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The copper, zinc and manganese status in opossum and gray fox

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1 Introduction

The mineral requirement of animals differs between species and is affected by the age and performance of the animals. If the mineral intake meets the requirement of the animals can be estimated by the mineral analysis of various organ tissues, which reflect a different mineral supply. However, the assessment of the mineral status of different species of wild animals depends on the knowledge of their normal status. The use of values of mineral concentration in organ tissues which are regarded as the limit values for a sufficient mineral supply in domestic animals may result in overestimating the incidence of mineral deficiency or toxicity in wild animals.

Because the Cu, Zn, Mn and Mo status is species specific, organ tissues and hair of opossum (*Didelphis virginiana*) and California gray fox (*Urocyon cinereoargenteus californicus*) were analyzed and compared with data of herbivores.

2 Material and Methods

Indicator organ tissues of the Cu-, Zn- and Mn-status

In previous studies various organ tissues and hair were tested for their suitability to reflect a different Cu, Zn or Mn supply in goats, bulls, and sheep (ANKE et al., 1978).

Under the condition of Cu deficiency, the Cu status was best reflected by the cerebrum of goat followed by liver, blood plasma and hair with decreasing reliability. On the other hand, the capacity of accumulating Cu is much higher in the liver of bulls, which received a high Cu amount in their diet than in their cerebrum and hair (ANKE et al., 1988).

The Zn status of the blood serum and the organ tissues is controlled homeostatically (KIRCHGESSNER, 1977). However, a Zn deficient diet affected the Zn concentration of ribs in goats (ANKE and RISCH, 1979) and rats (BERG and KOLLMER, 1987). It was found in cattle and particularly in sheep that the Zn concentration of ribs correlated with that of serum, liver and cerebrum (ANKE et al., 1988).

The Mn status of the body and its organ tissues is less controlled homeostatically than that of Zn. The liver was the best indicator to reflect a marginal Mn-supply (ANKE et al., 1978). Hair, kidneys, cardiac muscle, ovaries, bone marrow, spleen, and cerebrum also contained significantly less Mn than the corresponding tissues of control goats. The individual correlative calculation of the Mn content of different organ tissues of sheep demonstrated that a high Mn supply is reflected in the liver and kidneys (ANKE et al., 1988).

Animals
Wild living opossums and foxes were trapped at the San Diego Zoo (California) and euthanized due to diseases screening. The ruminants which were used for comparison came from the San Diego Zoo and Wild Animal Park. For the presentation of the results the various species of ruminants were classified as morphophysiological the ruminant feeding types concentrate selectors, intermediate mixed feeders, and grass and roughage eaters (HOFMANN and STEWART, 1972; HOFMANN, 2000).

Chemical Analysis
Tissues from the liver, kidney, cerebrum, rib, skeleton muscle, heart, lung, aorta, spleen, pancreas, and hair were obtained from clinical healthy animals at necropsy of San Diego Zoo Hospital. The samples were dried at 105°C to constant weight and dry ashed at 405°C. The ashes were dissolved in hydrochlorid acid (25 %) and thinned down with aqua bidest to 2.5 %. The Cu-, Zn-, Mn- and Mo-concentration was analysed by atomic absorption spectrometry (JarRell Ash 850) or optical emission spectroscopy with inductively coupled plasma (Spectroflame D, Spectro Analytical Instruments). The results were statistically assessed with the program SPSS for Windows (version 6.01, SPSS, Inc.).

3 Results

Copper
The cerebrum is regarded as the best indicator organ tissue for Cu deficiency (ANKE et al., 1978). The mean Cu concentration of the cerebrum averaged 19 mg/kg dry matter in opossum and 15 mg in foxes and remained in the same range compared to ruminants (Table1).

