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RESEARCH ARTICLE

Evaluation of the Relationship of Blood Heavy Metal, Trace Element Levels and Antioxidative Metabolism in Cattle Which Are Living Near The Trunk Roads

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Summary

In this study, detection of a possible relationship of some trace element levels of copper (Cu), zinc (Zn), and iron (Fe), toxic heavy metals, cadmium (Cd) and lead (Pb), and antioxidant functions in cattle, which are living near the trunk roads for at least 3 years, was aimed. Totally, 70 cattle, which were in different herds, were used. Fifty-four cattle (3-13 years), which were living near the trunk roads at about 0-500 m were used for the experiment group. Also, 16 cattle (3-12 years), which were living far from trunk roads at about 2-3 km were used for the control groups. Blood samples were collected and Cd, Pb, Cu, Zn, Fe levels, lipid peroxidation product malondialdehyde (MDA) and antioxidative defense system indicators, erythrocyte superoxide dismutase (SOD) activity, catalase (CAT) activity, and plasma ceruloplasmin (CP) level were measured. Cd and Pb levels of the experimental animals were determined to be higher than the control group ($P<0.01$ and $P<0.001$, respectively). However, Zn level of the experiment group was found lower than the control animals ($P<0.001$). MDA increased about three times and CP increased about two times more than the control group ($P<0.001$). Significantly correlations were observed among Pb, MDA and CP levels. Also, important correlations were found between Fe and CP, also Cu and MDA levels. Besides, MDA and CAT levels of the animals increased with CP and SOD together, respectively. At the end of the study, Cd and Pb accumulation was observed in cattle, which were living near the road with intensive traffic for at least three years when compared with the control group. In addition, the results showed significant interactions among toxic heavy metals, physiological trace elements, enzymatic and non-enzymatic antioxidants and the lipid peroxidation.

Keywords: *Heavy metals, Trace elements, Antioxidative metabolism, Cattle*

Anayol Yakınında Yaşayan Sığırlarda Kan Ağır Metal, İz Element Seviyeleri ve Antioksidan Metabolizma Arasındaki İlişkinin Değerlendirilmesi

Özet

Bu çalışmada, en az 3 yıldır anayol kenarında yaşayan sığırlarda bakır (Cu), çinko (Zn) ve demir (Fe) gibi bazı iz elementler, zehirli ağır metallerden olan kadmiyum (Cd) ve kurşun (Pb) ve antioksidan fonksiyonlar arasındaki olası ilişkinin tespit edilmesi amaçlandı. Çalışmada farklı ahırlarda yaşayan toplam 70 besi sığırı kullanıldı. Ana yola 0-500 metre mesafede yaşayan 54 (3-13 yaşlarında) sığır araştırma grubu olarak kullanıldı. Ana yola 2-3 km mesafede yaşayan 16 (3-12 yaşlarında) sığır ise kontrol grubunu oluşturdu. Hayvanlardan kan örnekleri toplandı ve Cd, Pb, Cu, Zn, Fe seviyeleri, lipid peroksidasyon göstergesi olan malondialdehid (MDA) ve antioksidasyon defans sistem indikatörleri olan eritrosit süperoksit dismutaz (SOD) aktivitesi, katalaz (CAT) aktivitesi ve plazma serüloplazmin (CP) seviyesi ölçüldü. Deneme grubu hayvanların Cd ve Pb seviyelerinin kontrol grubu hayvanlardan yüksek olduğu belirlendi (sırasıyla $P<0.01$ ve $P<0.001$). Bununla birlikte deneme grubunun Zn seviyesi kontrol grubundan düşük bulundu ($P<0.001$). MDA seviyesi kontrol grubundan yaklaşık üç kat, CP seviyesi ise yaklaşık iki kat daha yüksek tespit edildi ($P<0.001$). Pb ile MDA ve CP seviyeleri arasında gözle görülür şekilde bir ilişki olduğu gözlendi. Ayrıca, Fe ile CP ve Cu ile MDA arasında da önemli ölçüde korelasyon bulundu. Bunun yanında MDA ile CAT ve CP ile SOD seviyelerinin birlikte yükseldiği görüldü. Çalışma sonunda, anayol yakınında en az üç yıl yaşamış olan hayvanlarda, kontrol grubuyla karşılaştırıldığında Cd ve Pb birikimi olduğu gözlendi. Ayrıca, elde edilen sonuçlar zehirli ağır metaller, fizyolojik iz elementler, enzimatik ve enzimatik olmayan antioksidanlar ve lipid peroksidasyon göstergesi olan MDA arasında önemli düzeyde ilişki olduğunu gösterdi.

