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POSTNATAL CHANGES IN MINK (*Mustela vision*) MINERAL METABOLISM ASSESSED BY MICRO- AND MACROELEMENTS IN BLOOD AND FUR

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Abstract

Mineral deficiency remains relevant in fur farming. That is why researchers are still developing methods to test whether mineral supply of animals is sufficient. Fur, unlike blood which composition strongly depends on many factors, is convenient biomaterial to control mineral levels in animal body. In this paper, we revealed a relationship between the blood and hair mineral composition and studied for the first time whether these parameters reliably reflect mineral welfare of the standard male minks fed with commonly used diets. Standard male minks of Saltykovskii breeding farm (Moscow Province) were grouped by age. Male minks, due to sexual dimorphism, are twice as large as females and all changes in their body manifest more quickly and reliably. Blood and hair were sampled from healthy standard male minks during postnatal ontogenesis, i.e. in 30-day-old animals and in 90-day-old animals, additionally, hair samples were taken from male minks aged 2 months, 7 months and 12 months. Contents of macro- and microelements were measured by atomic emission and mass spectrometry using an optical emission spectrometer Optima 2000^{TM} DV and ELAN 9000 ICP-MS mass spectrometer (Hitachi, AIC, Inc, Japan). The skin development during ontogenesis was controlled histologically. Hair condition was studied by electronic scanning microscopy (Hitachi-S-520, Hitachi, AIC, Inc, Japan). It was found that reference contents of macro- and microelements in blood and hairs of standard male minks are characteristic of each phase of postnatal ontogenesis. In blood, the concentrations (mmol/l) averaged 1.6-4.2 for calcium, (2.7-9.9)×10^{-5} for cobalt, (0.23-1.00)×10^{-3} for chromium, (0.53-2.10)×10^{-2} for copper, 5.9-11.3 for iron, (1.5-6.3)×10^{-3} for iodine, 29-44 for potassium, 0.64-1.23 for magnesium, (0.35-2.70)×10^{-2} for manganese, 82-103 for sodium, 4.8-21 for phosphorus, (1.8-6.3)×10–3 for selenium, and 0.24-0.89 for zinc. The blood levels of all these elements were maximal in one-month and three-month old minks and minimal at sexual maturity and body maturation of the animals. In hairs, the average contents (mmol/kg) were 4.5-8.5 for calcium, (0.92-2.70)×10^{-4} for cobalt, (2.3-7.9)×10^{-3} for chromium, (0.20-0.73)×10^{-1} for copper, (1.02-4.10)×10^{-1} for iron, (1.58-7.20)×10^{-3} for iodine, 0.44-1.70 for potassium, 0.70-1.92 for magnesium, (0.38-1.70)×10^{-2} for manganese, 9.1-39 for sodium, 0.97-2.40 for phosphorus, (2.0-4.6)×10^{-3} for selenium, and 0.28-0.46 for zinc. Accumulation of the most important elements in the mink hair was maximal during 30 days of life and minimal in 7-month old animals. Mineral compositions of blood and hair of standard male minks correlate and depend on the animals' age. The strong and moderate (positive and negative) correlations are found for 8 elements, Ca, Mg, Na, P, Co, Cr, Se and I. For all 13 elements studied, there are reliable correlations between their levels in animal hairs and in the diets. Apparently, the mineral composition of hairs can be used as a test of dietary balance of mineral elements for each age of the standard mink.

Keywords: *Mustela vision*, mink, postnatal ontogenesis, mineral metabolism, mineral composition of blood, mineral composition of hair

Mineral substances enter animal body mainly with feed and are involved in numerous metabolic processes at different levels [1-5]. We would consider only some of main mineral elements (sodium, phosphorus, calcium, magnesium, iodine, potassium, chrome, cuprum, iron, cobalt, zinc, selenium, manganese) re-

quired for formation and maintenance of the pelage and skin structure and function in fur animals, which is important both for deepening of the basic knowledge about their mineral metabolism, as well as for practical work.

