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Comparing levels of certain heavy metals and minerals and antioxidative metabolism in cows raised near and away from highways*

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Abstract: The scope of this study was to investigate the concentrations of certain heavy metals, mineral levels, and antioxidative metabolism in cows raised near (300 m) and away from highways (2.5 km) in Çankırı Province. For this purpose, blood samples were collected from 100 cows (aged 3–5 years) raised near and away from highways in this province. Concentrations of certain heavy metals and minerals in the serum were analyzed on an ICP-MS device. Additionally, lipid peroxidation product malondialdehyde (MDA) in the plasma and antioxidative defense system indicators, erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities, were measured with a spectrophotometer. As a result, levels of certain heavy metals and minerals and antioxidative status were determined in cows raised near and away from highways. Aluminum, iron, nickel, and zinc levels in the cows raised near highways were significantly different from those of the cows raised away from highways. Furthermore, MDA concentrations and SOD activities were significantly different between the two groups. It is concluded that monitoring the concentration of these heavy metals periodically may be useful to increase the productivity of cows.

Key words: Antioxidant status, cows, heavy metals

1. Introduction

Heavy metals are elements with a density higher than 5 g/cm^3 or with an atomic weight larger than 50 (1). Heavy metals are known to be chemicals extremely dangerous to living organisms. Large quantities of heavy metals are discharged each day in various ways and reach the rivers, lakes, and seas (2).

Environment and soil in which the food consumed was grown and water involved in the cultivation are of importance regarding the intake of these metals. Tissue damage or even mortality might occur as a result of heavy metal accumulation in the biological tissues such as liver, kidney, and brain and body fluids of animals that are fed on plants grown in soils with dense industrial pollution and vehicle traffic (1,2).

Currently, it is known that heavy metal ions lead to serious health problems; they even cause death in some cases. Therefore, it is important to focus on heavy metal contamination, and it is necessary to examine various sources and concentrations of heavy metals in foods and to take effective precautions (3). Heavy metals, most of which are necessary for organisms, bind to cell structure in very low concentrations. However, higher concentrations of these heavy metals might produce toxic effects by inhibition of enzyme systems in organisms. Mercury (Hg), lead (Pb), cadmium (Cd), and copper (Cu), in particular, have greater toxicity (4,5).

Factors that affect the degree of heavy metal toxicity include the form of the metal, the presence of other metals and poisons, environmental factors (pH, temperature, oxygen, etc.), life-stage and sex of the organism, etc. (6). While some metals such as arsenic (As), Cd, Hg, molybdenum (Mo), Pb, and selenium (Se) are extremely toxic, some of them have carcinogenic [As, Cd, chromium (Cr), nickel (Ni), Pb, Se], mutagenic, and teratogenic effects. In addition, Cd stimulates free radical production by oxidative deterioration of lipids and Pb may lead to damage as a result of disruption of the equilibrium between oxidants and antioxidants in mammalian cells (7).

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Vehicle traffic, which is a significant source of heavy metals, contributes to air pollution. There is an increasing number of highways with a parallel increase in the number of vehicles using them. The increasing numbers of highways are significant sources of heavy metal. These factors can lead to contamination of human and animal food sources. The role of heavy metals in environmental pollution is gaining importance with each passing day (8,9).

Gurer et al. (10) and El-Demerdash et al. (11) have reported that some heavy metals (Cd, aluminium (Al), Ni) stimulate free radical production and affect the balance between oxidants and antioxidants in animals.

The economy of Çankırı Province is based on agriculture and animal husbandry. However, this province is rich in terms of mines, and industrialization is on the rise. In addition, Çankırı Province is an important transit route between the Central Anatolia and Black Sea regions (12). In this context, animals and animal products can be exposed to all these pollutants in time. The aim of this study was to detect and compare some heavy metals and mineral levels (Al, vanadium (V), chrome (Cr), manganese (Mn), iron (Fe), Ni, cobalt (Co), Cu, zinc (Zn), As, Se, Cd, Pb) as well as antioxidative metabolism (SOD, CAT activities, MDA concentration) in cows raised near (300 m) and away (2.5 km) from highways. In the light of the data obtained, the effects of the heavy metals, minerals, and antioxidant levels were determined in terms of animal and human health in cows breeding near and away from highways.

