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Zinc, Iron, Copper, Manganese, Calcium and Magnesium Supply Status of Free-living Bank Voles

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The content of Zn, Fe, Cu, Mn, Ca and Mg in the food (stomach contents) and in various tissues and organs of bank voles, *Clethrionomys glareolus* (Schreber, 1780) living in a deciduous forest in winter, spring, summer and autumn was determined. The highest Zn level in food was found from March to June (56—70 μg/g), while from July to December it was 37—43 μg/g. The Fe level in food was stable (250—330 μg/g) during all seasons except in animals caught in March (650 μg/g). The mean Cu concentration in food changed in various seasons from 5.0 μg/g to 15.4 μg/g, that of Mn ranged from 65 μg/g to 330 μg/g, Ca varied from 0.21‰ to 0.53‰ and Mg from 850 μg/g to 1530 μg/g. The concentration of Zn in voles' bone and liver indicated that Zn supply status of the body in spring and autumn was maintained at a higher level than in summer and winter. In six animals in August and in one in September the concentration of Zn was 2—5 times lower in all organs and tissues suggesting a Zn deficiency in these voles. Although the concentrations of Fe, Cu and Mn in certain organs showed seasonal variability, they were within the normal physiological range. The concentration of Ca and Mg in the tissues remained at a stable level over a whole year. It was shown that, with the exception of Zn, the mineral supply status of the bank vole was maintained throughout the whole year at a level indispensable for normal functioning of these rodents.

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1. INTRODUCTION

Zinc, iron, copper, manganese, calcium and magnesium, metals which are components or activators of many enzymes, play an important role in animal reproduction, growth and development (Underwood, 1971). No data are available on the supply status of these elements in wild animals. Thus it is not known, whether wild animals consume sufficient amounts of trace elements and macroelements throughout the whole year or whether their amount in the consumed food is too small to meet the requirements for these components. Deficiency of any of these elements may cause diseases and reproduction disorders (Rogers *et al.*, 1985; Stem-

mer *et al.*, 1985; Menino *et al.*, 1986), and may thus influence the animal populations numbers. It thus seems necessary to study minerals supply status of wild animals.

The purpose of our study was to establish the zinc, iron, copper, manganese, calcium and magnesium supply status of free-living bank voles, *Clethrionomys glareolus* (Schreber, 1780). Bank voles are common in Polish forests, and their biology and ecology are fairly well known (Petrusewicz, 1983). However, it is not known whether the quantity and quality of food available to these animals from the ecosystem are optimal throughout the whole year. Andrzejewski (1975) found that additional food (oats) increased population numbers of bank voles as a result of their winter reproduction. This author found that the population size was in principle regulated by food abundance in the ecosystem, although in a more complicated way than it would appear from the simple energy balance between the available food and the nutritional requirements of the population. It could be expected that food (especially in winter) would contain either too small amounts of certain nutrients, *e.g.* trace elements, or that these components are present in food in inappropriate proportions. In view of this the content of certain trace elements and macroelements in food and in various tissues of bank voles in winter, spring, summer and autumn was studied.

2. MATERIAL AND METHODS

Bank voles were caught in snap traps in a deciduous forest, *Tilio-Carpinetum* Tracz. 1962, in the Solnicki Forest near Białystok (53°06'N, 23°10'E), during the second half of each month from October 1985 to September 1986 (with the exception of January and February). The time from capture to examination of the animals in the laboratory was at most 12 hours. In pilot investigations the stomach content of Zn, Fe, Cu, Mn, Ca and Mg in various tissues of bank voles determined immediately *post mortem* was the same as 12 hours after death. No significant differences were found either between the concentrations of these elements in the consumed food (oats) and their concentrations in stomach contents.

In the laboratory the bank voles were weighed, their sex and physiological state were determined. There were animals both sexually inactive (immature) and sexually active (mature and pregnant females). Within sexually inactive males, maturating animals (imm+), animals in the stage of sexual regression (r) and young immature animals (imm) were differentiated. Imm+ males had advanced development of testes and seminal vesicles, while males in sexual regression had large epididymis and testicular atrophy. Imm males had small testes and seminal vesicles. Table 1 presents the characteristics of the different groups of bank voles.

After determination of the physiological state of the animals the stomach contents, liver, kidneys, heart, sceletal muscles, tibias and testes were dissected and removed. The food from the stomach and the tissues were dried to a constant

Table 1

Body and organ weights of bank voles under study. GA — group of animals, N — number of animals, M — males, F — females, ma — mature animals, imm — young immature animals, imm+ — maturing animals, r — testicular regression, p — pregnant.

