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Influence of antagonistic minerals in soil and pastures on the blood and liver copper in goats in Khuzestan province, Iran

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Summary

The copper level in the serum of 1280 and liver of 200 goats raised in the mountain areas of Khuzestan province, Iran were determined. Copper, molybdenum and iron content of soil and pastures as well as sulfur content of the pastures were seasonally estimated. Mean serum copper levels of goats in Behbahan, Ramhormoz, Eizeh and Masjed-Soleyman districts were 8.14 ± 0.21, 1.98 ± 0.12, 7.74 ± 0.11 and 6.97 ± 0.14 µmol/L, respectively. The results showed that the blood copper of goats in the area was significantly ($P<0.05$) low in autumn and winter in comparison with spring and summer. The liver copper concentration was low in Behbahan, Eizeh and Masjed-Soleyman and at a deficient level in Ramhormoz. The results also revealed a high level of molybdenum in the soil and pasture as well as high amounts of sulfur in pastures of the studied areas. In this survey the status of copper in many goat flocks around the studied towns was dangerously low. Many liver samples had a copper concentration below 30 mg/kg and many serum copper concentrations were below normal. The results of this study confirm the presence of a secondary copper deficiency in goats in the mountain areas of Khuzestan province.

Key words: Goat, Copper, Serum, Liver, Khuzestan

Introduction

Copper plays a number of important biological roles in animals through several Cu-dependent enzymes (Xin *et al*., 1991). Copper deficiency in ruminants occurs either as a primary or as a secondary deficiency. Most of the copper deficiencies in livestock which occur naturally are conditioned by the presence of dietary factors that interfere with the absorption or utilization of copper by the animal (Underwood and Suttle, 1999). These dietary factors, such as iron, molybdenum or sulfur, interfere with the absorption and metabolism of copper (Suttle, 1991). In the rumen, molybdenum combines with reduced sulfur to form tetrathiomolybdate that binds copper and prevents its absorption, while other thiomolybdates and molybdates are absorbed into blood and bind endogenous copper to render it unavailable for metabolic purposes (Mason, 1982).

Copper deficiency has been reported in grazing livestock in some parts of Iran (Nouri, 1998; Nouri *et al*., 2005). In the west and east Azerbaijan and Kurdistan provinces high molybdenum levels were responsible for copper deficiency among ruminants. Swayback in lambs and kids in the Khuzestan province of Iran has been observed frequently. Nouri *et al*. (2005) reported enzootic ataxia in lambs in this region. There is no information with regard to copper deficiency in goats in Khuzestan province, therefore the aim of this study was to determine serum and liver copper concentrations of goats in some parts of the area and to evaluate the effects of antagonistic minerals for the determination of primary or secondary copper deficiency.

Materials and Methods

This study was carried out in the mountain areas of Khuzestan province including Behbahan, Ramhormoz, Eizeh and

Masjed-Soleyman towns. A total of 1280 blood samples (80 samples in each town and season) were taken from non-lactating female native goats aged between 3-5 years raised in the local pastures. The samples were collected from the jugular vein by venoject and centrifuged at 3000 rpm for 10 min, and the serum separated to determine copper concentration.

A total of 200 liver samples were collected from goats slaughtered in different slaughterhouses in each town (50 samples in each town) to estimate their copper concentrations.

One hundred ninety two soil and pasture samples were taken seasonally (12 samples in each town and season) to differentiate the primary and secondary copper deficiency, and copper, molybdenum and iron content of the soil and pasture as well as the sulfur content of the pasture were determined.

To determine the concentration of copper, molybdenum and iron, an atomic absorption spectrophotometer (UNICAM-919) was used (Pesce and Kaplan, 1987) and the levels of sulfur were determined by turbidimetric method (Allen *et al.*, 1974).

The one-way ANOVA was used to determine any difference between means, and the pairwise comparison of means were done by LSD method. The values were expressed as mean concentrations ± SE. P-values <0.05 were considered to be significant.

Results

Mean seasonal serum copper concentrations (µmol/L) of goats in Behbahan, Ramhormoz, Eizeh and Masjed-Soleyman districts are presented in Table 1. The mean annual serum copper concentrations in Behbahan, Ramhormoz, Eizeh and Masjed-Soleyman districts for goats were 8.14, 1.98, 7.74 and 6.97 µmol/L, respectively. These values were lower in autumn and winter and higher in spring and summer (Table 1)

Mean seasonal copper, molybdenum and iron concentrations of soil and pastures as well as the sulfur concentration of pastures are shown in Tables 2, 3, 4 and 5.