2. Materials and methods

2.1. Animals

Approval for animal experiments was granted for the study by the Ethics Committee of Kırıkkale University (Decision no: 12/08). Blood samples were taken from the jugular veins of 100 cows (aged 3–5 years). The animals were selected in summer from clinically healthy cows that were fed by villagers by traditional techniques in farms near (300 m, 22 native breeds, 17 Holstein, 11 Simmental) and away from highways (2.5 km, 15 native breeds, 21 Holstein, 14 Simmental). The distance between a main road and a farm was determined as described by Arslan et al. (8). The samples were transferred to the laboratory in cold chain. In the laboratory, after coagulation, the samples were drawn and serum was separated by centrifugation (3000 rpm for 10 min). The obtained serum was kept at –20 °C until analysis.

2.2. Blood heavy metal and mineral analysis

For the analysis of certain heavy metals and minerals in blood, 1 mL of serum was taken and placed in the heat- and pressure-resistant Teflon cells of a microwave solubilization device and 5 mL of 65% HNO_3 was added. To prevent gas outlet and foaming, samples were kept uncovered for at least 20 min before placing into the microwave solubilization device. Then the covers were sealed and the appropriate temperature program was applied. When the solubilization was completed, the clear solutions obtained were transferred to 10-mL flasks and the volumes were completed with double distilled water. The samples were readied for measurement. In order to test the performance of the device, tune adjustment was conducted by using tune solution; this solution was composed of yttrium (Y), lithium (Li), cobalt (Co), thallium (Tl), and erbium (Er). The count values of these elements were checked during this adjustment and then the device was readied for the analysis. To correct the deviation in calibration line, the internal standard that represents the periodic table containing beryllium (Be), scandium (Sc), radium (Ra), and bismuth (Bi) elements was introduced to the ICP-MS instrument (Agilent 7500a, Agilent Technologies Japan) (13). The ICP-MS instrument conditions are given in Table 1. The selected element values were read from the samples and recorded as ppm. The method was validated by the parameters of accuracy, recovery (Al: 99.80%, V: 99.90%, Cr: 99.90%, Mn: 99.90%, Fe: 98.20%, Ni: 99.90%, Co: 99.90%, Cu: 99.80%, Zn: 99.90%, As: 99.70%, Se: 99.90%, Cd: 99.90%, Pb: 99.90%), specificity, limit of detection (Al: 1.158 ppb, V: 0.120 ppb, Cr: 0.730 ppb, Mn: 0.480 ppb, Fe: 10.860 ppb, Ni :0.33 ppb, Co: 0.320 ppb, Cu: 1.363 ppb, Zn: 2.305 ppb, As: 0.077 ppb, Se: 3.299 ppb, Cd: 0.070 ppb, Pb: 0.200 ppb), and limit of quantitation (Al: 3.82 ppb, V: 0.40 ppb, Cr: 2.41 ppb, Mn: 1.58 ppb, Fe: 35.840 ppb, Ni: 1.09 ppb, Co: 1.06 ppb, Cu: 4.50 ppb, Zn: 7.61 ppb, As: 0.25 ppb, Se: 10.89 ppb, Cd: 0.23 ppb, Pb: 0.66 ppb).

2.3. Analysis of antioxidative metabolism

The anticoagulated blood samples were centrifuged at 3000 rpm for 10 min so as to separate plasma and erythrocytes. The plasma and buffy coat were removed. Erythrocytes were washed three times by re-suspending in phosphate

Table 1. ICP-MS instrument conditions.

Parameters	Value
Nebulizer	0.11 rps
Spray chamber temperature	2 °C
RF generator	1220 W
Ar flow rate (L/min)	15 L/min
Auxiliary gas flow rate (L/min)	0.9 L/min
Nebulizer gas flow rate (L/min)	1.11 L/min
Sample uptake rate (µL/min)(speed)	0.3 rps
Number of replicates	3
Integration time (s)	3s
Internal standards	Be, Sc, Rh, Bi (200 ppb)

buffered saline (PBS, pH 7.4). The erythrocyte pellet was mixed with an equal volume of PBS (14). During the analysis, erythrocytes were lysed in ice-cold distilled water to prepare erythrocyte hemolysate. Erythrocyte SOD activities were determined in the hemolysates according to the methods described by Sun et al. (15). This involved inhibition of nitroblue tetrazolium (NBT) reduction by the superoxide anions. The resulting reduction of NBT was measured at 560 nm by the spectrophotometer. Malondialdehyde concentrations in erythrocytes were determined according to a procedure described for the thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (16). The optical densities were measured at 532 nm by spectrophotometer (Shimadzu UV-1700, Japan). The erythrocyte CAT activity was measured using the method of Aebi (17) with a spectrophotometer at 240 nm.