Season	Months	GA	N	Body wt (g)	Dry weight (mg±SEM)			
					Liver	Kidneys	Heart	Testes
Winter	Nov.	Mr	6	17.6±0.4	270±6	62±4	26±1.0	6.5±1.5
	Nov.-Dec.	Mimm	12	15.8±02	243±7	48±1	23±0.9	1.5±0.1
	Nov.	Fma	4	20.6±0.5	324±8	48±3	26±0.9	
	Nov.-Dec.	Fimm	7	15.8±0.1	235±2	44±4	21±1.3	
Early	March	Mimm+	5	16.5±0.8	242±12	53±5	24±2.0	50±9
		Fimm+	3	16.0±0.4	242±9	44±8	23±0.5	
spring	April	Mma	9	21.0±0.8	315±18	81±5	30±1.5	116±8
		Fma	3	18.4±2.0	290±20	73±11	23±2.0	
Late spring	May-Jun.	Mma	10	23.3±1.0	368±18	71±4	27±1.6	123±11
		Fp	4	26.9±1.3	380±10	79±2	26±2.9	
Summer	Jul.-Aug.	Mma	13	21.6±0.7	340±12	62±3	26±1 1	124±7
		Fma&Fp	9	23.3±1.3	327±26	67±9	24±1.4	
Autumn	Sep.	Mimm+	5	18.3±0.3	280±12	65±3	24±1.0	51±4
	Oct.	Mr	4	16.5±0.2	250±10	52±4	23±0.6	9.0±0.2
	Sep.-Oct.	Mimm	11	15.4±0.4	228±7	48±3	20±0.8	2 0±0.1
	Sep.-Oct.	Fma&Fp	8	22.8±1.5	343±26	56±7	28±1.3	
		Fimm	7	16.8±0.3	257±10	53±2	21±1.3	

weight at 100°C for 20 hours. After drying and weighing, food and tissue samples (maximal mass 120 mg) were placed in calibrated glass tubes with 1.5 ml of concentrated nitric acid. After 20 hours of samples digestion at room temperature, sulphuric (0.1 ml) and 62% perchloric (0.5 ml) acids were added. The mixture was mixed and heated at 100°C for 1 hour. Then the temperature was raised to 150°C (1 hour) and 200°C(1 hour). Digestion was carried out in an oven (lined with aluminium foil) connected to a vacuum water pump. The residue after digestion (about 0.3 ml) was made up with bidistilled water to 5.0 ml. The obtained solutions were kept in tightly closed polyethylene containers. The elements in these solutions were determined by means of atomic absorption spectrophotometry in air-acetylene flame using a AAS 3 Carl Zeiss Jena apparatus. The standard curve was prepared using Merck standard solutions. The recovery of the analysed elements by this method was 95—105%.

Data are reported as the mean ± SEM. Differences between individual means were determined by the Duncan's multiple-range test and by the Student's *t*-test.

3. RESULTS AND DISCUSSION

3.1. Zinc

The concentration of Zn in food (stomach contents) in a given season was not significantly different in both sexually active and inactive male and female voles. No significant differences were found either in the levels of this mineral in the tissues and organs of males or females, and thus the results for both sexes are presented together. Pregnant females had similar ($p>0.05$) Zn concentrations in the tissues as mature non-pregnant females.

The highest Zn content in the food was found from March to June (Table 2, 3). From July to December this content remained at a stable level which was, however, significantly lower ($p<0.05$) than in spring.

The highest Zn concentration in the liver of bank voles was noted in spring (Table 2). In the livers of maturating animals (imm+) caught in March a statistically similar ($p>0.05$) Zn level was found as in mature animals (ma) caught in April, May and June. The concentration of Zn in the liver of sexually mature voles examined in summer and winter was, however, significantly lower in comparison to adult animals caught in spring and autumn. In autumn the concentration of this mineral in the liver of young animals (imm) and males in the stage of sexual regression (Mr) was similar, and significantly lower than in ma and imm+ voles. In winter the Zn level in the liver of mature voles was low, similar to that in imm animals and in males in the sexual regression stage.

The Zn concentration in the bone of bank voles demonstrated a sig-

Table 2

Zinc concentrations of food and various organs and tissues of bank vole (μg/g d. wt±SEM). GA — group of animals, N — number of animals, M — males, F — females, ma — mature animals, imm — young immature animals, imm+ — maturing animals, r — testicular regression, p — pregnant, nd — not detected. When there were no statistically significant differences between voles with different physiological states, the mean value for all animals in a given season is presented. Mean values in columns with different superscript letters are significantly different (Duncan's multiple range test; $p<0.05$).