Anahtar sözcükler: *Ağır metaller, Iz elementler, Antioksidatif metabolizma, Sığır*

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INTRODUCTION

Road traffic contributes significantly to air pollution in urban areas, generating particulate matter, aerosols, and heavy metals around the roads. The problem of environmental pollution with heavy metals is becoming increasingly urgent [1]. Cd, Pb, Cu, and Zn are good indicators for some environmental pollution [2]. Calcium (Ca), Fe, and Zn deficiency, for instance, enhance susceptibility to Cd and Pb toxicity [3]. Cd and Pb enter food chains mostly through plants, which often accumulate heavy metals to concentrations exceeding their levels in the soil by many times [4]. Cd, which is a highly toxic metal, causes necrosis by accumulating especially in liver and kidney [5]. General clinical symptoms of Cd toxicity in mammals include anemia, retarded testicular development or degeneration, enlarged joints, scaly skin, liver and kidney damage, reduced growth, and increased mortality [6,7]. Cd also stimulates free radical production, due to oxidative deterioration of lipids [8]. Pb is the most common cause of heavy metal poisoning in cattle and signs include a depressed appearance, blindness, grinding of teeth, muscular twitching, snapping of eyelids, and convulsive seizures [6,7,9]. Pb-induced damage may result from disturbance of prooxidant and antioxidant balance that is found in the mammalian cells. These suggestions include disturbances in mineral metabolism and demyelination of nervous tissues [9]. Normal blood levels of Cd and Pb are 1 and 0-0.24 ppm, respectively in animals [7]. Also, blood Pb values 0.35 ppm is diagnostic of Pb poisoning in cattle [10].

Trace elements are essential in human and animal nutrition. For some, trace elements suffer from deficiencies; they are likely to produce milk and meat that are deficient as well. Clinical signs linked to trace element deficiencies are well-described in cattle [11]. There is an important interaction between trace elements and toxic metals such as Pb, Cd and Zn [12,13]. Exposure of livestock to either high levels of toxic metals (Cd and Pb) or less than optimal levels of the essential microelements (Cu, Co, and Zn) can trigger adverse effects such as reproductive impairment, physiological abnormalities, behavioral modifications, or even death [14]. In addition contribute to important developments both in treatment and control of diseases directly related to the lack of trace elements, as well as in the treatment and control of parasitary, bacterial and viral diseases caused as a consequence of trace element deficiency [15]. Nevertheless, many of the clinical signs are not pathognomonic of trace element deficiencies and their diagnosis is most commonly confirmed by blood analysis [11].

The aim of this study was the detection of possible correlation of some trace element levels (Cu, Zn, and Fe), toxic heavy metals (Cd and Pb), and antioxidant functions (superoxide dismutase [SOD] and catalase [CAT] activities, plasma malondialdehyde [MDA] and ceruloplasmin [CP]) in cattle which are living near the trunk roads.

MATERIAL and METHODS

Animals

Totally 70 female cattle, which lived in different herds in Samsun and Tekirdag Cities of Turkey, were used in the study. Fifty-four cattle (3-13 years), which were living near the expressway at about 0-500 m were used for the experiment group. The breeds of the experiment animals were local race (15), Jersey (11), Simental (2) and Holstein (26). In addition, 16 cattle (3-12 years), which were living far from the expressway at about 2-3 km were used for the control groups. The breeds of the control animals were local race (9), Jersey (5) and Holstein (2). All the animals were living for at least 3 years in the same region and were grazing at least 6 hours per day.

Blood Heavy Metal and Trace Elements Analysis

Blood samples were taken from the jugular vein in anticoagulant and empty tubes from both control and experiment groups. For the Cd and Pb measurements, an equal amount of 20% trichloroacetic acid (TCA) was added to the tubes with 3 ml anticoagulated blood and this mixture was centrifuged.

