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Determination of levels of some essential (iron, copper, zinc) and toxic (lead, cadmium) metals in the blood of sheep and in samples of water, plants and soil in Northwest Turkey

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ABSTRACT

The aim of this investigation was to determine the levels of trace elements (zinc, iron, copper), as well as of lead and cadmium, in both the soil and in organism in order to obtain more efficient economical results and healthier sheep breeds in Northwest Turkey. Based on the results of these analyses, we plan to make suggestions to veterinarians and sheep breeders in that region on how to improve their performance. The study was carried out on 400 sheep from different sites in Northwest Turkey and included the collection of water (n = 5), plant (n = 5) and soil (n = 5) samples from every district. When serum parameters were examined an important decrease in iron levels was found in the 8th, 9th and 10th districts, while the lowest zinc levels were found in the 1st and 7th districts, the lowest copper levels in the 1st, 3rd, 5th and 6th districts, the highest lead levels in the 9th and 10th districts, and the highest cadmium levels in the 2nd, 5th and 6th districts. Analyses of water, plant and soil samples showed significant differences in zinc and copper levels between districts. High levels of lead were detected in water and plant samples. We are of the opinion that important improvements will be obtained in the treatment and control of diseases due to deficiency of trace elements and of parasitic, bacterial and viral diseases due to related systems affected by such deficiencies if these results are appropriately communicated to animal breeders and veterinary surgeons operating in the regions in question.

Key words: sheep, trace element, zinc, iron, copper, lead, cadmium

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Introduction

The importance of many inorganic elements classified as macro- and micro-elements in the diet and on the growth of humans and animals has meanwhile been accepted and recognized beyond doubt (ALTINTAŞ et al., 1990). Diseases due to deficiencies or an excess of macro- and micro-elements in animals are of unquestionable importance. While the deficiency or excess of one or a few elements damages normal bodily functions, imbalance among elements impairs the regular function of organism (ÇAMAŞ et al., 1994).

While deficiencies or excess of trace elements in animals result in serious clinical disorders, the important economic losses they cause have been highlighted only in recent years. Clinical signs of trace element deficiencies in animals are diarrhoea, anemia, alopecia, depigmentation, development disorders in the osseus tissues, impaired movement, seborrhea, hyperkeratosis, parakeratosis, anorexia, fertility problems, development problems in young animals, decreased production, tetany, decrease in protein synthesis, insufficiencies in the immune system, abortions not related to infections and pica (ERGÜN, 1983; MARTIN et al., 1983; KUMARESAN and KAPIOH 1984; AĞAOĞLU, 1991; ÇAMAŞ et al., 1994; YILDIZ et al., 1995; UNDERWOOD, 1997; ZANTOPOULOS et al., 1999).

It has been reported that trace elements are of equal importance as hormone and vitamins and have an impact in all climatic zones (OZAN, 1985; ALTINTAŞ et al., 1990; AĞAOĞLU, 1991; ÇAMAŞ et al., 1994). It is also been pointed out that losses caused by the effects of trace elements are as important as the losses incurred as a result of infectious and parasitary diseases (ÇAMAŞ et al., 1994), because trace elements have a siginificant impact on resistance against diseases in living organisms.

Various systems are affected by a deficiency or excess of trace elements such as zinc, copper and iron, or by the negative effects of elements that contribute to metal pollution poisoning such as lead and cadmium. As a consequence, any of the specific diseases caused by any of the elements in question may occur, or they may contribute as important factors in the occurrence of various diseases (MARTIN et al., 1983; ALTINTAŞ et al., 1990; AĞAOĞLU, 1991; ÇAMAŞ et al., 1994).

The aim of this study was to determine the levels of trace elements in sheep and water, soil and plants in order to define the influence of trace elements in the natural environment on sheep in Northwest Turkey and consequently to provide recommendations to veterinary surgeons and sheep breeders in this region.

Materials and methods

The research involved a total of 400 sheep of the Kıvırcık breed, all between 2-3 years old, which were selected from different flocks in Northwest Turkey. The randomly chosen sheep were neither pregnant in a late state nor had they recently given birth. Sheep were regularly administered antiparasits after faeces examination. The study was carried out from April to June while the sheep were grazing in the pasture. As a first step, the northwestern region of Turkey was divided into 12 districts, as shown in Table 1, and water (n = 5), plant (n = 5) and soil (n = 5) samples were collected from every region.