Comparison of Cu:Mo ratio of pastures in different towns and seasons is shown in Table 6.

Copper concentrations in the liver samples obtained from slaughterhouses of Behbahan, Ramhormaz, Eizeh and Masjed-Soleyman towns were 50.35 ± 7.63, 23.83 ± 5.72, 75.26 ± 8.33 and 69.92 ± 6.81 mg/kg,

Table 1: Mean ± SE levels of copper in serum of goats in different towns and seasons (µmol/L)

Town	Season				
	Spring n = 80	Summer n = 80	Autumn n = 80	Winter n = 80	Total n = 320
Behbahan	9.59 ± 0.51^{a}	8.33 ± 0.46^{b}	7.67 ± 0.31^{bc}	6.98 ± 0.35^{c}	8.14 ± 0.21
Ramhormoz	2.92 ± 0.28^{a}	2.85 ± 0.23^{a}	0.86 ± 0.08^{b}	1.28 ± 0.20^{b}	1.98 ± 0.12
Eizeh	8.28 ± 0.22^{a}	7.81 ± 0.25^{ab}	7.55 ± 0.26^{b}	7.30 ± 0.16^{b}	7.74 ± 0.11
Masjed-Soleyman	7.98 ± 0.17^{a}	9.81 ± 0.18^{b}	4.86 ± 0.14^{c}	5.21 ± 0.13^{c}	6.97 ± 0.14

In each row different letters show significant difference (P<0.05)

Table 2: Mean ± SE levels of copper in pasture and soil in different towns and seasons (mg/kg)

Town	Sample type	Season				Total n = 48
		Spring n = 12	Summer n = 12	Autumn n = 12	Winter n = 12	
Behbahan	Pasture	14.50 ± 3.17	15.62 ± 2.07	12.75 ± 0.63	13.12 ± 0.43	14 ± 0.91
	Soil	35.75 ± 0.32	35.5 ± 1.62	35.25 ± 0.48	36.78 ± 2.60	35.84 ± 0.71
Ramhormoz	Pasture	13.00 ± 0.54^{a}	12.75 ± 0.82^{ab}	10.22 ± 0.49^{c}	11 ± 0.54^{bc}	11.74 ± 0.41
	Soil	37.25 ± 2.49	37.25 ± 2.31	35.5 ± 1.37	35.5 ± 1.04	36.37 ± 0.88
Eizeh	Pasture	12.25 ± 0.43	11.87 ± 0.85	11.57 ± 0.63	11.25 ± 0.32	11.74 ± 0.28
	Soil	36.50 ± 0.20	38.62 ± 1.46	32.87 ± 1.39	36.00 ± 2.07	36.00 ± 0.84
Masjed-Soleyman	Pasture	11.87 ± 0.55	12.37 ± 0.85	12.12 ± 0.77	12.00 ± 0.89	12.09 ± 0.35
	Soil	35.75 ± 0.66	35.00 ± 0.41	34.25 ± 0.97	34.75 ± 0.48	34.94 ± 0.33

In each row different letters show significant difference (P<0.05)

Table 3: Mean ± SE levels of molybdenum in pasture and soil in different towns and seasons (mg/kg)

Town	Sample type	Season				Total n = 48
		Spring n = 12	Summer n = 12	Autumn n = 12	Winter n = 12	
Behbahan	Pasture	11.70 ± 1.46	12.77 ± 1.90	12.97 ± 0.63	14.45 ± 1.93	12.97 ± 0.75
	Soil	17.80 ± 0.08[ab]	20.00 ± 0.41[bc]	21.47 ± 0.34[c]	15.00 ± 2.29[a]	18.57 ± 0.82
Ramhormoz	Pasture	13.00 ± 0.54[b]	12.75 ± 0.82[ab]	10.22 ± 0.49[a]	11.00 ± 0.54[a]	11.74 ± 0.41
	Soil	37.25 ± 2.49	37.25 ± 2.31	35.5 ± 1.37	35.5 ± 1.04	36.37 ± 0.88
Eizeh	Pasture	11.60 ± 0.81[ab]	10.07 ± 0.45[a]	14.10 ± 0.91[c]	12.65 ± 0.68[bc]	12.11 ± 0.50
	Soil	20.07 ± 0.97	20.70 ± 0.99	21.70 ± 1.67	16.12 ± 1.90	19.65 ± 0.85
Masjed-Soleyman	Pasture	10.27 ± 1.37	11.92 ± 1.67	14.35 ± 1.30	12.12 ± 0.44	12.17 ± 0.68
	Soil	17.05 ± 1.03[a]	18.12 ± 0.65[ab]	20.07 ± 0.88[b]	16.75 ± 0.48[a]	18.00 ± 0.49