Various pathological processes occur in animal if mineral substances in feed are misbalanced or if their metabolism is disturbed. We would analyze only those that are accompanied by changes in dermal and hair coat. Interesting, many adverse environmental factors may not render significant effect on vital processes since animal body has physiological and biological mechanisms ensuring homeostasis provided the presence of all necessary bioactive and mineral components [6-10]. Growth, development, pelt size and quality are directly dependent on strict adherence to the feeding and housing conditions [11, 12], as metabolic processes, homeostasis, and functions of antioxidant system are influenced by these conditions [13-16]. Essential mineral substances which ensure metabolic reactions and state of skin and hair coat corresponding to animal age and season are needed in specific quantities. Comparison of the concentrations of mineral elements in blood, organs and tissues, and in feed mixtures reflects animal body supply with minerals [6, 7, 15, 17]. Unfortunately, number of publications on the topic with regard to fur animals is quiet small, although the issue on the root causes of mineral deficiencies and reliable methods for identification thereof is most acute in the global fur farming practice [6, 11, 18-20]. It could be assumed that the content of mineral components in the blood provides a more informative description of the state of mineral metabolism in animals, but this indicator is rather labile, depends on many factors and changes even daily. Therefore, more convenient and reliable estimates of mineral status are required to learn more about the character and relationship of metabolic processes in different organs and tissues. Such control is also important to ensure welfare and productive performance of the animals [19, 21, 22].

Changes in the mineral content during growth of fur animal and their hair coat reflect longstanding changes in mineral metabolism in the body, and, thus, hair could be conveniently used as a biomaterial for analysis and also, its removal would not cause stress in animal [19-22].

In present paper we have analyzed age-related dynamics of the quantitative changes in 13 most important micro- and macroelements and have assessed to what extent mineral composition of animal blood and hair coat objectively reflects whether mineral nutrition is enough. Our findings show that these indicators in standard minks are significantly interrelated with eight elements (sodium, phosphorus, calcium, magnesium, iodine, chrome, selenium, and cobalt) out of 13 studied and depend on the age of animals, whereas mineral concentration in hair coat and diet correlates for all 13 elements. Accordingly, mineral content of hair for each pubertal group of minks could be assessed as indicator indirectly characterizing quantity of mineral substances in their feed.

Purpose of our research was to compare age-related dynamics of morphological and biochemical indicators reflecting the state of mineral metabolism, upon analysis of blood, hair coat and skin samples in physiologically healthy minks during different periods of postnatal ontogenesis.

Techniques. Experiments were carried out in 2006-2014 (pedigree farm Saltykovskii, Balashikha District, Moscow Province) on physiologically healthy standard male minks. Groups of 5 animals each were formed for each age based on pair-analogues principle. Experimental animals were kept in sheds and fed depending on the age, size, and season [15, 16]. Feeding was of meat-and-fish type as recommended by Afanas'ev All-Russia Research Institute of Fur Animal and Rabbit Breeding (Moscow Province); mineral composition of the diets was analyzed according to the recommendations [16]. Physiological state of animals

was assessed by commonly accepted clinical methods (by body weight, quality of fur coat and blood morphological and biochemical parameters, i.e. hemoglobin, total protein, proteins of blood plasma and their fractions) [23].

Whole blood and hair were analyzed at transitional phase (age of 30 days), at natural alimentation phase (age of 90 days), and in animals aged 7 and 12 months. Additionally, hair samples were taken from animals aged 60 days; skin samples were analyzed at the same age. Blood was collected on empty stomach from the end of tail or finger by single-use syringes, then placed in sterile vials and kept at 0-4 °C for 3-5 days the most or at –18 °C in single-use polypropylene vials with air-proof covers. After slaughter, a 2 cm^2 skin cutout was taken from scrapings of ramp part of pelt (hair was pulled out) for histological study; hair samples from 1-month-old mink were collected from the whole pelt. Hairs for mineral and histological analysis were carefully decontaminated by washing in soap solution, triple rinse in distilled water, and drying on filter paper. Afterwards, hairs were passed through ethylic alcohol in ascending concentrations (50, 75, and 96 %), defatted by acetone (Chemme, Russia), rinsed in distilled (deionized) water and dried at of 60 °C. Until analysis, hair samples were kept in paper bags.