Table 1: The copper content of liver and cerebrum in Northern Opossum and Gray Fox compared to ruminants (mg/kg dry matter)

Species/Feeding types (n;n)	Cerebrum		Liver		p	Ratio[1)]
	SD	mean	mean	SD		
Opossum (9;14)	4.2	19	14	2.0	< 0.01	0.7
Fox (6;6)	3.0	15	24	10	< 0.05	1.6
Ruminants						
Conc.Sel. (11;14)	6.8	18	41	46	n.s.	2.3
Int.Mix Feed (96;101)	8.1	19	262	269	< 0.001	14
Grass Rough. (29;28)	9.8	17	249	195	< 0.001	15
Fp	< 0.05		< 0.01			
Ratio[2)]	1.3		22			

[1)] Cerebrum = 1, Liver = x; [2)] Lowest value = 1, Highest value = x

Contrary, the liver can store much higher Cu amounts than the cerebrum. Ruminants which were classified as intermediate mixed feeders and grass and roughage eaters accumulated 14 and 15 times more Cu in the liver than in the cerebrum, respectively. It seems, that this rule might not be true for opossums and foxes. The Cu content was only 1.6 times higher in the liver than in cerebrum of foxes. Furthermore, opossums contained even less Cu in the liver than cerebrum.
When animals take in high Cu amounts the Cu concentration of liver as well as of kidney can be excessive. However, due to the relatively low Cu concentration in the liver of opossums and foxes compared to ruminants no Cu accumulation was expected in the kidney, that indeed contained similar Cu concentrations as in livers of opossums and foxes (Table 2).

Table 2: The copper content of liver and kidney in Northern Opossum and Gray Fox compared to ruminants (mg/kg dry matter)

Species/Feeding types (n;n)	Kidney		Liver		p	Ratio[1)]
	SD	mean	mean	SD		
Opossum (8;14)	7.2	22	14	2.0	< 0.01	0.6
Fox (6;6)	3.6	14	24	10	< 0.05	1.7
Ruminants						
Conc.Sel. (13;14)	5.9	17	41	46	n.s.	2.5
Int.MixFeed (114;101)	7.2	20	262	269	< 0.001	13
Grass Rough. (29;28)	9.4	21	249	195	< 0.001	12
Fp	< 0.05		< 0.01			
Ratio[2)]	1.6		22			

Even though there are significant differences between the opossums, foxes and ruminants the Cu concentration of the kidney remained in the same range among the animals.

The sex does not effect the Cu concentration of several organ tissues of the opossum (Table 3). However male opossums tended to store more Cu in a few organ tissues but the difference remained insignificant except in the skeleton muscle. This tendency is not influenced by the status of lactation of the female opossums because the mean Cu concentrations of the organ tissues did not differ between lactating and non lactating females.

Table 3: The copper content of several organ tissues in Northern Opossum (mg/kg dry matter)

Organ/tissue (n;n)	Female		Male		p	%[1)]
	SD	mean	mean	SD		
Liver (8;6)	1.7	13	14	2.4	n.s.	108
Kidney (5;3)	5.6	24	19	9.7	n.s.	79
Cerebrum (6;4)	2.3	17	22	4.6	n.s.	129
Rib (7;6)	5.1	7.6	11	6.6	n.s.	145
Skeletal muscle (7;5)	0.90	6.5	8.4	2.3	< 0.05	129
Heart (6;2)	3.0	16	16	3.8	n.s.	100
Lung (7;5)	2.6	6.8	6.8	1.6	n.s.	100
Aorta (2;-)	3.2	8.3	-	-	-	-
Spleen (4;3)	1.3	4.4	6.4	3.0	n.s.	145
Pancreas (6;3)	3.7	8.2	11	5.1	n.s.	134
Hair (7;5)	4.4	10	11	3.4	n.s.	110

[1)] Female = 100%, Male = x%

Zinc

The Zn contents of the ribs of opossums and foxes were about 1.5. times higher than in ruminants (Table 4). This result was expected because the ribs of omnivores like domestic pigs contained more Zn than cows and sheep (ANKE et al., 1986). However, the Zn concentration of ribs and livers correlated in cows (ANKE et al., 1988), which accumulated 2 times more Zn in their livers than in ribs (ARNHOLD et al., 1991).

The various feeding types wild ruminants follow this rule, but not opossums and foxes. They stored 26 and 21 % less Zn in the livers than in the ribs, respectively.