In each row different letters show significant difference (P<0.05)

Table 4: Mean ± SE levels of iron in pasture and soil in different towns and seasons (mg/kg)

Town	Sample type	Season				Total n = 48
		Spring n = 12	Summer n = 12	Autumn n = 12	Winter n = 12	
Behbahan	Pasture	468.75 ± 62.39[b]	646.25 ± 10.28[d]	490.00 ± 14.72[bc]	266.25 ± 49.55[a]	467.81 ± 39.36
	Soil	1172.37 ± 164.74	1466.00 ± 139.71	1310.00 ± 33.42	1463.75 ± 268.35	1353.03 ± 83.55
Ramhormoz	Pasture	143.75 ± 23.57[a]	525.00 ± 137.73[c]	422.50 ± 109.32[bc]	188.75 ± 19.83[ab]	320.00 ± 57.22
	Soil	1375.50 ± 268.56	1163.12 ± 210.90	989.62 ± 204.27	1464.37 ± 200.63	1248.16 ± 110.51
Eizeh	Pasture	453.75 ± 20.55[a]	668.75 ± 12.31[a]	1117.50 ± 205.35[b]	1087.50 ± 174.76[b]	831.87 ± 94.55
	Soil	1524.12 ± 340.74	1104.75 ± 207.61	1374.12 ± 140.61	1337.50 ± 103.13	1335.12 ± 104.81
Masjed-Soleyman	Pasture	318.75 ± 25.85[a]	478.75 ± 27.86[b]	743.75 ± 54.14[c]	752.50 ± 24.62[c]	573.44 ± 49.97
	Soil	1031.87 ± 129.79	1280.62 ± 125.24	975.37 ± 118.17	919.50 ± 135.07	1051.84 ± 67.12

In each row different letters show significant difference (P<0.05)

Table 5: Mean ± SE levels of sulfur in pasture in different towns and seasons (mg/kg)

Town	Sample type	Season				Total n = 48
		Spring n = 12	Summer n = 12	Autumn n = 12	Winter n = 12	
Behbahan	Pasture	481.50 ± 51.65	495.00 ± 44.81	491.25 ± 58.54	483.00 ± 38.28	487.69 ± 21.92
Ramhormoz	Pasture	511.00 ± 34.51	463.00 ± 30.88	492.50 ± 39.45	492.50 ± 20.97	489.75 ± 15.06
Eizeh	Pasture	440.00 ± 14.14	462.00 ± 19.76	494.00 ± 19.98	445.50 ± 14.31	460.37 ± 9.45
Masjed-Soleyman	Pasture	429.50 ± 14.86	413.00 ± 13.13	477.50 ± 19.31	455.00 ± 23.27	443.75 ± 10.28

Table 6: Mean ± SE, Cu: Mo ratio of pastures in different towns and seasons

Town	Season				Total
	Spring	Summer	Autumn	Winter	
Behbahan	1.30 ± 0.29[a]	1.37 ± 0.32[a]	0.98 ± 0.02[b]	0.95 ± 0.12[b]	1.15 ± 0.11
Ramhormoz	1.14 ± 0.05[a]	1.02 ± 0.10[a]	0.76 ± 0.04[b]	0.77 ± 0.06[b]	0.22 ± 0.05
Eizeh	1.08 ± 0.10[ab]	1.18 ± 0.06[a]	0.84 ± 0.10[b]	0.90 ± 0.05[b]	1.00 ± 0.05
Masjed-Soleyman	1.22 ± 0.17[a]	1.06 ± 0.06[ab]	0.85 ± 0.05[b]	0.99 ± 0.07[ab]	1.03 ± 0.05

In each row different letters show significant difference (P<0.05)

respectively.

Discussion

The copper levels of soil and pastures in most parts of the country are unknown. Copper bioavailability can be low in ruminant diets, especially when molybdenum, sulfur and (or) iron are presented in moderate to high concentrations (Humphries *et al.*, 1983; Suttle *et al.*, 1984; Ward *et al.*, 1993). Therefore determination of copper in the diet or pasture has no diagnostic value in ruminants unless other elements which interact with copper are determined (Underwood and Suttle, 1999).