Upon studying the mineral composition of blood, hair, and diets [16], mineralization and decomposition of biomaterial was done according to applied methodologies [6, 16]. During the experiments, only biomaterial of animals with normal fur formation was used. Blood, hair, and diet samples were analyzed for 13 elements (Fe, I, K, Ca, Co, Mg, Mn, Cu, Na, Se, P, Cr, and Zn) by inductively coupled plasma mass spectrometry (a quadrupole mass-spectrometer ELAN 9000 ICP-MS, PerkinElmer, Inc., USA) and by atomic emission spectrometry (an Optima 2000^{TM} DV, PerkinEImer, Inc., USA). Manipulations (insertion of samples, measurements, and statistical processing of outcomes) were fully automated by WinLab32 software (PerkinElmer, Inc, USA) in OS Windows 2000.

In histological control of hair coat, guiding hair cuticle (bed and grain) was examined (an electronic scanning microscope Hitachi-S-520, Hitachi, AIC, Inc., Japan). After defattening, hair samples dried on filter paper were glued on copper plate with polystyrene, samples were 10 min vacuumed (at 4 mPa), followed by gold sputtering by an Eiko lB-3 Ion Coater (Eiko Engineering Co., Ltd., Japan). Morphology of hair cuticles was studied by scanning microscope Hitachi-S-520 (Hitachi, AIC, Inc., Japan; ×15-3000 magnification, 4 μm resolution), the results were documented [24].

Once collected, skin samples were placed for 1 day in 10 % formalin, followed by dehydration and defatting by G.A. Merkulov [25]. Longitudinal and transversal sections were prepared using microtome with freezing chamber (TOC-1, Russia), staining with hematoxylin and eosin and celloidin embedding were according to common methods. Preparations were viewed under microscope (BIOLAM, LOMO, Russia) at 10×10 magnification without filter and documented with SONY camera (Japan; 20 s hold, 0.2 diaphragm).

Five repeated measurements of sample (5 analytic repeats) were done at analysis of each element. Tables 1 and 2 contain means (M) and standard error of means (±SEM) (defined subject to GOST 8.207). Correlations were analyzed by t-Student criterion. Correlations deemed to be weak at $r < 0.3$ and $r > –0.3$, medium at $0.3 < r < 0.69$ and $–0.69 < r <– 0.3$, and high at $r > 0.69$ and $r < –0.69$. Medium and high correlations were statistically significant at $p < 0.001$, $p < 0.01$, and $p < 0.05$.

Results. Only mink males were used in the experiment since due to sex dimorphism they are twice bigger than females, and all changes in their body are more apparent and could be detected earlier. Hair sampling at ages of 30 days, 60 days, 90 days, 7 and 12 months was due to the fact that these periods

correspond to main morphophysiological stages in formation of organs, tissues, body systems, and hair coat [26].

Age-related changes in the mineral content of the blood in standard mink males. Mineral substances in the blood are the body resources used for growth and life activities. Blood levels of the mineral substances optimal for metabolism is maintained due to water-salt metabolism regulation mechanisms based on response generated in nervous center of hypothalamus in response to signals from vessel and tissue receptors [3, 27]. Homeostasis of chemical elements is maintained due to mineral substance release from a labile bound form and deposition in relevant organs (bone tissue, liver, muscles, spleen, skin, subcutaneous fibre, etc.) or by regulation of absorption at digestion and by excretion.

Neurohumoral regulation mechanisms of water-salt metabolism are imperfect in immature mink body at early postnatal and transitional phases. At this age, animals rapidly accumulate and loose water with mineral substances [7, 28, 29]. According to our studies (Table 1), blood concentrations of Na (97±7 μmol/l) and K (34±2 μmol/l), involved in intracellular metabolism, regulation of osmotic pressure, and protein synthesis [3, 28, 30] were higher during more intensive growth of standard minks (transitional phase, 1 month age). Besides, concentration of the most important intracellular macroelement magnesium (1.23±0.07 μmol/l, see Table 1), a cofactor of oxidation phosphorilation enzyme, which is also involved in protein biosynthesis, carbohydrate and nucleic acid metabolism [3, 30], is maximum in the blood of 1 month aged minks (as compared to other ages). Evidently, this level of magnesium in 1-month old animals is required for intensive metabolism during intensive growth. Further formation and maturing of organs and tissues were accompanied by decrease in blood magnesium, whereas content of potassium and sodium was high (see Table 1) and slightly changed with age.