Table 1. Sampling districts in Northwest Turkey

District Number	Name of the District	Number of Collected Blood Samples
1	Babaeski, Sofuhalil, Pehlivanköy and surrounding villages	25
2	Kırklareli, Kofçaz and surrounding villages	35
3	Istanbul University Veterinary Faculty Farm	35
4	Çorlu, Çerkezköy and surrounding villages	35
5	Kırklareli, Dereköy and surrounding villages	30
6	Çatalca and surrounding villages	30
7	Babaeski, Lüleburgaz and surrounding villages	25
8	Tekirdağ, Malkara and surrounding villages	35
9	Tekirdağ, Keşan and surrounding villages	40
10	Saray, Vize, Pınarhisar and surrounding villages	40
11	Kırcasalih, Uzunköprü and surrounding villages	35
12	Edirne and surrounding villages	35

Samples were collected in sterile plastic containers in order to prevent any contamination. Water, plant and soil samples were evaluated by the Department of Biophysics at the Cerrahpaşa Faculty of Medicine of Istanbul University. 10 cc blood were taken from the v. jugularis of sheep selected from each region, both with and without anticoagulant. Seven cc anticoagulated blood was centrifuged at 5000 rpm for 3-5 minutes for zinc, iron and copper measurements. These sera were stored at -18 °C until analysis. For the lead and cadmium measurements an equal amount of 20% TCA (Triclor asetic acide) was added to the tubes with 3 cc anticoagulated blood and this mixture was then centrifuged. Trace elements (Zn, Fe, Cu) as well as lead and cadmium measurements were carried out with a Shimadzu Atomic Absorbtion Spectrometry model AA-680 in accordance with the technique described in the references (BROWN et al., 1986; BROWN and TAYLOR, 1995).

Mean values, standard deviations and statistical differences were calculated using the Duncan F Test (DUNCAN, 1955).

Results

The mean values and standard deviations of the levels of non-toxic (zinc, iron, copper) and toxic (lead and cadmium) elements in the serum of 400 sheep, as well as the examined water, plant and soil samples taken in Northwest Turkey are shown in Tables 2, 3, 4 and 5.

Results summarized in Table 2 show the lowest serum zinc levels in the 1st and 7th districts, a slight decrease in serum iron levels in the 8th, 9th and 10th districts, the lowest copper levels in the 1st, 3rd, 5th and 6th districts, the highest lead levels in the 9th, 10th and 11st districts, and the highest cadmium levels in the 2nd, 5th and 6th districts.

Results presented in Table 3 reveal the lowest zinc levels in the 1st and 2nd districts, a significant decrease in the iron level in the 2nd district, the lowest copper levels in the 1st and 7th districts, the highest lead levels in the 11th and 12th districts, and the highest cadmium level in the 7th district.

When plant samples were examined, the lowest zinc level was found in the 3rd district, the lowest copper levels in the 3rd, 8th and 9th districts, the highest lead levels in the 4th and 7th districts, and the highest cadmium level in the 1st district.

Table 2. Mean values and standard deviations of essential elements (Zn, Fe, Cu) in serum and toxic metal (Pb, Cd) levels in full blood of 400 sheep from different districts of Northwest Turkey