Season	Months	Ga	N	Food	Liver	Bone	Testes	Kidneys	Heart	Muscle
Winter	Nov.	Fma	4		84 ± 3^{ac}	155 ± 10^{a}	—			
	Nov.-Dec.	MFimm	19	39 ± 3^{a}	80 ± 4^{ac}	160 ± 7^{a}	nd	79 ± 2^{a}	71 ± 2^{a}	47 ± 1.5^{a}
	Nov.	Mr	6		78 ± 8^{a}	145 ± 10^{a}	nd			
Early spring	March	MFimm+	8	59 ± 7^{bc}	103 ± 7^{b}	160 ± 7^{b}	126 ± 4^{a}	91 ± 2^{b}	70 ± 2^{a}	51 ± 2^{a}
	April	MFma	12	70 ± 5^{b}	102 ± 4^{b}	192 ± 10^{b}	163 ± 7^{b}			
Late spring	May-Jun.	MFmap	14	56 ± 6^{c}	102 ± 4^{b}	199 ± 7^{b}	159 ± 4^{b}	86 ± 3^{ab}	70 ± 6^{a}	38 ± 3^{b}
Summer	Jul.-Aug.	MFmap	16	43 ± 2^{a}	90 ± 5^{c}	167 ± 6^{a}	154 ± 4^{b}	87 ± 4^{ab}	74 ± 5^{a}	44 ± 4^{ab}
Autumn	Sep.-Oct.	Fmap	8		101 ± 5^{b}	187 ± 9^{b}	—			
	Sep.	Mimm+	5	37 ± 3^{a}	99 ± 5^{bc}	152 ± 7^{a}	127 ± 7^{a}	80 ± 2^{a}	69 ± 2^{a}	45 ± 2^{ba}
	Sep.-Oct.	MFimm	18		86 ± 3^{ac}	154 ± 8^{a}	nd			
	Oct.	Mr	4		87 ± 3^{ac}	186 ± 12^{b}	nd			

nificant relationship to the season and the physiological state of animals (Table 2). The highest Zn level in the bone was found in mature animals examined in spring and autumn. The lowest level of this element in bone tissue was noted in adult voles caught in summer and winter, and in imm and imm+ animals. The Zn content in the bone of the animals in sexual regression stage caught in October was high, statistically similar ($p>0.05$) to that in adult animals caught in spring and autumn. In November the Zn level in the bone in this group of animals was significantly decreased to the level characteristic of imm voles.

In contrast to the liver and bone the heart Zn level in bank voles remained stable throughout the whole year, independently of the physiological state of the animals (Table 2). In the kidneys the highest concentration of Zn was found in voles caught in March and April, while in muscles the lowest level was in May and June.

Zn concentration in the testes of imm+ males caught in March and September was significantly lower ($p<0.05$) than in adult males caught from April to August (Table 2). In the testes of males caught in October, November and December the concentration of Zn could not be determined due to the small mass of this organ (Table 1).

In six out of 14 voles caught in August and in one caught in September the Zn concentration was several times lower in the stomach contents and in all tissues and organs (Table 4). This group of seven animals comparised four adult males and three females, two of them pregnant. The mean body weight of six voles caught in August was 21.0 ± 0.7 g, and it was significantly lower (by 2.5 g, Student t-test, $p<0.05$) than the body weight of the other animals caught in the month. This decreased Zn level in all tissues and organs, and lower body weight might indicate a deficiency of Zn in the body of these animals.

The Zn level in various mammalian tissues is affected by many factors,

Table 3

Percentage of bank vole individuals with different zinc concentrations of stomach contents.

Season	Months	Number of animals	Zn (μg/g)				
			<20	20—30	30—40	40—50	>50
Winter	Nov.-Dec.	29	26	10	20	20	24
Early spring	March-Apr.	20	—	9	9	13	69
Late spring	May-Jun.	14	—	8	12	25	55
Summer	Jul.-Aug.	22	20	15	15	30	20
Autumn	Sep-Oct.	35	25	17	17	14	27

among them the most important is: the total content of the mineral in the diet (Roth & Kirchgessner, 1983), the presence of components inhibiting its absorption from the intestine, *e.g.* phytate or fiber, and coexistence of antagonistic elements (Davies & Nightingale, 1975; Ismail-Beigi *et al.*, 1977; Keen *et al.*, 1985), the source and level of protein in the diet (Roth & Kirchgessner, 1985; Cossack, 1986), and the age and physiological state of the animals (Kennedy *et al.*, 1986). It may be supposed that all these factors influenced the Zn level in the tissues of wild bank voles.

According to the American Institute of Nutrition (1977) the optimal Zn level in the diet for the animals should be about 30 μg/g dry wt. In the diet of wild bank voles the mean total Zn concentration from March to June was about twofold higher, and in voles caught from July

Table 4

Zinc concentrations of food and various organs and tissues of bank vole suspected of Zn deficiency (μg/g d. wt±SEM). Mean values with different superscript letters are significantly different (unpaired Student's *t*-test; $p<0.001$).