1. Concentration (μmol/l) **of macro- and microelements in blood of standard mink males (*Mustela vison*) of different age** (*M*±SEM, pedigree farm Saltykovskii, Balashikha District, Moscow Region)

Element	Age, months			
	1 (n = 25)	3 (n = 25)[a]	7 (n = 25)[b]	12 (n = 25)[c]
Ca	3,5±0,4	4.2±0.5$^{\#}$	1.6±0.2***,***	2.0±0.3$^{\#}$,***,**
Co	(9.9±0.5)×10^{-5}	(4.5±0.2)×10^{-5}***	(2.7±0.2)×10^{-5}***,***	(8.6±0.4)×10^{-5}***,***,$^{\#}$
Cr	(3.9±0.4)×10^{-4}	(10.0±1.0)×10^{-4}***	(2.3±0.2)×10^{-4}***,**	(3.7±0.3)×10^{-4}***,***,$^{\#}$
Cu	(12.3±0.7)×10^{-3}	(21.0±1.0)×10^{-3}***	(8.3±0.6)×10^{-3}*,$^{\#}$	(5.3±0.3)×10^{-3}***,***,***
Fe	7.9±0.5	11.3±0.7***	7.8±0.5***, $^{\#}$	5.9±0.4**,***,**
I	(3.9±0.4)×10^{-3}	(6.3±0.5)×10^{-3}**	(2.1±0.2)×10^{-3}***,***	(1.5±0.2)×10^{-3}*,***,***
K	34±2	36±2$^{\#}$	44±3*,*	29±2***,*,$^{\#}$
Mg	1.23±0.07	0.64±0.04***	0.67±0.04$^{\#}$,***	0.70±0.03$^{\#}$,$^{\#}$,***
Mn	(11.4±0.7)×10^{-3}	(9.3±0.6)×10^{-3}*	(3.5±0.2)×10^{-3}***,***	(27.0±2.0)×10^{-3}***,***,***
Na	91±7	82±7$^{\#}$	103±7*,$^{\#}$	94±7$^{\#}$,$^{\#}$,$^{\#}$
P	21±2	11±1***	18±2**,$^{\#}$	4.8±0.5***,***,***
Se	(4.8±0.5)×10^{-3}	(6.3±0.6)×10^{-3}*	(2.8±0.3)×10^{-3}***,**	(1.8±0.2)×10^{-3}*,***,***
Zn	0.24±0.02	0.89±0.06***	0.58±0.04***,***	0.44±0.03*,***,***

N o t e. [a] — difference between 3 months and 1 month; [b] — between 7 months and 1 months, 7 month and 3 month; [c] — between 12 months and 1 month, 12 months and 3 months, 12 months and 7 months.
*, **, *** Differences are statistically significant at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively; $^{\#}$ — unreliable differences.

Body weight increases with linear growth of minks. In this, mineral substances serve as construction material [29, 31] which is required for intensive development of skeleton, muscles and teeth [20] terminated by transition to definitive diet [32]. Our research showed that blood concentration of Ca in minks aged 3 months, and concentration of P and Mg at age of 1 month were maximum. Higher Ca (3.5-4.2 μmol/l), P (11-21 μmol/l) and Mg (0.64-1.23 μmol/l) values at age of less than 3 months are, evidently, also explained by the fact that these elements as a part of muscle tissue are required for more intensive development of young animals. The fact that blood levels of Mg, Ca,

Na and K ensuring functioning of muscle and nervous tissues at transitional phase and by beginning of natural nutrition was high is possibly related to more intensive formation and development of these functional systems during early ontogenesis [33]. By 3 months blood concentration of zinc also increased (from 0.24 to 0.89 μmol/l) stimulating (along with macroelements) formation of bones [21, 34], and of copper (from 0.0123 to 0.021 μmol/l) which is required for osteogenesis and activity of osteoblasts. During the intensive growth, the need for such elements is very high [21, 29, 34].