Table 4: The zinc content of liver and rib in Northern Opossum and Gray Fox compared to ruminants (mg/kg dry matter)

Species/Feeding types (n;n)	Rib		Liver		p	%[1]
	SD	mean	mean	SD		
Opossum (13;14)	19	132	98	22	< 0.01	74
Fox (6;6)	16	138	109	34	n.s.	79
Ruminants						
Conc.Sel. (13,14)	20	105	146	59	< 0.05	139
Int.Mix.Feed (84;113)	23	91	169	109	< 0.001	186
Grass Rough. (29;28)	23	94	215	218	< 0.001	229
Fp	< 0.05		< 0.01			
%[2]	152		219			

[1] Rib=100%, Liver = x%; [2] Lowest value = 100%, Highest value = x%

Table 5: The zinc content of hair and kindney in Northern Opossum and Gray Fox compared to ruminants (mg/kg dray matter)

Species/Feeding types (n;n)	Hair		Kidney		p	%[1]
	SD	mean	mean	SD		
Opossum (12;8)	64	182	129	32	n.s.	71
Fox (3;4)	80	247	74	22	< 0.05	30
Ruminants						
Conc.Sel. (8;13)	19	127	138	36	n.s.	109
Int.Mix.Feed (42;113)	75	145	115	59	< 0.01	79
Grass Rough. (15;26)	75	152	150	113	n.s.	99
Fp	< 0.05		< 0.01			
%[2]	194		203			

[1] Hair = 100%, Kidneys = x%; [2] Lowest value = 100%, Highest value = x%

Both, opossums and foxes accumulated less Zn in kidneys than in hair (Table 5). This finding is in accordance with results obtained in cattle, in which the Zn concentrations in hair and kidneys correlated significantly (ANKE et al., 1988). Contrary to the relation of Zn in ribs and liver, this rule of ratio of Zn in hair and kidneys might be true also for opossums and foxes

The liver of female opossums contained a significant lower Zn amount than in males (Table 6) whereas the Zn concentration of kidneys was significantly higher in females than in males. The status of lactation had no effect on the Zn status.

Table 6: The zinc content of several organ tissues in Northern Opossum (mg/kg dry matter)

Organ/tissue (n;n)	Female		Male		p	%[1]
	SD	mean	mean	SD		
Liver (8;6)	7.2	84	115	24	< 0.01	137
Kidney (5;3)	12	147	99	36	< 0.05	67
Cerebrum (6;4)	36	89	72	7.5	n.s.	81
Rib (7;6)	20	139	124	15	n.s.	89
Skeletal muscle (7;5)	17	151	142	13	n.s.	94
Heart (7;2)	16	84	80	12	n.s.	95
Lung (7;5)	12	70	75	10	n.s.	107
Aorta (2;-)	25	125	-	-	-	-
Spleen (4;3)	15	91	120	33	n.s.	132
Pancreas (6;3)	17	122	110	19	n.s.	90
Hair (7;5)	76	166	205	41	n.s.	123

1) Female = 100 %, Male = x %

Table 7: Manganese content of liver and kidney in Northern Opossum and Gray Fox compared to ruminants (mg/kg dry matter)

Species/Feeding types (n)	Kidney		Liver		p	%[1]
	SD	mean	mean	SD		
Opossum (8;14)	0.86	2.8	8.3	1.4	< 0.05	296
Fox (6;6)	1.6	4.7	8.4	2.0	< 0.05	179
Ruminants						
Conc.Sel. (13;14)	1.5	4.6	11	3.8	< 0.01	239
Int.MixFeed (113;116)	1.5	4.4	9.2	4.7	< 0.001	209
Grass Rough. (26;28)	0.90	3.8	6.6	1.7	< 0.001	174
Fp	< 0.05		< 0.01			
%[2]	168		167			

1) Kidney = 100%, Liver = x% 2) Lowest value = 100%, Highest value = x%

Manganese

The Mn accumulation was significantly higher in the liver of opossums and foxes than in kidneys (Table 7). In comparison to the various feeding types of ruminants the mean Mn concentrations remain in the same range for both livers and kidneys. Furthermore, there are also no differences between the ratio of Mn contents of kidneys and liver among the animal species.

Female and male opossums as well as lactating and non lactating female opossum did not differ regarding there mean Mn concentrations in the various organ tissues.

The Mn as well as the Cu and Zn concentrations of various organ tissues can be considerably higher in new born than in adults (ANKE and RISCH, 1979; ARNHOLD et al., 2001). These differences disappear during the growing period of the animals up to their adult status. Whereas the age of the animals did not take a significant effect on the Cu and Zn status of opossums juveniles accumulated significantly more Mn in kidneys, cerebrum and spleen (Table 8).