Food & organ	Bank voles	
	Suspected of Zn deficiency N=7	Others N=16
Food	15±3[a]	43±2[b]
Liver	44±5[a]	90±5[b]
Bone	63±15[a]	167±6[b]
Testes	64±6[a]	154±4[b]
Kidneys	42±4[a]	87±4[b]
Heart	36±7[a]	74±5[b]
Muscle	8.0±1.0[a]	44.0±4.0[b]

to December this concentration was about 30% higher than that recommended by the AIN (1977) (Table 2). However, on the basis of the total Zn content in food only, no unequivocal conclusions can be reached concerning the Zn supply status of the body. Research has shown that one of the best indicators of the Zn status is its level in bone (Momcilović *et al.*, 1975). Stuart *et al.* (1986) demonstrated that Zn absorbed from the digestive tract is preferentialy used first in the soft tissues where it is needed for many essential zinc-dependent enzymes, and its excess is incorporated into the bone matrix, forming a poorly available zinc pool. This is confirmed by our results. Bank voles reaching maturity in March, that is immediately after winter, incorporate Zn mainly into the soft tissues (liver, kidneys, testes) and after reaching sexual maturity (April,

May, June) a significant increase of the Zn concentration occurs also in the bone (Table 2). These data indicate that high amounts of Zn were absorbed from the gut and that the Zn status of the bank voles in spring was maintained at a level ensuring intensive growth and reproductive activity of the animals.

This high supply of Zn from March to June was primarily due to a high level of the mineral in food (56—70 μg/g). However, the absorption of Zn and its subsequent incorporation into tissues are significantly affected by the source and level of dietary protein (Roth & Kirchgessner, 1985; Cossack, 1986; Dibi & Reber, 1987). In rats, for instance, a high protein level in the diet caused a significant increase of Zn in the plasma, liver and bone, suggesting a better utilization of dietary Zn (Cossack, 1986). It might be supposed that in the diet of bank voles in spring the level of protein of animal or plant origin is high (Drożdż & Osiecki, 1973; Gębczyńska, 1976). Perhaps in the studied animals in spring this factor was important in Zn absorption and retention.

As compared with spring, in summer, autumn and winter a significant decrease in the total content of dietary Zn was observed (Table 2, 3). The level of plant protein (Drożdż & Osiecki, 1973) and animal protein (Gębczyńska, 1976) in the diet was reduced. Although the Zn level in the food of bank voles was identical in summer, autumn and winter, the content of this mineral in the liver and bone of adult voles caught in autumn was similar to that in the animals caught in spring, and it was significantly higher than in the voles caught in summer and winter. These data suggest that Zn utilization from the food provided to bank voles by the ecosystem in summer and winter is poorer in comparison to autumn. One may suppose that in summer and winter the food of bank voles contains more (in comparison to autumn) components inhibiting Zn absorption, *e.g.* phytate and fiber, or that the amount of food components enhancing this process, *e.g.* protein, is lower. It cannot be excluded also that the lower Zn level in the liver and bone of adult voles in summer and winter was due to an unfavourable ratio of calcium and zinc molar concentrations in their food. Excess calcium in the diet inhibits Zn absorption in rodents (Kirchgessner & Weigand, 1983). The present study showed that the Ca molar concentration in the food of voles in summer and winter was 155—222 times higher than the Zn molar concentration, while in spring and autumn this concentration was only 85—130 times greater.

The Zn level in rodent tissues depends also on the age and physiological state of animals (Kennedy *et al.*, 1986). Our results confirm reports in the literature, and point out that sexual maturation of bank voles is associated with a raise of Zn in the body.

It is difficult to determine why the Zn concentration in the liver and bone of the animals in sexual regression was reduced. Perhaps food components inhibiting Zn uptake from the gut played a role. Another possibility was the presence of specific homeostatic mechanisms ensuring a definitely lower Zn level in the tissues. Since the regression of sexual organs is caused by a short photoperiod (Steger *et al.*, 1985), one may suppose that changes in the levels of certain hormones associated with this process cause either a limitation of Zn absorption or increase the excretion of endogenous Zn.

Also it is not known what caused the low Zn levels in the tissues of six voles caught in August and one caught in September (Table 4). Although the total Zn content in the food of these animals (mean 15 μg/g) was insufficient to completely meet the requirements for this mineral (Stemer *et al.*, 1985), this could not be the only cause of a several fold decrease of Zn in all organs and tissues. Dreosti *et al.*, (1986) kept laboratory mice for three weeks on a diet containing Zn in concentrations below 1 μg/g and found a reduced concentration of Zn in the liver by only 15% and in the bone by about 20% as compared to control animals. Thus it seems that this drastic reduction of Zn level in the tissues of seven voles must have had another cause. Perhaps, it was due to stress connected to a large population usually occurring in this season (Petrusewicz, 1983). This hypothesis requires verification in further studies, since it may be important for the physiological and population related consequences of Zn deficiency in the bodies of animals with various metabolic disturbances resulting in reduced resistance to infections, infertility of males, and disturbances in the development of fetuses and newborns (Chesters, 1983; Rogers *et al.*, 1985).

