
<td></td>
<td></td>
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<td>A</td>
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<td>39.6</td>
<td>5.42</td>
<td>0.14</td>
<td>43.6</td>
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<tr>
<td></td>
<td>Protein food</td>
<td>0.06</td>
<td>61.2</td>
<td>7.68</td>
<td>0.39</td>
<td>112.9</td>
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<tr>
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<td>Potatoes</td>
<td>0.02</td>
<td>17.3</td>
<td>2.93</td>
<td>0.15</td>
<td>27.3</td>
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<td>Straw</td>
<td>0.01</td>
<td>16.8</td>
<td>1.67</td>
<td>0.10</td>
<td>20.4</td>
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<tr>
<td></td>
<td>Hay</td>
<td>0.03</td>
<td>41.2</td>
<td>6.71</td>
<td>0.16</td>
<td>51.3</td>
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<td>35.2</td>
<td>4.88</td>
<td>0.19</td>
<td>51.1</td>
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<tr>
<td>B</td>
<td>Pasture grass</td>
<td>0.06</td>
<td>61.4</td>
<td>8.99</td>
<td>0.27</td>
<td>279.2</td>
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<td>Protein food</td>
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<td>98.4</td>
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<td>0.63</td>
<td>407.9</td>
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<tr>
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<td>5.22</td>
<td>0.27</td>
<td>41.8</td>
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<td>22.1</td>
<td>3.42</td>
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<td></td>
<td>Hay</td>
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<td>73.8</td>
<td>9.11</td>
<td>0.31</td>
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<tr>
<td></td>
<td>Mean</td>
<td>0.07</td>
<td>56.6</td>
<td>8.27</td>
<td>0.32</td>
<td>211.0</td>
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</tbody>
</table>
The selected animals were clinically examined. Samples of blood were taken from the jugular vein using individual sets. Mineral analysis of pasture grass, protein food, potatoes, and straw had been made before the experiment started. The concentrations of Zn, Cu, Mo, Se, and Mn were checked. The following blood parameters were determined: SOD activity, erythrocyte count, haemoglobin, and haematocrit. The blood was taken three times: 1 - 3 d before calving, 2 – 2 d after calving, 3 – 42 d after calving (lactation peak period). For the detection of Cu, Zn, Mo, and Mn the method of atomic spectrophotometry was applied. Se was detected with the use of automated hydride generation atomic absorption technique. Haematological tests were performed with semiautomatic analyser HC-510 (Hycel). SOD activity in the blood was assayed by Ransod kit. This method uses xanthine and xanthine oxidase to generate superoxide radicals (18). The final results were subjected to statistical calculations by means of the Student’s t-test.

Results

Average activity of SOD in the erythrocytes of cows from groups A (experimental) and B (control) during the 1st, 2nd and 3rd periods were: 57.71±14.2, 76.85±14.6, 62.15±13.7 and 71.53±15.5, 96.37±15.0, 76.68±14.0 U/g Hb, respectively. Average number of erythrocytes in cows from group A (experimental) and B (control) in the particular periods were: 4.40±1.04, 5.73±1.13, 4.70±1.09, and 5.24±1.01, 6.51±0.85, 5.52±0.81x10^12/dm³, respectively. Average contents of haemoglobin and haematocrit values in the blood of cows from both groups during the 1st, 2nd and 3rd periods were as follows: haemoglobin: 62.8±16.5, 85.7±16.2, 68.5±18.6, 79.9±20.6, 98.5±40.1, 85.1±28.7 g/dm³ and haematocrit: 0.24±0.06, 0.32±0.07, 0.26±0.06, 0.29±0.07, 0.40±0.05, 0.32±0.07 l/l, respectively (Figs 1-3).

The results of the investigations showed that the fodder from group A had lower concentrations of all minerals than that from group B. The SOD activity in erythrocytes and haematological indices in cows from group A were also lower than in cows from group B. The SOD activity differences between groups A and B were statistically significant in the 1st (P ≤ 0.01), 2nd (P ≤ 0.001) and 3rd period (P ≤ 0.05). The highest values of indices were observed in the 2nd period (2 d after calving). There were statistically significant differences in SOD activity between the 1st and 2nd period in the groups A (P ≤ 0.001) and B (P ≤ 0.05).