The copper status of sheep was evaluated in Behbahan, Ramhormoz, Eizeh and Masjed-Soleyman towns. The serum copper concentration of sheep in Behbahan, Ramhormoz, Eizeh and Masjed-Soleyman districts were 8.01, 3.77, 7.38 and 7.06

µmol/L, respectively. Khan *et al*. (2006) conducted a study to determine the copper status of grazing sheep. Although an adequate level of plasma copper was found, it was higher in winter than that in summer. The normal ranges of plasma and serum copper are wide and vary between species. The norms for serum copper are 9-15 µmol/L for ruminants (Underwood and Suttle, 1999). Serum copper levels between 3 and 9 µmol/L represent marginal deficiency, and levels below 3 µmol/L represent functional deficiency or hypocuprosis (Radostits *et al*., 2007).

Liver copper values above 200 mg/kg DM in sheep are considered to be normal, levels of less than 80 mg/kg DM are accepted as low, and less than 35 mg/kg DM is deficient (Radostits *et al*., 2007). The present results indicated that goats in Behbahan, Eizeh and Masjed-Soleyman were in a state of marginal deficiency, but in Ramhormoz functional copper deficiency was seen. These results are consistent with our previous finding of the occurrence of clinical cases of enzootic ataxia in lambs in Ramhormoz (Nouri *et al*., 2005).

The mean seasonal and annual copper concentrations of pastures in all of the studied towns were above 10 mg/kg DM (Table 2). Pastures containing less than 3 mg/kg DM of copper will result in signs of deficiency, levels of 3-5 mg/kg DM can be considered as dangerous, and levels greater than 5 mg/kg DM are safe unless complicating factors cause secondary copper deficiency (Radostits *et al*., 2007). Therefore, the low serum copper levels were not due to the copper content of diet, so our study rejected primary copper deficiency.

In all of the towns, molybdenum and sulfur content of pastures were above 10 and 400 mg/kg DM, respectively. This level for iron was between 150-1150 mg/kg DM. Pastures containing below 3 mg/kg DM of molybdenum are considered to be safe, but disease may occur at 3-10 mg/kg DM if the copper intake is low. Pastures containing levels above 10 mg/kg DM of molybdenum are dangerous unless the diet is supplemented with enough copper (Radostits *et al*., 2007). It has been shown that a high sulfur content of diet (>400 mg/kg DM) can prevent copper absorption in the digestive system whenever the diet molybdenum is high (Church, 1988). High dietary intake of iron can interfere with copper metabolism (Radostits *et al*., 2007). Dietary levels of iron in the range of 500-1500 mg/kg DM are safe and higher levels can induce copper deficiency in ruminants (Radostits *et al*., 2007). Hypocupraemia and reduced copper concentration of liver in cattle supplemented with molybdenum and sulfur and cattle supplemented with iron and sulfur have been clearly demonstrated (Humphries *et al*., 1983; Phillippo *et al*., 1987a), however, only the molybdenum and sulfur supplemented cattle had clinical signs of deficiency. Ivan *et al*. (1990) showed that in lambs with posterior paralysis serum copper levels were 0.29-0.67 µmol/L and evaluation of diet determined secondary copper deficiency due to excessive amounts of molybdenum and sulfur. In southern Australia, associations between bovine hypocupraemia and iron-rich soils and pastures have been reported (McFarlane *et al*., 1990). Nouri (1998) and Nouri *et al*. (2005) reported secondary copper deficiency in some parts of Iran due to high amounts of molybdenum in soil and pastures.

In the present study, the mean Cu:Mo ratio in diet was 1.15, 0.92, 1 and 1.03, respectively in Behbahan, Ramhormoz, Eizeh and Masjed-Soleyman districts. It has been shown that Cu:Mo ratio below 1.0 in the diet induces a high risk of disorder and ratios of 1.0-3.0 indicate a marginal risk of past or future problems (Underwood and Suttle, 1999). However, some authors suggested 2:1 ratio and for safety in sheep 5:1 and in cattle 10:1 (Smith and Group, 1973).

The present study is the first investigation concerning the copper status of goats in the high altitude areas of Khuzestan province and indicates that a secondary copper deficiency due to the high molybdenum content of soil and pasture exists in the area.

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