Coordination of metabolic processes is due to participation of the central nervous system [27] via control of synthesis and penetration of hormones, containing macro- and microelements, into the blood. In our research, upon active growth all mineral elements (except for manganese) (see Table 1) increased in whole blood until 3 months of age as compared to other age groups. Our results are coherent with the fact that intensive protein metabolism (mostly its anabolic phase) is characteristic of this period, with positive balance of nitrogen due to Na- and K-containing enzymes.

Upon formation of organs, tissues, and functional systems, the intensive metabolism needs compounds containing cobalt, the essential element catalyzing transamination and synthesis of amino acids for structural, transport, receptor proteins, hormones, enzymes, etc., required for growing body of minks aged from 1 to 3 months [14, 18, 37]. Transaminases, the aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are especially active in minks during growth and development period [36]. We believe that total protein in blood serum during intensified growth and development of minks increases sufficiently not only due to Co reaching $(0.045\text{-}0.099)\times10^{-3}$ μmol/l, but also due to Cu (0,0123-0,021 μmol/l), since hematopoietic processes and metabolism of many proteins require these elements [18, 37].

The need for metabolic energy changes during postnatal development. This is mainly influenced by animal age, metric parameters (body weight and size), seasonality and physiological state (sexual behavior, pregnancy, parturition, etc.). Earlier development of minks (from birth to 3 months) is accompanied by great energy consumption and, possibly, the need for Cu, Fe, Mn, and Zn, which are related to enzymes of oxidation metabolism [29]. Apparently, this explains significant blood levels of cooper, manganese, and iron, as components of enzymes performing tissue respiration, redox reactions, and also as factors of stimulation of hematopoiesis [29]. Our research showed that blood levels of zinc, chrome, iodine, and selenium required for 3-month aged typical minks significantly exceed the values in 1-month aged animals and is maximum compared to other age groups. We explain this by improvement of digestion, immune, endocrine, and central nervous systems. By the 3 month age, standard young minks reach the sizes of adult animal, which results in the need for iodine, the microelement in charge for main types of metabolism and homeostasis, as well as for selenium which, being part of selenium-proteins, increases immunoreactivity [26, 27, 29]. Enzymes including zinc and chrome are important for metabolism of fatty acids and fat utilization [22, 29]. It should be noted that the need for fat in early phases of minks' postnatal ontogenesis is especially high since fat serves the source of linolic acid required for structure formation and functioning of organs and tissues [22]. Also, Fe-, Cu- and Zn-binding oxidoreductases participate in carbohydrate and fat metabolism closely related to protein metabolism. The same elements play an important role in synthesis of glycoproteins and mucopolysaccharides required for formation of connective tissue. All these explain the highest blood concentration of Fe, Cu, and Zn in 3-month aged young minks.

Hormonal function of testicles is intensified at pubescence phase of males (7-8 months), which is accompanied by growth of blood concentration of sex hormones (progesterone, estradiol, and testosterone) [38]. Our research shows that blood level of manganese, iodine, and zinc in 7-month aged minks is low compared to 1 and 3-month aged minks. By this age, growth decelerates, metabolic pathways mainly become rather stable, all organs and tissues, as well as winter hair coat, have fully completed their formation. Apparently, the need for such elements decreases. At the same time, the need in iodine for function of thyroid hormones which ensure timely pubescence of young animals, in manganese as an activator of many enzymes on which function reproductive organs depend, and in zinc involved in production of male sex hormone testosterone and maintenance of reproductive function is associated with intensive establishment of reproductive system [16, 19, 38].

In minks, metabolism decreases during autumn and winter [16, 18] because of its seasonal nature in fur animals and dependence on duration of the light day [35].

At 7-12-month aged minks, intensity of digestion, metabolism and energy exchange, load on circulatory and respiratory systems and kidneys decreases due to termination of formation of organs and regulation mechanisms [18, 19]. However, blood concentration of copper, $(8.3\pm0.6)\times10^{-3}$ μmol/l, and zinc, 0.58±0.04 μmol/l, remains high to support functioning of skin and hair coat (formation of skin and hair pigments, synthesis of keratin and collagen) [16], while levels of other elements were significantly lowered or became minimal as in case of Ca, Co, Cr (see Table 1). Fur shedding occurs during the maturity phase (12-month age). Apparently, mink body does not any more need such amounts of minerals as during intensive growth, and concentration of all micro- and macroelements (except for Zn, Co, and Mg) becomes minimal or slightly differs from indicators during pubescence period (7-month age) (see Table 1).

