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Copper, Zinc, Cadmium, and Lead in Sheep Grazing in North Greece

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The industrial evolution, the intense use of raw material and the agricultural technology have all some how improved man's living while simultaneously have polluted the natural environmen with consequences on his health. Some of the more toxic elements are heavy metals which are absorbed through food by people not involved with this by profession (Schuhmacher et al 1991). It is also necessary to point out the fact that some of these heavy metals (Pb, Cd) are implicated in causing cancer or mutations (Jacobson and Turner, 1980) while at the same time man's load of these elements in comparison to the last century has quadrupled (Ellinder and Kjelstrom 1977).

The aim of this study is to investigate the levels of Cu, Zn, Cd and Pb in sheep in connection to the area of housing and grazing. The reason for choosing sheep for this investigation is that these animals live and feed for the greatest part of the year outdoors and, thus, the concentration of heavy metals in this species reflects the environmental load more accurately than in other productive animals (Antoniou et al 1995).

MATERIALS AND METHODS

In this study sheep tissue samples (70 liver and 70 kidney samples) were examined in respect to the concentration of copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb). The samples were taken from sheep 3-7 years old and from three areas of Northern Greece is shown in figure 1.
Area A: this area is a part of the county of Veria, has large areas of fruit-tree cultures and vegetable cultures where many incidents of copper poisoning occur.
Area B: this area is included in the county of Aridea and is also characterised by many fruit-tree cultures but, due to the presence of sulphur radicals in the soil, is considered poor in copper.
Area C: this area is in the county of Grevena, is mostly highlands and human intervention on its ecosystem is minimal.
The samples were stored at -20° C until examination. For the measure-

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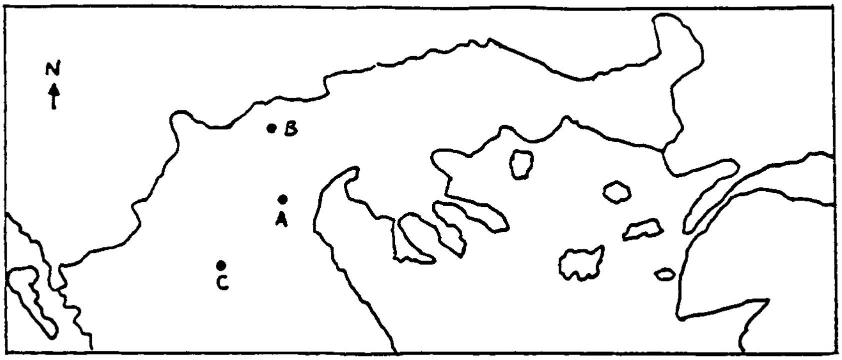

Figure 1. Map of Northern Greece showing the sampling sites

ments, 20 g of tissues were homogenised and wet digested with nitric and perchloric acid (7:3 ratio). For the Cu and Zn assay, direct measurement by Atomic Absorption Spectrometry (A.A.S.) was performed. For Pb and Cd determinations, pH values were adjusted to 4, complexes with ammonium pyrrolidinum dithiocarbonate were formed and extracted in methyl-isobutyl-ketone. Finally, the organic layer was aspirated in flame AAS at appropriate wave lengths with cathode lamps (Tsalev and Zaprianov 1983; Evans et al 1978; AOAC 1980).

The spectrophotometer used was a Perkin-Elmer, model2380, with dual beam, background correction of deuterium (DBC). Hollow cathode tubes from Perkin-Elmer were used. Wavelengths for each element were: Pb 283.3 nm, Cu 324.8 nm, Cd 228.8 nm and Zn 213.9 nm. Recovery ranged from 82-90% for Pb, 88-92 for Cd, 95-100% for Cu and 92-101% for Zn. Method's detection limits were as follows: for Pb 0.1 mg/kg, Cd 0.05 mg/kg, Cu 0.2 mg/kg, Zn 0.2 mg/kg.

Statistical analysis was performed using the method of one-way analysis of variance (ANOVA) (Armitage 1971).

RESULTS AND DISCUSSION

Copper (Cu) an the ingredient in a multitude of enzymes and plays an important role in many physiological functions of man and animals. Frequently though pathological conditions known as chalkoses with a characteristic high concentration of Cu, mainly in liver, kidneys and blood occur. These pathological states are caused by either an uptake of an excessive amount of Cu or by food containing normal amounts of Cu butlow amounts of Mo or sulphur radicals (Doyle and Spaulding 1978; Bartik and Piscac 1981).In contrast, lack of copper causes disturbances with a characteristic example being swayback in sheep (Schuhmacher et al 1991; Koh and Judson 1988).