		Zn (μg/dL)		Fe (μg/dL)		Cu (μg/dL)		Pb (μg/dL)		Cd (μg/dL)	
District	N	X	Sx	X	Sx	X	Sx	X	Sx	X	Sx
1	25	22.5^{e}	4.6	88.1^{cb}	11.8	29.9^{ed}	4.6	4.7^{cba}	2.9	0.2^{f}	0.1
2	35	31.1^{c}	5.3	92.2^{b}	14.6	30.1^{ed}	12.2	4.2^{fe}	1.4	1.8^{ba}	0.5
3	35	28.8^{d}	4.8	88.6^{cb}	12.7	29.6^{ed}	5.6	3.9^{f}	0.5	1.2^{c}	0.7
4	35	29.1^{d}	4.1	87.1^{dcb}	13.3	30.4^{ed}	5.7	4.4^{fedcb}	0.5	0.9^{dc}	0.4
5	30	32.1^{cb}	3.5	89.9^{cb}	7.3	29.1^{e}	3.1	4.2^{fed}	0.3	2.1^{a}	0.4
6	30	28.6^{d}	3.8	89.6^{cb}	10.4	27.7^{e}	4.7	4.3^{fedcb}	0.4	1.8^{ba}	0.4
7	25	20.9^{e}	1.7	88.3^{cb}	11.7	32.6^{dc}	8.1	4.4^{edcb}	0.3	0.3^{f}	0.1
8	35	27.7^{d}	3.4	85.9^{edc}	2.5	35.2^{c}	2.2	4.8^{cba}	0.2	0.9^{edc}	0.5
9	40	34.1^{b}	2.5	81.4^{e}	2.2	35.7^{c}	2.7	5.1^{a}	0.2	1.1^{c}	0.5
10	40	36.4^{a}	3.7	82.9^{ed}	3.8	35.6^{c}	3.3	5.0^{a}	2.6	0.9^{ed}	0.4
11	35	28.9^{d}	3.9	89.2^{cb}	4.4	41.2^{b}	3.1	4.9^{ba}	0.3	0.9^{edc}	0.5
12	35	32.8^{cb}	4.9	97.2^{a}	6.7	44.9^{a}	5.7	4.3^{fedc}	0.6	0.7^{e}	0.6

There is a statistically significant difference (P<0.001) between the mean values (X), which are indicated with different letters in each column. N = Number of animals, X = Mean values, Sx = Standard deviation.

Table 3. Mean values and standard deviations of essential (Zn, Fe, Cu) and toxic (Pb, Cd) metal levels in water samples from different districts of Northwest Turkey

		Zn (μg/L)		Fe (μg/L)		Cu (μg/L)		Pb (μg/L)		Cd (μg/L)	
District	N	X	Sx	X	Sx	X	Sx	X	Sx	X	Sx
1	5	19^{g}	3	29^{d}	5	7^{h}	2	1.45^{i}	3	0.05^{f}	1
2	5	7^{h}	3	4^{g}	1	39^{e}	5	1.24^{h}	0.6	0.78^{dcb}	3
3	5	140^{c}	5.3	32^{c}	0.9	40^{ed}	2.9	2.45^{e}	0.2	0.54^{ed}	0.2
4	5	125^{e}	5.8	21^{f}	0.5	64^{a}	4.2	1.78^{g}	0.1	0.56^{edc}	4
5	5	135^{d}	5.2	36^{b}	0.5	47^{c}	0.3	2.65^{d}	0.2	0.85^{cb}	0.2
6	5	120^{f}	5.4	35^{b}	0.8	32^{f}	2.6	2.1^{f}	0.1	0.45^{e}	0.3
7	5	148^{b}	5.3	39^{a}	0.8	27^{g}	1.1	2.03^{f}	0.2	1.23^{a}	0.6
8	5	135^{d}	4.9	32^{c}	1.2	41^{d}	2.3	2.8^{c}	0.1	0.89^{b}	0.3
9	5	120^{f}	5.2	30^{d}	0.6	59^{b}	3.3	2.48^{e}	0.1	0.56^{edc}	0.6
10	5	170^{a}	4.6	33^{c}	3.2	63^{a}	1.6	2.05^{f}	0.1	0.74^{dcb}	0.3
11	5	150^{b}	3.2	27^{e}	0.8	48^{c}	1.3	5.2^{a}	0.1	0.85^{cb}	0.5
12	5	140^{c}	5.6	32^{c}	1.2	40^{d}	1.8	4.5^{b}	0.1	0.12^{f}	0.6

There is a statistically significant difference ($P<0.001$) between the mean values (X), which are indicated with different letters in each column.