2.4. Statistical analysis

SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical calculations. Study data were expressed as mean ± standard deviation (X ± SD). The differences between the parameters of the two groups were determined by Mann–Whitney U-test. P values of less than 0.05 were considered statistically significant.

3. Results

Al, V, Cr, Mn, Fe, Ni, Co, Cu, Zn, As, Se, Cd, and Pb levels of blood samples obtained from cows raised near and away from highways are given in Table 2. Al, Fe, Ni, and Zn levels of cows raised near and away from highways were significantly ($P < 0.05$) different. The values were 105.2 ± 35.04 (50.33–211.8 ppm) for Al, 122.6 ± 34.76 (68.69–190.4 ppm) for Fe, 2.37 ± 1.21 (0.71–5.72 ppm) for Ni, and 0.22 ± 0.15 (0.02–0.684 ppm) for Zn in cows raised near highways and 91.29 ± 31.14 (43.61–205.5 ppm) for Al, 103.9 ± 19.20 (61.22–164.3 ppm) for Fe, 1.46 ± 0.54 (0.57–2.88 ppm) for Ni, and 0.14 ± 0.08 (0.004–0.36 ppm) for Zn in cows raised away from highways. In contrast, there were no statistically significant differences between the two groups with respect to V, Cr, Mn, Co, Cu, As, Se, Cd, and Pb levels.

Lipid peroxidation and some antioxidant parameters are shown in Table 3. MDA concentrations were higher in cows raised near highways than those raised away from highways ($P < 0.05$), although SOD activities were higher in cows raised away from highways ($P < 0.05$). There was no significant difference between the groups in terms of CAT activity ($P > 0.05$).

Table 2. Some heavy metals and mineral levels in cows raised near and away from highways.*

Group	Near highways n: 50		Away from highways n: 50	
Elements	Mean ± SD	Min.–Max.	Mean ± SD	Min.–Max.
Al	105.2 ± 35.04[a]	50.33–211.8	91.29 ± 31.14[b]	43.61–205.5
V	0.136 ± 0.05	0.04–0.31	0.14 ± 0.04	0.08–0.27
Cr	4.62 ± 1.10	3.07–7.36	4.31 ± 0.78	3.10–7.11
Mn	0.55 ± 0.21	0.26–1.20	0.54 ± 0.17	0.29–1.05
Fe	122.6 ± 34.76[a]	68.69–190.4	103.9 ± 19.20[b]	61.22–164.3
Ni	2.37 ± 1.21[a]	0.71–5.72	1.46 ± 0.54[b]	0.57–2.88
Co	0.056 ± 0.02	0.02–0.10	0.056 ± 0.02	0.03–0.17
Cu	33.55 ± 11.38	16.97–78.03	29.73 ± 8.78	11.55–46.45
Zn	0.22 ± 0.15[a]	0.02–0.68	0.14 ± 0.08[b]	0.004–0.36
As	0.0004 ± 0.0002	0.000–0.001	0.0008 ± 0.003	0.000–0.001
Se	0.005 ± 0.005	0.000–0.023	0.005 ± 0.004	0.001–0.012
Cd	0.002 ± 0.016	0.000–0.11	0.001 ± 0.0009	0.000–0.00655
Pb	0.0002 ± 0.0009	0.0000–0.007	0.0006 ± 0.002	0.0000–0.012

*as ppm: mean ± standard deviation and range

[a,b] : Means within the same row with different letters are significantly different ($P < 0.05$).

Table 3. Lipid peroxidation and some antioxidation parameters of the groups.

	Group	
	Near highways n: 50	Away from highways n: 50
Parameters	Mean ± SD	Mean ± SD
MDA (μmol/L)	1.19 ± 0.16[a]	0.70 ± 0.12[b]
SOD (U/gHb)	186.094 ± 12.11[a]	221.013 ± 12.68[b]
CAT (k/gHb)	92.35 ± 26.76	103 ± 18.17

[a,b]: Means within the same row with different letters are significantly different ($P < 0.05$)

4. Discussion

Today, all living organisms and their living environment are constantly exposed to environmental pollutants. Industrial activities are on the rise in parallel with increasing population, and air, soil, and water pollution is endangering the life on earth. Recently built highways and the growing number of vehicles also cause environmental pollution in Turkey. It has been reported that significant amounts of heavy metals and minerals are discharged into the environment as a result of such industrial activities, and pose a risk to living organisms (9,18).