Trace elements (Zn, Fe, and Cu) were detected from sera, as well as Cd and Pb measurements were carried out with Atomic Absorption Spectrometry (Shimadzu AA-680) based on comparison with external standards. The standards were freshly prepared from standard stock metal solutions (Titrisol 1.000 8 0.002 mg/l, Merck) just prior to analysis, and were used for initial calibration for each substance. These solutions were also used as internal quality standards Hollow Cathode Lamp and Background Correction (with Deuterium Lamp) modes were selected to element analyses. Each result was corrected for the appropriate reagent used and matrix blanks. For the accuracy of trace element concentrations, each sample was measured twice for each element analysis and the differences 5% between two measurements were accepted. Deionized water was used to clean the chamber and zero control for each analysis. The stock standard metal solutions for every metal (Merck, 1.000 mg/l) were used for positive control and tested for every 25 samples, for the reliability of measurement [16-18].

Assay of Lipid Peroxidase and Antioxidant Metabolism Parameters

SOD and CAT activities, MDA and CP levels were determined to evaluate lipid peroxidation and antioxidant metabolism. The anticoagulated blood samples were centrifuged at 3.000 rpm for 15 min to separate the plasma and erythrocytes. Plasma was harvested by aspiration. Prior to analysis, erythrocytes were washed the three times with an equal volume saline. The plasma specimens used for the determination of MDA and CP, and the erythrocyte samples used for the determination of SOD and CAT.

MDA levels were determined using the method described by Yoshoiko et al.[19], based on thiobarbituric acid (TBA) reactivity. The optical densities were measured at 535 nm by spectrophotometer (Schimadzu UV 1208). The erythrocyte CAT activity was measured using the method of Aebi [20] with a spectrophotometer at 240 nm. Erythrocyte SOD activities were determined with the method of Sun et al.[21]. This involved an inhibition of nitroblue tetrazolium (NBT) reduction by the superoxide anions. The resulting reduction of NBT was measured at 560 nm by the spectrophotometer. Plasma CP level was determined using P-Phenilen diamin dichloride (PPD). The optical density was measured at 546 nm by the spectrophotometer [22].

Data Evaluation

Independent samples of t-test or Mann Whitney U-test was used for statistical comparison of the groups. Also, pearson correlation test was used for determination of correlations among heavy metals, trace elements, lipid peroxidation and antioxidation parameters. All data were expressed as mean ± standard deviation (SD). Differences were considered as significant when *P* values were less than 0.05.

RESULTS

Heavy metal and trace element levels and lipid peroxidation and antioxidation parameters are shown in *Tables 1* and *2*, respectively. Cd and Pb levels increased in the experimental animals, which were living at 0-500 m near the road for at least 3 years and this increasing is statistically important compared with the control animals ($P<0.01$ and $P<0.001$ for Cd and Pb, respectively). On the other hand, serum Zn level decreased in the experiment group when compared with the control group ($P<0.001$).

Average MDA level of the experiment group was increased about three times than the control group ($P<0.001$). In addition, a statistical difference was determined in mean CP levels between the groups ($P<0.001$). However, no statistical differences were detected at SOD and CAT activity levels ($P>0.05$).

Table 1. *Blood trace element and heavy metal concentrations (µg/dl)*
Tablo 1. *Kan iz element ve ağır metal konsantrasyonları (µg/dl)*

Parameters	Groups	
	Control (n=16)	Experiment (n=54)
Cu	57.53±6.98	53.89±12.45
Zn	72.40±15.11	64.57±9.76**
Fe	131.55±22.20	120.72±35.94
Cd	1.308±0.691	2.379±0.871*
Pb	11.89±5.227	19.33±9.535**

** Statistically different from the control group (*P<0.01, **P<0.001)*

Table 2. *Lipid peroxidation and antioxidation parameters of the groups*
Tablo 2. *Grupların lipid peroksidasyon ve antioksidasyon parametreleri*

Parameters	Groups	
	Control (n=16)	Experiment (n=54)
MDA (µmol/L)	25.74±13.13	77.07±36.81*
CP (mg/dL)	5.17±3.07	11.57±4.67*
CAT (K/ghb)	0.31±0.24	0.38±0.29
SOD (U/ghb)	2.82±0.87	2.90±1.01