Table 4. Mean values and standard deviations of essential (Zn, Fe, Cu) and toxic (Pb, Cd) metal levels in plant samples from different districts of Northwest Turkey

		Zn (μg/g)		Fe (μg/g)		Cu (μg/g)		Pb (μg/g)		Cd (μg/g)	
District	N	X	Sx	X	Sx	X	Sx	X	Sx	X	Sx
1	5	167.4^{a}	9.8	399.3^{a}	26.4	47.3^{a}	3.8	1.99^{b}	0.29	1.04^{a}	0.9
2	5	95.2^{c}	3.7	250^{c}	21.4	8.1^{c}	2.4	1.26^{d}	0.21	0.68^{e}	0.2
3	5	39.6^{hj}	4.6	115.3^{k}	11.7	5.5^{d}	1.6	0.85^{f}	0.04	0.76^{d}	0.6
4	5	85.4^{f}	4.7	185.5^{h}	14.7	7.6^{c}	0.8	2.14^{a}	0.26	0.56^{g}	0.5
5	5	90.2^{d}	2.6	245.5^{cd}	26.7	9.6^{b}	1.4	1.49^{c}	0.16	0.6^{f}	0.1
6	5	87.6^{e}	4.8	178^{i}	13.7	7.2^{c}	0.8	1.05^{e}	0.32	0.75^{d}	0.7
7	5	85.5^{f}	5.4	135^{j}	8.9	7.8^{c}	0.8	2.15^{a}	0.24	0.95^{b}	0.2
8	5	80.2^{g}	3.2	225^{e}	15.5	5^{d}	0.8	1.35^{cd}	0.06	0.53^{h}	0.3
9	5	96.4^{c}	5.4	265^{b}	11.3	5.9^{d}	0.5	1.12^{e}	0.21	0.45^{i}	0.2
10	5	78.2^{h}	2.1	200^{f}	12.6	7.7^{c}	0.8	1.07^{e}	0.07	0.82^{c}	0.9
11	5	74^{i}	1.8	195^{g}	10.8	7.4^{c}	0.5	1.48^{c}	0.11	0.46^{i}	0.2
12	5	112.5^{b}	17.8	175^{i}	8.7	7.5^{c}	0.4	1.35^{cd}	0.14	0.55^{gh}	0.5

There is a statistically significant difference ($P<0.001$) between the mean values (X), which are indicated with different letters in each column.

Table 5. Mean values and standard deviations of essential (Zn, Fe, Cu) and toxic (Pb, Cd) metal levels in soil samples from different districts of Northwest Turkey

		Zn (μg/g)		Fe (μg/g)		Cu (μg/g)		Pb (μg/g)		Cd μg/g)	
District	N	X	Sx	X	Sx	X	Sx	X	Sx	X	Sx
1	5	58.7^{b}	2.1	162.5^{fg}	10.8	13.7^{j}	1.6	1.16^{g}	0.4	0.66^{e}	0.2
2	5	61.1^{a}	4.2	159.4^{g}	9.6	39.7^{b}	2.7	2.46^{a}	0.2	0.88^{c}	0.4
3	5	41.5^{cd}	5.8	195.7^{c}	12.8	20.9^{h}	1.3	2.39^{ab}	0.3	0.21^{j}	0.4
4	5	38.8^{ef}	3.6	125.4^{k}	11.3	26.8^{e}	1.6	1.76^{de}	0.1	1.23^{b}	0.8
5	5	35.5^{g}	5.1	225.5^{b}	21.4	35.4^{c}	2.1	0.98^{h}	0.2	0.52^{gh}	0.3
6	5	41.4^{cd}	4.2	149.5^{h}	11.6	27.8^{e}	1.3	1.45^{f}	0.1	0.48^{h}	0.4
7	5	40.5^{de}	5.4	135.8^{j}	14.3	32.5^{d}	2.4	1.87^{d}	0.1	1.25^{b}	0.5
8	5	36.9^{fg}	4.2	165.3^{f}	18.7	39.5^{b}	2.1	2.15^{c}	0.1	0.35^{i}	0.4
9	5	38.5^{ef}	3.1	145.4^{i}	14.8	17.8^{i}	1.1	1.74^{e}	0.09	0.78^{d}	0.5
10	5	42.7^{cd}	3.2	178.3^{d}	10.4	25.4^{f}	1.3	2.28^{b}	0.1	0.54^{fg}	0.7
11	5	40.5^{de}	4.2	256.2^{a}	11.4	41.6^{a}	2.6	2.05^{c}	0.1	1.45^{a}	0.6
12	5	43.4^{c}	4.2	174.6^{e}	9.8	22.5^{g}	1.6	0.94^{h}	0.07	0.58^{f}	0.4

There is a statistically significant difference ($P<0.001$) between the mean values (X), which are indicated with different letters in each column.