Table 8: The manganese content of several organ tissues in adult and juvenile Northern Opossum (mg/kg dry matter)

Organ/tissue (n;n)	Adult		Juvenile		P	%[1]
	SD	mean	mean	SD		
Liver (14;3)	1.4	8.3	8.7	2.4	n.s.	105
Kidney (8;2)	0.86	2.8	5.4	0.63	< 0.05	193
Cerebrum (10;3)	0.69	2.2	3.8	1.2	< 0.05	173
Rib (13;3)	3.0	3.1	11	13	n.s.	355
Skeletal muscle (12;1)	0.17	0.78	1.0	-	-	128
Heart (9;3)	0.58	1.8	2.1	0.45	n.s.	117
Lung (12;1)	0.66	1.0	0.97	-	-	97
Aorta (2;2)	1.8	2.6	3.7	0.81	n.s.	142
Spleen (7;3)	0.24	1.3	3.5	2.3	< 0.5	269
Pancreas (9;2)	1.4	6.4	5.5	0.36	n.s.	86
Hair (12;3)	3.4	5.0	5.2	4.7	n.s.	104

1) Adult = 100%, Juvenile = x%

Discussion

The Cu, Zn and Mn status depends on species, age and trace element intake (ANKE et al., 1980; DAVIS and MERTZ, 1987). The mean Cu concentration of the liver averaged 14 mg/kg dry matter in opossum and 24 mg in foxes and remained in the same range as the Cu content of the cerebrum. Contrary, herbivores and carnivores accumulated considerably higher Cu amounts in livers than in the cerebrum (ANKE et al., 1983; KOSLA, 1985; ARNHOLD et al., 2001). This may depend on species and may be related to the nutrition category because further omnivores like wild boars, domestic pigs and humans also did not show big differences regarding the Cu concentration of liver and cerebrum (ANKE and SCHNEIDER, 1971; ANKE et al., 1983) (Table 9). Thus the Cu accumulation is more distinct in carnivores and herbivores especially in intermediate mixed feeders and grass and roughage eaters than in omnivores. The influences of sex and age can be ignored in adult animals.

100 mg Zn/kg rib dry matter in ribs and 150 mg Zn/kg hair dry matter are regarded as a limit value for sufficient Zn supply in domestic pigs (ANKE et al., 1986). The ribs of opossum and foxes contained more Zn than these limit values. Furthermore, both animal species accumulated more Zn in the ribs than herbivores but considerably less than carnivores like minks (ANKE et al., 1983).

Additionally beside the effect of species the Zn status depends also on sex and age. Female opossums stored less Zn in their ribs than male. This is contrary to minks and humans in which females accumulated more Zn than males (ANKE and SCHNEIDER, 1971; ANKE et al., 1983). Liver and cerebrum of horses at the age of 16 to 20 years contained significantly more Zn than younger adult horses (KOSLA 1988). Furthermore, in elder humans the Zn concentration of liver, rib and hair was significantly higher than in younger persons (BAUMANN et al., 1986).

Table 9: The copper content of liver and cerebrum in omnivores, carnivores and herbivores (mg/kg dry matter) (ANKE and SCHNEIDER, 1971; ANKE et al., 1983; KOSLA, 1988; ARNHOLD et al., 2001)

Species/Nutrition categories (n;n)	Cerebrum		Liver		p	Ratio[1)]
	SD	mean	mean	SD		
Omnivores						
Wild boar (18;20)	4.7	13	16	4.1	n.s.	1.2
Domest. pig (79;73)	4.0	15	21	9.0	< 0.05	1.4
Human (59;58)	4.3	17	18	7.0	n.s.	1.1
Opossum (9;14)	4.2	19	14	2.0	< 0.01	0.7
Fox (6;6)	3.0	15	24	10	< 0.05	1.6
Carnivores						
Mink (41;42)	2.2	13	60	37	< 0.001	4.6
Herbivores						
Dom.horse (154;164)	2.7	8.6	21	8.3	< 0.001	2.4
	6.8	18	41	46	n.s.	2.3
Conc.Sel. (11;14)	8.1	19	262	269	< 0.001	14
Int.Mix Feed (96;101)	9.8	17	249	195	< 0.001	15
Grass Rough. (29;28)						

100 mg Zn/kg rib dry matter in ribs and 150 mg Zn/kg hair dry matter are regarded as a limit value for sufficient Zn supply in domestic pigs (ANKE et al., 1986). The ribs of opossum and foxes contained more Zn than these limit values. Furthermore, both animal species accumulated more Zn in the ribs than herbivores but considerably less than carnivores like minks (ANKE et al., 1983).