** Statistically different from the control group (P<0.001)*

Correlations between the parameters are shown in *Table 3*. Mean blood Pb levels were significantly correlated with MDA and CP levels ($r = 0.252, P = 0.036$ and $r = 0.276, P = 0.022$, respectively). Also, Fe and Cu levels were significantly correlated ($r = 0.245, P = 0.05$). A negative correlation was determined between Fe and CP levels ($r = -0.272, P = 0.028$) and also Cu and MDA levels ($r = -0.242, P = 0.046$). Furthermore, positive correlations were detected between MDA and CP ($r = 0.271, P = 0.023$) and CAT and SOD levels ($r = 0.264, P = 0.031$). Although a negative correlation was observed between Cd and Zn levels, this correlation was not statistically important ($r = -0.084, P = 0.492$).

DISCUSSION

Heavy metals may come from many different sources in urbanized areas, including vehicle emissions, industrial discharges, and other activities. Traffic exhaust is an important influence on Pb and Cd pollution, leading to a concern on their ecological impact, as indicators of these pollutants [23]. Heavy metals are among the most widespread potential chemical contaminants in the environment and are transferred to man and animals through diet and other routes [9]. Cd has a long biological half-life (10-30 years) in animals [24]. Accumulation of Cd and Pb in ruminants causes toxic effects (characterized by neurological signs, which include depression, ataxia, blindness, and seizures, gastrointestinal, haemopoietic, liver and kidney failures) in cattle, but also in humans consuming meat contaminated with toxic metals [10,25]. In this study, plasma Pb and Cd levels of the experiment animals, which were living and feeding near the trunk road at least three years, were determined importantly increased compared with the control group. Although these values are under the acute toxicity levels, heavy metals cause serious health effects in animals in long-term period [7]. Furthermore, these animals were used for milk and meat production, and therefore toxic metal accumulation is also a health risk for humans.

The absorption, accumulation, and toxicity of each metal may be affected by diverse factors, including interactions with other metals. Indeed, interactions between toxic and essential metals are central to mineral balance. It has been demonstrated that toxic metals can disrupt trace element metabolism [3].

Table 3. *Correlations between the parameters*
Tablo 3. *Parametreler arasındaki ilişkiler*

Parameters	Correlation Analysis	Cd	Zn	Fe	Cu	MDA	CP	CAT	SOD	Pb
Cd	Pearson Correlation	1	-0.084	0.268	0.182	0.219	0.133	0.149	0.129	0.011
	Sig. (2-tailed)		0.492	0.051	0.134	0.071	0.274	0.234	0.302	0.927
	N	69	69	65	69	69	69	66	66	69
Zn	Pearson Correlation	-0.084	1	0.078	-0.132	-0.225	-0.197	0.168	-0.074	-0.150
	Sig. (2-tailed)	0.492		0.538	0.281	0.063	0.105	0.178	0.554	0.218
	N	69	69	65	69	69	69	66	66	69
Fe	Pearson Correlation	0.268	0.078	1	0.245*	0.169	-0.272*	0.113	0.062	-0.220
	Sig. (2-tailed)	0.051	0.538		0.050	0.178	0.028	0.380	0.632	0.079
	N	65	65	65	65	65	65	63	63	65
Cu	Pearson Correlation	0.182	-0.132	0.245*	1	-0.242*	0.059	-0.215	0.067	0.014
	Sig. (2-tailed)	0.134	0.281	0.050		0.046	0.631	0.084	0.596	0.907
	N	69	69	65	69	69	69	66	66	69
MDA	Pearson Correlation	0.219	-0.225	0.169	-0.242*	1	0.271*	-0.064	0.076	0.252*
	Sig. (2-tailed)	0.071	0.063	0.178	0.046		0.023	0.607	0.542	0.036
	N	69	69	65	69	69	69	67	67	69
CP	Pearson Correlation	0.133	-0.197	-0.272*	0.059	0.271*	1	0.085	0.150	0.276*
	Sig. (2-tailed)	0.274	0.105	0.028	0.631	0.023		0.495	0.224	0.022
	N	69	69	65	69	69	69	67	67	69
CAT	Pearson Correlation	0.149	0.168	0.113	-0.215	-0.064	0.085	1	0.264*	-0.019
	Sig. (2-tailed)	0.234	0.178	0.380	0.084	0.607	0.495		0.031	0.877
	N	66	66	63	66	67	67	67	67	66
SOD	Pearson Correlation	0.129	-0.074	0.062	0.067	0.076	0.150	0.264*	1	-0.115
	Sig. (2-tailed)	0.302	0.554	0.632	0.596	0.542	0.224	0.031		0.357
	N	66	66	63	66	67	67	67	67	66
Pb	Pearson Correlation	0.011	-0.150	-0.220	0.014	0.252*	0.276*	-0.019	-0.115	1
	Sig. (2-tailed)	0.927	0.218	0.079	0.907	0.036	0.022	0.877	0.357	
	N	69	69	65	69	69	69	66	66	69