3.2. Iron

The Fe concentration in the food of bank voles, with the exception of the animals caught in March, was not related to season, sex or physiological state of the voles (Table 5). In March the Fe content in the food consumed by voles was 650 μg/g and it was 2—3 times higher in comparison to the Fe content in the food of animals caught in the other months.

Among the examined tissues and organs the greatest variability of Fe concentrations was found in the liver (Table 5). In a given season the concentration of this mineral in the liver of sexually active females (mature and pregnant) was significantly higher than in sexually inactive females. In males a similar situation was observed only in early spring

Table 5

Iron concentrations of food and various organs and tissues of bank voles (μg/g d. wt±SEM). When there were no statistically significant differences between voles with different physiological states the mean value for all animals in a given season is presented. Mean values in columns with different superscript letters are significantly different (Duncan's multiple range test; $p<0.05$).

Season	Months	Group of animals	Number ♀♀	Number ♂♂	Liver ♀♀	Liver ♂♂	Kidneys	Heart	Muscle	Food
Winter	Nov.	Sex. active	4	—	893±90[a]	—	320±18[a]	330±12[a]	68±3[a]	330±20[a]
	Nov.-Dec.	Sex. inactive	7	18	652±68[b]	673±60[b]				
Early spring	March	Sex. inactive	3	5	355±80[c]	388±25[c]	334±20[a]	340±20[a]	66±4[a]	650±80[b]
	April	Sex. active	3	9	900±120[a]	640±50[b]				250±35[a]
Late spring	May-Jun.	Sex. active	4	10	851±100[a]	679±54[b]	324±22[a]	300±16[a]	68±4[a]	270±30[a]
Summer	Jul.-Aug.	Sex. active	8	14	875±75[a]	540±63[b]	360±20[a]	345±14[a]	60±4[a]	260±15[a]
Autumn	Sep.-Oct.	Sex. active	8	—	598±45[b]	—	310±15[a]	323±10[a]	65±3[a]	250±15[a]
		Sex. inactive	7	20	351±74[c]	740±60[b]				

(March, April). From April to August the liver of females contained more Fe than the liver of males; in autumn a reverse situation was found. In March the Fe concentration in the liver of males and females was similar, without statistically significant differences. A similar observation was made in winter, however, Fe concentration in the liver of sexually inactive animals caught in November and December was twice as high as in the animals caught in March.

The concentration of Fe in the kidneys, heart and muscles was stable, independent of the season, sex and physiological state of animals (Table 5).

The fall of Fe level in the liver in voles caught in March to one half of the value found in November and December was probably due to the depletion of the stores of this mineral during the severe winter in February 1986. Most probably, these stores were used for the synthesis of haemoglobin, since its level increased considerably in the blood of bank voles in winter (Kostelecka-Myrcha, 1967). If this was true, then it can be assumed that the amount of Fe actually absorbed from the gastrointestinal tract was insufficient to fully meet the Fe requirements of voles. In April the concentration of Fe in the liver of voles was 2—3 times higher than in the animals caught in March. This indicated that the stores of Fe were increasing quickly in the liver of overwintered voles. This increase might be due to a very high concentration of Fe (650 μg/g) in the food consumed by voles in March. This high Fe level in the tissues of plants growing in an environment not contaminated with iron is found only in mosses (Czarnowska, 1980). It seems that in early spring the main component of food of bank voles is this group of plants, which is replaced later on by other herbaceous plants.

The obtained data show that the Fe supply status of bank voles is optimal for normal functioning of these rodents throughout the whole year. This is evidenced by the Fe content in food which exceded several times the amount recommended by AIN (1977) (about 35 μg/g), and by the concentration of this element in the examined tissues (kidneys, heart, muscles) which remained stable during the whole year.

3.3. Copper

The concentrations of Cu in food and in the examined organs of males and females, both sexually mature and immature, caught in any month were not significantly different ($p>0.05$). Therefore results were combined for all voles examined in various months.

The highest Cu level in food and in the liver, kidneys and heart was

observed in the animals caught in October and November (Fig. 1). In December the content of this mineral in the food and organs of the voles was several times smaller than that in the animals caught in October and November. In March the concentration of copper in food and tissues increased again. This concentration was stable until August, and in September a significant ($p<0.05$) decrease of Cu in food and liver was seen. The highest Cu level in the testes was found in voles caught in March (10.0 ± 0.8 μg/g) and April (7.2 ± 0.5 μg/g). From May to Sep-