Table 1. Mean copper concentrations (μg/g w/w*) in sheep liver and kidney.

Area		A		B		C
Tissue	n	⊠**	n	⊠	n	⊠
Liver***	26	107.80±8.47	24	52.40±4.50	20	73.50±8.50
Kidney	26	3.80±0.18	24	3.70±0.25	20	3.80±0.09

* wet weight.

**⊠ = mean value.

± = standard error.

***p<0.01 statistically significant between areas.

Table 2. Distribution (%) of copper concentrations (μg/g w/w) in sheep liver.

Area		A		B		C
μg/g	n	%	n	%	n	%
10-100	13	50.0	23	95.8	16	80.0
100-150	8	30.8	1	4.2	4	20.0
> 150	5	19.2	-	-	-	-
Total	26		24		20	

Table 3. Mean zinc concentrations (μg/g w/w) in sheep liver and kidney.

Area		A		B		C
Tissue	n	⊠	n	⊠	n	⊠
Liver*	26	24.00±2.17	20	26.50±2.17	20	14.40±0.47
Kidney*	26	18.50±1.01	24	23.40±1.86	20	10.30±0.69

*p<0.01 statistically significant between areas.

Mean concentration of Cu in liver in area A (⊠=107.8 μg/g) is more than double that in area B and significantly higher than area C (Table 1).

Furthermore, in area A, a percentage of 19.2 has over 150 μg/g of Cu in liver. This value borders the limit values for chalkosis, while in the other two areas there is no sample approaching these concentrations (Table 2). This is probably due to the spraying of fruit -tree cultures with copper-containing compounds during the season the samples from these sheep were obtained (Koh and Judson 1986).

In area B, as mentioned above, there were no samples having high Cu concentrations even though there was also spraying with copper-containing chemicals. This must be attributed to the high sulphuric radical content in these soils which prevents copper from being absorbed.

In area C copper concentration ranged within normal levels.
In goat slaughtered in India, copper concentrations of 10.5 ppm in liver and 5.2 ppm in kidney were found (Agyadurai and Krishnasamy 1986). Copper concentrations in Canadian sheep & goats were 78.7 ppm in liver and 4.7 ppm in kidney (Salisbury and Chan 1991). In Poland, copper concentrations were found to be 29 and 3.7 ppm in the liver and kidney, respectively (Falandysz 1991).

Zinc (Zn) is a co-factor in many enzymes and plays a role in carbohydrate, lipid and protein metabolism. It is considered to be necessary for growth and development, in wound healing and in DNA synthesis (Hammond and Belites 1980; Peppa-Papasteriadou 1986). It is comparatively non-toxic (Underwood 1977) but pathological conditions often occur caused by lack of Zn as a result of consumption of food poor in Zn. Copper and phosphorus affect zinc's absorption because they form non-absorbable complexes. A typical example of lack of zinc is parakeratosis in swine.

If we assume that normal concentrations of Zn in the liver are 35-45 µg/g (Bartik and Piscac, 1981; Underwood, 1977) it is apparent that in all three areas zinc concentration in sheep tissues is low (Table 3). Similarly low concentrations of zinc were found in the area of Olympiada and Arnea of Halkidiki in 1992 (Antoniou et al 1995). Investigators in India, Canada, Poland and USA report values of zinc concentration in liver and kidney of goat and sheep, ranged from 39 to 82.2 ppm and 23 to 147.2 ppm respectively (Agyadurai and Krishnasamy 1986; Falandysz 1991; Salisbury and Chan 1991; Khan et al 1995a).
It must be emphasised that lack of zinc in ruminants in our country is very common. In particular, previous work done at the Pathology Clinic of the Veterinary School, Aristotelian University of Thessaloniki, shows that 56.3% of blood samples obtained from ruminants coming also from the areas that we examined indicate lack of zinc (Papasteriadis 1973).

Cadmium (Cd) is considered to be one of the most toxic heavy metals. In addition, cadmium is implicated in high blood pressure (Perry et al., 1979) prostate cancer, mutations, dysplasy and foetal (embryonic) death (Schuhmacher et al 1991; Pitot III and Dragan 1996). Man and animals take up cadmium through air, water and mainly by food. Some foodstuff, in particular of animal origin, like liver and kidney, contain higher levels of Cd (Andersen and Hansen 1982; Spierenburg et al 1988; Antoniou et al 1989).
Mean concentrations of Cd in liver were found to be 0.5 µg/g and in kidney below 1 µg/g (Table 4). These concentrations are considered to be at desirable levels (Bartik and Piscac, 1981; Andersen and Hansen, 1982; Spierenburg et al., 1988) except the ones in kidney from area B (1.23 µg/g) that marginally exceed these levels but are well below the

tolerable limits posed for human consumption (3 μg/g) (Spierenburg et al., 1988). It must, also, be noted that the values of Cd are below the ones measured at an earlier study carried out by our lab in the area of Halkidiki during 1992-93 (Antoniou et al., 1995).