** Correlation is significant at the 0.05 level (2-tailed)*

Cd metabolism is closely related to Zn metabolism. A well-known example of this is that pretreatment with Zn or the simultaneous administration of Zn with Cd reduces Cd toxicity [26]. Both metals are absorbed in the intestinal canal connecting the same metallothionein. In experimental studies, even low doses of Cd intake have shown to inhibit the absorption of Zn from the intestine [27]. In addition, it has been reported that high Cd levels lead to Cu deficiency [28]. Spierenburg et al.[29] reported that while mean Cd organ concentrations were increased about 2.5 times, Cu levels decreased, but Zn levels did not differ from the control group in the cattle, which lived near the industrial area.

However, the obtained data from the present study do not completely confirm with previous studies. Because, mean Cu and Fe levels of the experiment group were not found different from the control group when Cd level increased in this study. Though, serum Zn level decreased in the experiment group compared with the control group. A correlation was observed between Cu and Fe levels, but although a negative correlation was seen between Cd and Zn levels, it was not statistically significant.

Pandya et al.[30] reported that when Pb and Cd are accumulated and presented together in similar concentrations, the antioxidant enzyme activities are affected. Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins, and DNA, and initiating various pathological conditions in humans and animals [8]. In addition, oxidative damage has been proposed as another possible mechanism involved in Pb toxicity [9]. Antioxidant defenses are composed by nonenzymatic hydro and liposoluble compounds, like vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols, cellular thiols, and enzymes like SOD, CAT, and glutathione peroxidase (GPX) [31]. Furthermore, MDA is a by-product of lipid peroxidation and used as an index of the rate of tissue reaction chain.

MDA is also used as an indicator of oxidative stress in cells and tissues [32]. Also, CP is a copper-containing ferroxidase that oxidizes toxic ferrous iron to its nontoxic ferric form. It protects tissues from iron-mediated free radical injury and is involved in various antioxidant and cytoprotective activities [33]. Extracellular nonenzymatic antioxidants, such as CP, neutralize the free radicals by binding oxygen free radicals and prevent the formation of new radicals [34].

In this study, average MDA level of the experiment group increased about three times, and CP levels increased about two times than the control group. Especially, it was concluded that Pb accumulation was affected the lipid peroxidation and antioxidative metabolism. Because, a significantly correlations were observed among Pb, MDA and CP levels. Also, the increasing of lipid peroxidation may be depending on reduced mineral concentration, as well as heavy metals, in blood. Because, trace elements, such as Fe, Cu and Zn, are very important factors in antioxidant defence system [35] and important correlations were found between Fe and CP, also Cu and MDA levels. Besides, another important finding was MDA-CP and CAT-SOD levels of the animals were increased together and there were significant correlations between these lipid peroxidation and antioxidative metabolism parameters.

Traffic exhaust is a very important factor and Cd and Pb are good indicators of the toxic effects of environmental pollution. In this study, Cd and Pb accumulation was seen in cattle, which were living near the intensive traffic road for at least three years when compared with the control group. In addition, the results showed significant interactions among toxic heavy metals (Cd and Pb), physiological trace elements (Fe, Cu, Zn), enzymatic and non-enzymatic antioxidants and the lipid peroxidation (SOD, CAT, CP and MDA). Disruption of trace element metabolism and antioxidant defence system by toxic metals could decrease of productivity and contribute to the pathogenicity of other diseases. Also, due to food residues, toxic metal accumulations may affect human health. Therefore, periodically monitoring systems for toxic metals should be constituted and the breeders should be educated about hazardous of heavy metals.

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