Additionally beside the effect of species the Zn status depends also on sex and age. Female opossums stored less Zn in their ribs than male. This is contrary to minks and humans in which females accumulated more Zn than males (ANKE and SCHNEIDER, 1971; ANKE et al., 1983). Liver and cerebrum of horses at the age of 16 to 20 years contained significantly more Zn than younger adult horses (KOSLA 1988). Furthermore, in elder humans the Zn concentration of liver, rib and hair was significantly higher than in younger persons (BAUMANN et al., 1986).
The differences of the mean Mn concentration in the liver is less distinct among the animal

species than that of Cu. It varied between 6.6 and 11 mg/kg dry matter among the investigated species and was in the same range as that in omnivores and carnivores (HURLEY and KEEN, 1987; ARNHOLD et al., 1991).

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Summary

The assessment of the mineral status of different species of wild animals depends on the knowledge of their normal status. Using the values of mineral concentrations in organs which are regarded as the limit values for sufficient mineral supply in domestic animals results in overestimating the incidence of mineral deficiency in wild animals. Due to the fact that the mineral status is species-specific, organ tissues and hair of species of opossum and gray fox were analysed and compared with ruminants of different feeding types.

Tissues from liver, kidney, cerebrum, rib, skeletal muscle, heart, lung, aorta, spleen, pancreas, and hair of opossum and gray fox were obtained at necropsy of the Zoological Society of San Diego. After dry ashing of samples the Cu, Zn and Mn concentration were analysed by optical emission spectroscopy with inductively coupled plasma.

The trace element status of opossum and gray fox depends on species, age, sex, and mineral intake. Compared to cerebrum the livers of opossum and gray fox accumulate Cu to the same extend as omnivores but to a fewer extend than carnivores and omnivores. Whereas the Zn concentration of the liver in ruminants is higher than that of ribs which are regarded as the best indicator tissue to reflect the Zn status, opossums and gray foxes do not follow this rule.
However no differences were found between the animal species and nutrition categories regarding the Mn status.

Key words: Opossum, Gray Fox, Copper, Zinc, Manganese

Zusammenfassung

Der Kupfer-, Zink- und Manganstatus bei Opossum und Graufuchs

Die Einschätzung des Mineralstoffstatus der verschiedenen Wildtierarten hängt von der Kenntnis ihres Normalstatus ab. Die Verwendung von Mineralstoffkonzentrationen in Organen als Grenzwerte für eine ausreichende Mineralstoffversorgung, die für landwirtschaftliche Nutztiere gültig sind, kann zu einer Überschätzung von Mangelsituationen bei Wildtieren führen. Auf Grund der Artspezifität des Mineralstoffstatus wurden Organgewebe und Haar vom Opossum und Graufuchs analysiert und mit den Werten der verschiedenen Ernährungstypen von Wiederkäuern verglichen.

Gewebe von Leber, Nieren, Großhirn, Rippen, Skelettmuskel, Herz, Lungen, Aorta, Milz und Pankreas sowie Haar wurden von Tieren in der Pathologie der Zoologischen Gesellschaft

von San Diego entnommen. Nach trockener Veraschung der Proben erfolgte die Cu-, Zn- und Mn-Analyse mit ICP-OES.

Der Spurenelementstatus von Opossum und Graufuchs wird von Tierart, Alter, Geschlecht und Mineralstoffaufnahme beeinflusst. Im Verhältnis zum Großhirn speichern die Lebern von Opossum und Graufuchs ähnlich hohe Mengen an Cu wie Omnivoren, aber zu einem geringerem Ausmaß als Fleisch- und Pflanzenfresser.

Während bei Wiederkäuern die Leber mehr Zn als die Rippe speichert, welches als das beste Zn-Indikatorgewebe angesehen wird, enthalten sie bei Opossum und Fuchs weniger Zn. Hinsichtlich des Mn-Status bestanden zwischen den Tierarten und Ernährungstypen keine Unterschiede.

Schlüsselwörter: Opossum, Graufuchs, Kupfer, Zink, Mangan

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