Table 6. Correlation between essential (Zn, Fe, Cu) and toxic (Pb, Cd) metal levels in water, soil and plant samples collected from different districts of Northwest Turkey

	Zn (n = 60)	Fe (n = 60)	Cu (n = 60)	Pb (n = 60)	Cd (n = 60)
Water	117.41 ± 49.0**	29.16 ± 8.89	42.25 ± 15.56**	2.56 ± 1.13	0.63 ± 0.36
Plant	91.01 ± 28.59^{++}	214.05 ± 71.06	10.55 ± 11.26^{++}	1.44 ± 0.4^{+}	0.67 ± 0.36
Soil	43.27 ± 7.91$^{\theta\theta}$	172.79 ± 36.37	28.63 ± 8.88	1.76 ± 0.5	0.74 ± 0.37$^{\theta}$

*Water-Plant, $^{\theta}$Water-Soil, $^{+}$Plant-Soil, *$P<0.05$, ** $P<0.01$. ($^{\theta}$, $^{+}$ similar)

Soil analyses results produced the lowest zinc levels for the 5th and 8th districts. In the 4th district a dramatically low iron level was detected. The lowest copper levels were found in the 1st and 9th districts. Lead levels were higher in the 2nd, 3rd, 8th and 10th districts, while cadmium levels were somewhat elevated in the 4th, 7th and 11th districts.

Discussion

When the number of animals with economical value is compared with those of other countries, Turkey is placed rather high up in the list. However, it would be closer the bottom of the list if products such as meat, milk, wool and leather obtained from these animals are considered (AĞAOĞLU, 1991; ÇAMAŞ et al., 1994). In this respect, deficiencies of trace elements play a role which is as important as infectious and parasitary diseases (UNDERWOOD, 1997). A similar condition became obvious in northwest Turkey, where the study was carried out. After interviews with animal breeders and veterinary surgeons it emerged that the level of diseases due to parasitary and trace element deficiencies was very high. In particular, animal health problems such as abortion, trouble in walking in new-born lambs, fertility problems, weight loss, lack of putting on weight despite proper rations, diarrhoea, growth disorders, susceptibility to infectious diseases and decrease in quality of wool were in conformity with data reported in the literature (ALTINTAŞ et al., 1990).

While NELSON et al. (1987) reported serum zinc levels of 0.32-0.40 μg/ml in a flock which suffered from cases of anorexia, wool eating, allopecia, hyperkeratosis and parakeratosis, ÇAMAŞ et al. (1994) have reported a level of 25-48 μg/dL for similar symptoms. Our zinc values were within the above levels, with the exception of the 1st and 7th districts. The low levels found only in the 1st and 7th districts, and especially in Babaeski, Lüleburgaz and Pehlivanköy, are an indication of the problem. In particular, the zinc level in those districts was found to be lower in water and plant samples as compared to other districts. This indicates that sheep there do not benefit from water and plants as a source of zinc. Moreover, we are of the opinion that high cadmium levels in plant samples from those districts are associated with zinc deficiency, as has been suggested by researchers (MARTIN et al., 1983; AĞAOĞLU, 1991).

While normal serum copper levels for sheep as reported by various researchers (MARTIN et al., 1983; GHOSAL and MATHUR, 1992) are 59-101 μg/dL and 80-120 μg/dL, respectively, the lowest values determined in our studies prove how serious the problem is in northwestern Turkey. The correlation between blood Cu levels below 50 μg/dL level and the increased risk of enzootic ataxia risk constitutes an important threat to the region where this study was carried out (WILSON et al., 1991). To increase this risk, zinc levels in plant sheep grazing areas in the 1st and 12th districts were high, while copper levels were low in plants of some districts (3rd, 8th and 9th). Clinical signs such as depigmentation, weight loss, growth retardation, diarrhoea and ataxia due to low blood serum level of trace elements which correlated with the low levels found in soil, plant and water samples, conform to previously reported results (FERM and HANLON, 1974; ERGÜN, 1983; WILSON et al., 1991).