No liver samples contain cadmium more than 1 μg/g, while a small percentage has concentrations of 0.5 - 1 μg/g (Table 5). Only one kidney sample contains more than 3 μg/g, while the percentage at 1 - 3 μg/g (58.3%) only at the area B, is considered to be high (Table 6). A study of Khan et al (1995b) reports concentrations of Cd in goat liver and kidney of 0.32 and 0.51 ppm ,respectively, while Salisbury and Chan (1991) have found concentrations of 0.06 and 0.17 ppm, in tissues of goat and sheep, respectively.

The toxicity of lead (Pb) is attributed to the fact that it interferes with the normal function of a number of enzymes. Bipolar Pb forms strong bonds with enzymes bearing sulfhydryl groups thus inhibiting their action. Lead is toxic to blood and the nervous, urinary, gastric and genital systems. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis and teratogenesis in experimental animals (Bryce-Smith and Stephens, 1983; Pitot III and Dragan 1996). The mean concentration of Pb in both tissues, in all areas, is at about the same level (0.71 to 0.96 μg/g) and this is considered to be a desirable level as it is less than 1μg/g (Table 7). It must be noted that these concentrations are higher than the ones found in sheep and goat in Greece and other countries (Falandysz, 1991; Polizopoulou, 1991; Salisbury and Chan, 1991; Zantopoulos et al 1992; Khan et al 1995b). Finally, it is found that 16 to 20% of liver samples exceeded the desirable levels of 1 μg/g while the percentage in kidney is 15% in area C and is as high as 34.6% in area A (Table 8, 9).

Sheep tissue concentration of Cu in area A is high, while in the other two areas it is at normal levels. This high concentration must be attributed mainly to the spraying with copper compounds at fruit-tree cultures. It is therefore wise to avoid grazing at these areas during the season of spraying. Zinc concentration in sheep liver and kidney is considered to be at low levels. Therefore, addition of Zn in feed is recommended. Cadmium concentration is at desirable levels. Finally, lead concentration is at about the same level in all three areas. These values, although considered desirable, are still higher than the ones found in sheep and goat from other areas in Northern Greece.

Table 4. Mean cadmium concentrations (μg/g w/w) in sheep liver and kidney.

Area		A		B		C
Tissue	n	⊠	n	⊠	n	⊠
Liver*	26	0.17±0.01	24	0.32±0.03	20	0.18±0.03
Kidney*	26	0.64±0.09	24	1.23±0.15	20	0.60±0.06

*p<0.01 statistically significant between areas.

Table 5. Distribution (%) of cadmium concentrations (μg/g w/w) in sheep liver.

Area		A		B		C
μg/g	n	%	n	%	n	%
< 0.5	25	96.1	20	83.3	19	95.0
0.5-1	1	3.9	4	16.7	1	5.0
> 1	-	-	-	-	-	-
Total	26		24		20	

Table 6. Distribution (%) of cadmium concentrations (μg/g w/w) in sheep kidney.

Area		A		B		C
μg/g	n	%	n	%	n	%
< 1	23	88.5	9	37.5	18	90.0
1-3	3	11.5	14	58.3	2	10.0
> 3	-	-	1	4.2	-	-
Total	26		24		20	

Table 7. Mean lead concentrations (μg/g w/w) in sheep liver and kidney.

Area		A		B		C
Tissue	n	⊠	n	⊠	n	⊠
Liver*	26	0.80±0.06	24	0.71±0.07	20	0.82±0.07
Kidney*	26	0.96±0.07	24	0.72±0.06	20	0.87±0.04

*p>0.01 not statistically significant between areas

Table 8. Distribution (%) of lead concentrations (μg/g w/w) in sheep liver

Area	A		B		C	
μg/g	n	%	n	%	n	%
< 0.5	4	15.4	8	33.3	5	25.0
0.5-1	17	65.4	12	50.0	11	55.0
> 1	5	19.2	4	16.7	4	20.0
Total	26		24		20	

Table 9. Distribution (%) of lead concentrations (μg w/w) in sheep kidney.

Area	A		B		C	
μg/g	n	%	n	%	n	%
< 0.5	3	11.5	8	33.3	1	5.0
0.5-1	14	53.9	12	50.0	16	80.0
> 1	9	34.6	4	16.7	3	15.0
Total	26		24		20	

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