In their study to investigate the levels of traffic-borne heavy metal pollution in soil along the Viranşehir–Kızıltepe highway, Bilge and Cimrin (18) found that Pb, Cd, Ni, Cr, and Cu levels decreased with increasing distance from the highway. Another study reported that traffic-borne heavy metal pollution in mullein leaves collected from the Black Sea coastal road extending between Samsun, Ordu, Giresun, and Rize provinces and Hopa District increased in parallel with traffic intensity (19). These studies are important indicators of heavy metal pollution in plants growing at roadsides. It is also possible to find such residues in animals raised close to highways. Arslan et al. (8) studied trace elements such as Cu, Zn, and Fe and levels of heavy metals such as Cd and Pb in cattle living at roadsides for at least 3 years and found accumulation of Cd and Pb in cattle living near highways. In the present study, Cu and Fe accumulation in cattle raised near highways were higher whereas Zn, Cd, and Pb accumulation levels were lower than those reported by Arslan et al. (8).

In the present study, the levels of Al, Fe, Ni, and Zn in cows raised near highways were higher than those in cows raised away from highways. Tools and equipment used in barns, drinking water, feed additives, vaccine adjuvants, drugs and parenteral solutions, raw materials and packing materials, Al powder inhalation from Al processing plants, and other similar factors have a significant role in the accumulation of Al in animals. Al is stored in various tissues but mainly in the bones and lungs, and excess Al is eliminated by the kidneys. Excessive Al in the body causes oxidative stress (20). In the present study, high levels of Al in animals may be associated with these conditions.

Trace elements such as Fe and Zn are important factors in the antioxidant defense system (21). Unlike in Arslan et al. (8), both Fe level and MDA concentration in this study were higher in cows raised near highways. In this study, high Fe levels in animals' blood may be due to a possible hereditary problem (2).

Burning fuel oil and its residues, processing and refining nickel materials, municipal waste incinerators, and nickel sulfate emission due to coal burning are responsible for 20%–80% of nickel sulfate emission in the air. Ni enters the body through inhalation, drinking water, and food (22). Hence, the high levels of Ni determined by the analysis can be considered an indication of sheltering animals in this metal-rich air, high levels of Ni in their drinking water, or feeding animals with Ni-rich plants.

MDA concentration is a commonly used parameter in the assessment of lipid peroxidation caused by oxidative stress. In this study, similar to Arslan et al.'s (8) research, MDA concentrations of the group raised near highways was higher than the group raised away from highways. Unlike SOD, CAT activities of the group raised away from highways were higher than those of the group living near highways. Significant differences were found in MDA concentration and SOD activity between the two groups. However, no significant difference was detected in CAT activity. The increase in MDA concentration, which is an indicator of lipid peroxidation, in the group living near roads, may be as a result of the effects of traffic pollution and heavy metals on living creatures. The activity of the antioxidant enzyme SOD, which causes cell damage, catalyzes the conversion of hydrogen peroxide and oxygen. Catalase has peroxidase activity and splits hydrogen peroxide into oxygen and water (23). In the group living near roads, lipid peroxidation disrupted the balance between oxidants and antioxidants in favor of oxidants.

Previous studies showed that excessive Al and V in the blood increase the MDA concentration, which is in agreement with our results in cows raised near highways (20,24). Martinez-Finley et al. (25) reported that Mn accumulation may be related to the production of free radicals and depletion of cellular antioxidant defense systems. A previous study has shown that As induces oxidative stress in rat erythrocytes (26). We observed no relationship between MDA concentration, Mn, and As levels in our study.

The risk among humans and animals of exposure to metals has greatly increased due to a variety of agricultural production methods, misuse and/or unconscious use of pesticides, and exhaust gases from vehicles. Moreover, human and animal food chains can be contaminated by unsanitary storage of solid wastes and waste water (9,18). In addition, disruption of the antioxidant defense system by toxic levels of heavy metals may affect human health (24–26).

In our study, Al, Fe, Ni, and Zn levels and MDA concentration were higher in the group living near highways than they were in the group living away from highways. This may consequently lead to a decrease in the quality of food of animal origin. Thus, toxic levels of heavy metals in livestock should be periodically monitored and livestock owners should be educated about the risk and toxicity of heavy metals. Additionally, various common antioxidants can be added to the diets of domestic livestock.

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