For normal sheep UNDERWOOD (1997) stated that serum iron levels should be in the order of 102-304 μg/dL, while NAZKI and RATTAN (1990) suggested a level of 115-234 μg/dL for the same trace element. Although serum iron level below 40 μg/dL, as reported by GHOSAL and MATHUR (1992), was not observed in any district of the region, in our opinion, the existence of a potential risk for the region should not be disregarded. In this connection, it is well known that lack of the trace element copper together with deficiency in the trace element iron leads to serious anaemia, which in consequence results in predisposition to parasitary diseases. It has been reported (PIRKLE et al., 1985; AĞAOĞLU, 1991) that high levels especially of of cobalt, zinc, cadmium, manganese and copper were reducing the absorption of iron. In parallel with industrial development in the districts of Tekirdağ, Keşan and Kırklareli, increased levels of lead and cadmium in sheep have been observed in those districts and their rations correlated with their content in plants in the region. High lead levels in sheep, especially in the districts of Edirne, Kırcasalih and their surroundings, and the level of the same element in plants from Çorlu, Çerkezköy and Lüleburgaz districts constitute the undesirable side effects of industrialisation such as environmental pollution, serious metalic toxication risks and negative effects on trace elements. As has been reported (MEREDITH, 1978; KUMARESAN and KAPIOH, 1984), the presence of excessive levels of lead and cadmium negatively affect primarily the hemopoietic system but also the urogenital system, the gastrointestinal system and the neurologic system, as well as the absorption of other trace elements.

We are of the opinion that by informing veterinary surgeons in the region about the results of soil, water and plant analyses, they would be in a position to prescribe supplements of those elements where deficient; the results would also contribute to the treatment of abortus cases which are regularly observed and which are not due to any infectious or parasitary diseases. And finally, the results could help to improve quality of wool.

Similar studies within the scope of the existing possibilities in the same region comprising other trace elements, as well as extended studies in other regions, would help to determine the levels of trace elements in soil, water and plants everywhere in Turkey and would thus provide an important tool to reduce incidences of disease, morbidity and mortality ratios.

This study, which found important differences in trace element levels in the investigated region, allows a certain generalisation as to the solution of problems regarding sheep breeding, which is an important contributor to the country's economy in the form of meat, milk, wool and leather, with respect to the effects of environmental factors.

If the results of this study are communicated to animal breeders and to local veterinary surgeons, they may, in our opinion, 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.

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SAŽETAK

Cilj istraživanja bio je odrediti razinu elemenata u tragovima (cink, željezo, bakar), te olova i kadmija u tlu i organizmu ovaca kako bi se polučili bolji gospodarski rezultati i uzgajale zdravije pasmine ovaca u sjeverozapadnoj Turskoj. Na temelju rezultata, za navedena područja planiraju se preporuke veterinarima i uzgajivačima na osnovi kojih bi se unaprijedio njihov rad. U istraživanje je bilo uključeno 400 ovaca iz različitih područja sjeverozapadne Turske. Uzimani su uzorci vode (n = 5), biljaka (n = 5) i tla (n = 5). Utvrđena je značajno niža razina željeza u 8., 9. i 10. području. Najniža razina cinka ustanovljena je u 1. i 7. području, najniža razina bakra u 1., 3., 5. i 6. području, dok su najviše razine olova utvrđene u 9. i 10. području, a kadmija u 2., 5. i 6. području. Analiza uzoraka vode, biljaka i tla pokazala je da između različitih područja postoje značajne razlike u razini cinka i bakra. Visoke razine olova ustanovljene su u uzorcima vode i biljaka. Prijenosom ovih rezultata do veterinara i uzgajivača iz promatranih područja, ostvarit će se značajna poboljšanja u liječenju i kontroli bolesti uzrokovanih deficijencijom elemenata u tragovima. Isto se može očekivati i za bolesti uzrokovane parazitima, bakterijama i virusima u kojih navedene deficijencije imaju određenu ulogu.

Ključne riječi: ovca, elementi u tragovima, cink, željezo, bakar, olovo, kadmij