The statistically significant differences between the 1st and 2nd period in the number of erythrocytes (P ≤ 0.001) and values of haemoglobin (P ≤ 0.001) and haematocrit (P ≤ 0.001) were observed. Moreover, the statistically significant differences in haemoglobin content between the 1st and 3rd period (P ≤ 0.001) were confirmed. Statistically significant differences in erythrocyte count and haemoglobin in the particular periods between group A and B were found.
Fig. 1. Superoxide dismutase activity in erythrocytes.

Explanations for figures 1 - 3: SD-significant differences, Hb=haemoglobin, differences statistically significant between: period 1 and 2 or 1 and 3 (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001), group A and B (***P ≤ 0.05, ****P ≤ 0.01, *****P ≤ 0.001).

Fig. 2. Number of erythrocytes.

Explanations as in Fig. 1.
Fig. 3. Value of haemoglobin.
Explanations as in Fig. 1.

Fig. 4. Value of haematocrit.
Explanations as in Fig. 1.
Discussion

The analysis of the results shows that mineral nutrition of dairy cows plays an important role in the protection of enzymatic defence mechanisms against free radicals. Especially connection between metal metabolism and CuZnSOD is well documented (14). We can show two principle forms of CuZnSOD. First of them – the intracellular dimeric form is present in cytosol and nucleus, and the second tetrameric form is present in the interstitial tissue fluid. Many experiments show dietary copper and zinc deficiency. It is clear that low SOD activity can indicate the dietary deficiency of copper and zinc (15). It was confirmed in many experiments that the SOD activity in lysates from copper deficient cells was low. However, CuZnSOD exists as both a metal pool and a metal-deficient pool. A diet low in copper increases also the inflammatory reaction in which a lower SOD activity may be observed (22). It seems that in the cells of particular systems of cows from preserved area of the Biebrza National Park (experimental group A) are generated lower levels of reactive oxygen species than in cows from the control group. It is confirmed that in lower SOD activity is a response to oxidative metabolism (13).

On the other hand, low SOD activity in erythrocytes of cows from the experimental group can be a result of adaptation processes and can indicate the increase of sensibility to different oxidative damage including neurodegenerative diseases. Adaptive processes are especially well described when excess of selected heavy metals is concerned. However, adaptive response to deficiency of minerals has been less described. Different concentration of trace elements in the fodder for cows influences lipid peroxidation and is correlated with enzymes activity such as: SOD, catalase, and glutathione transferase. But oxidative results are dependent on the period of observation. It can be an initial oxidative stage, an adaptive response, and a secondary stage where antioxidants are suppressed. There was confirmed different SOD activity in the blood of cows in particular periods, including physiological stages (Fig. 1.). We can observe in feeding practice the prevalence of a combined exposure to two or more trace elements. It can have an opposite effects on lipid peroxidation. Metabolic interactions between Cu, Zn, Mo, Se, and Mn participate in the mentioned above processes. For instance, zinc can inhibit copper-induced lipid peroxidation by competing with copper; zinc and cadmium can inhibit nickel-induced lipid peroxidation (17). The mechanism of antioxidant protection may include metallothioneins. The metallothioneins are antioxidant proteins of low molecular weight and high sulphur content, binding Cu, Zn, Cd, Hg, and Fe. The function of metallothionein in the internal organs of ruminants is not quite clear. The binding of trace elements can be important for the homeostasis of Cu and Zn.

Low concentration of Cu and Fe in the particular elements of feed chain induces an excessive absorption of Mn in cattle. Then oxidation of Mn++ increases into its oxidative species - Mn+++ . Mn+++ accumulates in mitochondria of brain cells of cattle in MnSOD (Mn-dependent superoxide dismutase) deficient genotypes. Lack of a scavenger of free radicals as co-factors such as Se, Cu, Zn, and Fe in the astrocytes is very dangerous for the central nervous system. It can lead to initiation of chain reactions of auto-oxidant neuronal degeneration, and expression of the Cu-metalloprotein, prion protein (PrP). When a rate of PrP turnover and its requirement for Cu exceeds depleted supply of Cu within the brain cells, PrP can not longer bind Cu. Thus PrP binds Mn+++ . Mn+++ through lethal auto-oxidative activity can lead to modifications of subcellular structures of central the nervous system including nucleic
acids, and mineral disturbances of cattle SOD activity can be also dependent on Cu and can help in the diagnosis of its deficiency (8, 11, 20, 21 ).

The mentioned above investigations and considerations show multiple molecular mechanisms between trace elements and SOD activity. Further researches are needed to explain the role of trace elements n pathogenesis of many diseases of cows, including neurodegenerative processes, caused by deficiency of minerals and possibility of treatment by various antioxidants.

References