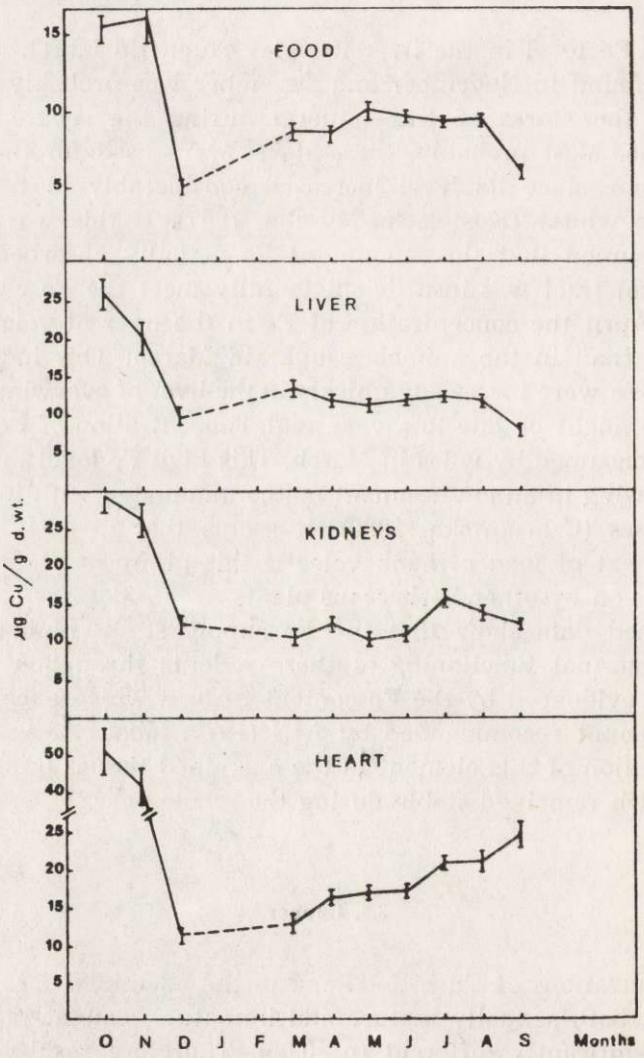

Fig. 1. Seasonal changes in copper concentrations of food and selected organs of bank voles (mean±SEM).

tember the concentration of Cu in this organ was stable (about 6.0 μg/g) but it was significantly lower ($p<0.05$) than in March. In the testes of males caught from October to December the concentration of Cu could not be determined since during that time the weight of both testes was at most 9 mg dry wt (Table 1).

Statistical analysis showed a positive linear correlation between the average Cu concentration in the food of bank voles, from 5.0 μg/g to 15.4 μg/g, and its average concentration in the liver ($y=0.28+1.68x$; $r=0.90$, $p<0.01$) kidneys ($y=1.15+1.62x$; $r=0.84$, $p<0.01$) and heart ($y=-5.7+3.14x$; $r=0.86$, $p<0.01$).

Cloutier *et al.* (1986) supposed that a similar relationship existed also in *Microtus pennsylvanicus* (Ord, 1815). On the other hand no such correlation was found in rats (Stuart & Johnson, 1986) in which, however, a positive linear correlation existed between the Cu level in the diet (from 2.5 μg/g to 20.0 μg/g) and its overall balance in these animals. This means that Cu is absorbed from the gastrointestinal tract of rats in amounts directly proportional to the Cu level in the diet but is deposited in tissues other than the liver, kidneys or heart. The above authors believe that in rats this element is incorporated mainly into muscles and bone. In light of these data one may assume that Cu absorption in various rodent species is linearly related to the Cu concentration in the diet, while species-specific differences exist in the mechanism of Cu distribution in the body.

Despite a significant seasonal variability of the Cu level in the food and tissues of bank voles it seems that the Cu supply status is optimal for normal functioning of these animals throughout the whole year. Data to date show that growth disturbances in rodents are observed only when the Cu level in the diet below is 2.5 μg/g (Kincaid & Carlton, 1982; Stuart & Johnson, 1986), while reproduction disturbances appear at Cu levels in the diet below 4.0 μg/g (Menino *et al.*, 1986).

3.4. Manganese

Mn concentration in food and in the liver and kidneys of bank voles also showed only a seasonal variability (Fig. 2). The highest Mn content in the food was found in December and March (over 300 μg/g) and the lowest in April and October, 86 and 65 μg/g respectively. In the remaining months the mean concentration of Mn in food was 110—184 μg/g. In the liver the highest Mn content was found in voles caught from October to March. In April the Mn level fell by one half in comparison to March, and after that a significant ($p<0.05$) rise of Mn concentration

was observed in the liver of the animals caught between May and September. In the kidneys three Mn levels were seen. The highest level (15—16 μg/g) was observed in October-December, the lowest (about 7 μg/g) in March-June. The mean level of Mn in the kidneys (12—13 μg/g) was found in voles caught from July to September. The Mn concentration in the testes of males caught between March and September was stable with a mean value of 7—8 μg/g dry wt. In the testes of males caught from October to December the concentration of this element was not determined due to their very small mass.

No correlation was observed between the Mn level in food and its

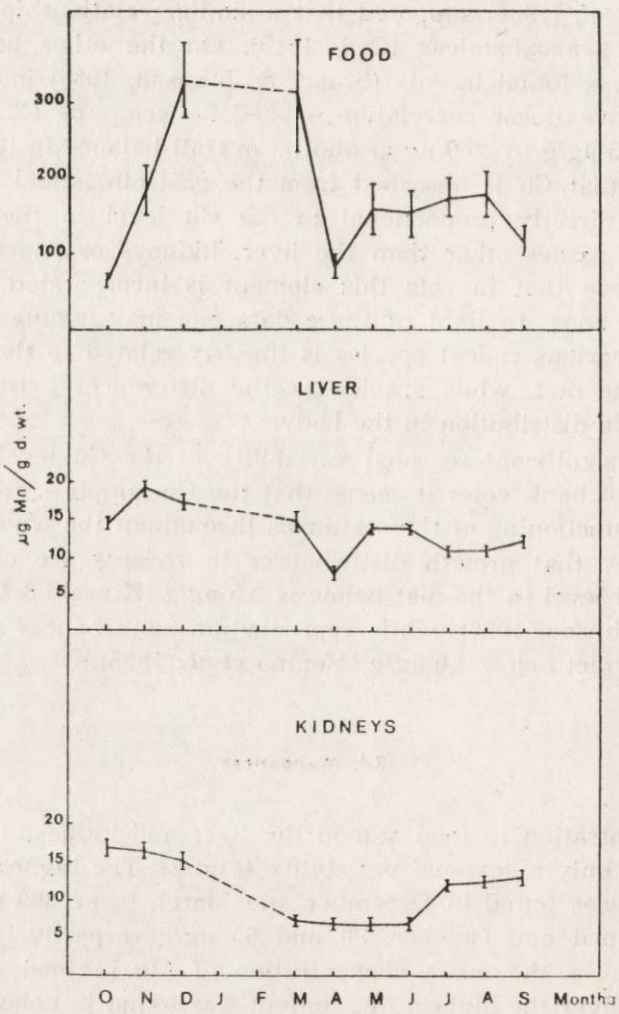

Fig. 2. Seasonal changes in manganese concentrations of food and selceted organs of bank voles (mean±SEM).

Table 6

Calcium and magnesium concentrations of food and various organs of bank voles (mean±SEM). nd — not detected. Mean values in rows with different superscript letters are significantly different (Duncan's multiple range test; $p < 0.05$).

Organ	Element	Winter Nov.-Dec. n=29	Early spring March-Apr. n=20	Late Spring May-Jun. n=14	Summer Jul.-Aug. n=22	Autumn Sep.-Oct. n=35
Food	Ca (%)	0.53±0.04^{a}	0.34±0.03^{b}	0.45±0.03^{a}	0.40±0.02^{ab}	0.21±0.01^{c}
	Mg (μg/g)	990±90^{a}	1340±140^{b}	1530±160^{b}	1100±100^{ab}	850±80^{a}
Liver	Ca (μg/g)	196±8^{a}	193±6^{a}	190±7^{a}	198±6^{a}	220±5^{a}
	Mg (μg/g)	950±35^{a}	900±33^{a}	870±25^{a}	940±30^{a}	940±32^{a}
Kidneys	Ca (μg/g)	207±5^{a}	193±9^{a}	222±11^{a}	230±6^{a}	230±7^{a}
	Mg (μg/g)	1020±34^{a}	1060±39^{a}	980±35^{a}	1030±36^{a}	1050±33^{a}
Heart	Ca (μg/g)	109±3^{a}	110±5^{a}	105±3^{a}	97±4^{a}	90±3^{a}
	Mg (μg/g)	1130±35^{a}	1180±40^{a}	1050±35^{a}	1150±37^{a}	1170±38^{a}
Testes	Ca (μg/g)	nd	250±12^{a}	238±10^{a}	220±9^{a}	200±15^{a}
	Mg (μg/g)	nd	1100±35^{a}	1160±39^{a}	1080±38^{a}	1100±40^{a}

concentration in the liver and kidneys of bank voles. These results confirm other research (Weigand *et al.*, 1986). Since the Mn level in the bank voles diet is without any significant effect on its tissue concentration, one may assume that the seasonal variability of Mn in the examined organs is related to other causes.

Mn stimulates cellular oxidation (Lindberg & Ernestr, 1954) and also participates as a component of one (mitochondrial) from of superoxide dysmutase (Dubick & Keen, 1983) in the intracellular system protecting against the toxic action of free oxide radicals (O_2^-) appearing during oxidation processes in the cells (Hiraishi *et al.*, 1987). In winter, *i.e.* at a low ambient temperature, the uptake of oxygen by voles is increased (Grodziński & Górecki, 1967) since this element is indispensable for the production of thermal energy. The high Mn level in the tissues of bank voles in winter (Fig. 2) may thus stimulate cellular oxidation and heat production on the one hand, and may participate in the detoxification of free radicals produced in this process on the other hand. This hypothesis requires verification in further studies.

Despite a significant seasonal variability of the Mn level in the food and tissues of bank voles it seems that the Mn supply status of the body is optimal for normal functioning of these rodents throughout the whole year. This was shown, by the level of this element in food which was in each month higher than the amount recommended by the American Institute of Nutrition (1977) (about 50 μg/g).

3.5. Calcium and Magnesium

In the food of bank voles only a seasonal variability of the concentrations of Ca and Mg was found (Table 6). The highest Ca level in the food was detected in winter (0.53%), the lowest in autumn (0.21%). The highest Mg content in the food of voles was found in spring. Its concentration in the food of voles caught in summer, autumn and winter was stable but was, however, significantly lower ($p<0.05$) than in spring.

Although the levels of Ca and Mg in the food of bank voles showed seasonal variability, the concentration of these elements in the liver, kidneys, heart and testes (excluding October-December period) was stable during all seasons (Table 6). These data point out that the Ca and Mg supply status of the body is maintained at a level indispensable for normal functioning of the bank voles throughout the whole year.

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STAN ZAOPATRZENIA DZIKO ŻYJĄCEJ NORNICY RUDEJ W CYNK, ŻELAZO, MIEDŹ, MANGAN, WAPŃ I MAGNEZ

Streszczenie

Zbadano zawartość Zn, Fe, Cu, Mn, Ca i Mg w pokarmie (treść żołądkowa) oraz w różnych tkankach i narządach nornicy rudej, *Clethrionomys glareolus* (Schreber, 1780), żyjącej w grądzie (Las Solnicki koło Białegostoku) w okresie zimy, wiosny, lata i jesieni.

Najwyższy poziom Zn w pokarmie stwierdzono od marca do czerwca (56—70 μg/g), natomiast od lipca do grudnia wynosił on 37—43 μg/g Zn/g (Tabela 2, 3). Stężenie Fe w pokarmie utrzymywało się na stałym poziomie (200—250 μg/g) we wszystkich sezonach, z wyjątkiem nornic odłowionych w marcu (650 μg Fe/g) (Tabela 5). Średnie stężenie Cu w pokarmie wahało się sezonowo od 5.0 μg/g do 15.4 μg/g (Ryc. 1), Mn od 65 μg/g do 330 μg/g (Ryc. 2), Ca od 0.21% do 0.53% a Mg od 850 μg/g do 1530 μg/g (Tabela 6).

Największej sezonowej i osobniczej zmienności podlegało stężenie Zn w wątrobie i kościach, natomiast w sercu, nerkach i mięśniach utrzymywało się ono na ogół na stałym poziomie (Tabela 2). Wiosną i jesienią poziom Zn w wątrobie i kościach osobników dojrzałych płciowo był istotnie wyższy w porównaniu z nornicami badanymi latem i zimą. Ponieważ stężenie Zn w kościach jest dobrym wskaźnikiem stanu zaopatrzenia całego organizmu w ten pierwiastek, dlatego też uzyskane dane wskazują, że stan zaopatrzenia nornic w Zn w okresie wiosny i jesieni jest utrzymywany na poziomie wyższym niż latem i zimą. U sześciu spośród czternastu osobników odłowionych w sierpniu i u jednego we wrześniu stwierdzono drastyczne (2—5 krotne) obniżenie koncentracji Zn we wszystkich tkankach i narządach (Tabela 4). Być może świadczy to o niedoborze Zn w organizmie tych siedmiu nornic.

Stężenie Fe w sercu, nerkach i mięśniach utrzymywało się na stałym poziomie u wszystkich nornic, natomiast koncentracja tego pierwiastka w wątrobie podlegała istotnej zmienności zależnie od sezonu, płci i stanu fizjologicznego zwierząt (Tabela 5).

Koncentracja Cu i Mn w badanych narządach podlegała jedynie sezonowej zmienności (Ryc. 1, 2). Stężenie Cu w wątrobie, nerkach i sercu było dodatnio skorelowane z zawartością tego metalu w pokarmie. Największe stężenie Mn w wątrobie i nerkach zanotowano zimą, najmniejsze zaś wiosną. Stężenie Ca i Mg w badanych narządach utrzymywało się na stałym poziomie we wszystkich sezonach (Tabela 6).

Uzyskane dane wskazują, że z wyjątkiem Zn, stan zaopatrzenia nornicy rudej w składniki mineralne jest utrzymywany na poziomie niezbędnym dla normalnego funkcjonowania tych gryzoni w ciągu całego roku.