Thus, reference blood concentrations of mineral elements are stable at each age of standard minks. Correlation analysis shows that mineral content of blood and diets used for each age of typical minks [16, 39] are interrelated. We have got the following correlation coefficients (r): K — 0.19^a, Na — 0.78^c, I — -0.48^b, Ca — 0.59^b, Mg — 0.83^c, Cu — -0.26^a, Zn — 0.65^b, Co — 0.21^a, Mn — -0.17^a, Fe — -0.62^b, Cr — -0.55^b, Se — 0.82^c, P — 0.56^b; at $r < 0.3$ and $r > -0.3$ the correlation is weak (a), at $0.3 < r < 0.69$ and $-0.69 < r < -0.3$ it is medium (b), and at $r > 0.69$ and $r < -0.69$ high (c); correlation coefficients marked by b and c letters are statistically significant ($p < 0.05$; $p < 0.01$ or $p < 0.001$). Although, medium and high correlations were generally detected for nine elements (P, Mg, Na, Ca, Fe, Cr, Zn, I, and Se), with close positive and negative correlations observed only for several elements (Mg, Na, and Se). Thus, we believe that blood mineral composition suits to assess whether the diet provides animal mineral needs.

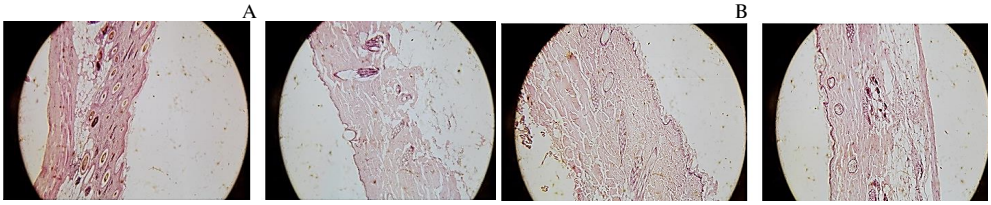


Fig. 1. Skin of a standard mink (*Mustela vison*) male: A — longitudinal sections, 1-month age (left) and 3-month age (right); B — transversal sections, age 7-month age (left) and 12-month age (right). H&E staining, ×100 magnification.

Age-related changes of mineral composition of hair coat

in standard minks. Morphostructure of skin and its derivatives shows (Fig. 1-3) that used feeding and housing provided skin and hair coat formation during postnatal ontogenesis without pathologies.

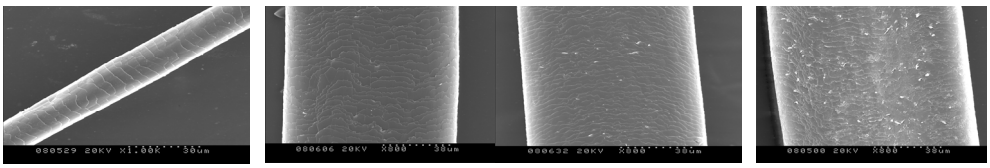
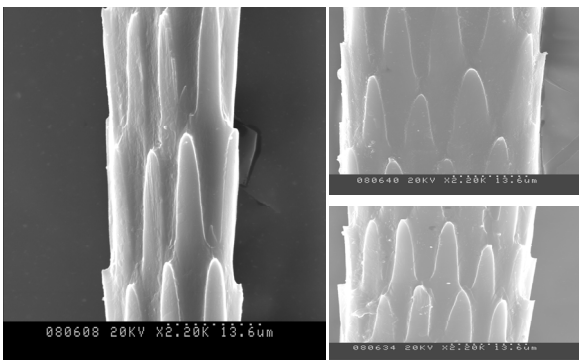


Fig. 2. Histological structure of grain in directional hair of standard mink (*Mustela vison*) males aged 1 month, 3 months, 7 months and 12 months (from the left to right). Scanning electron microscopy (Hitachi-S-520, Hitachi, AIC, Inc., Japan; ×800 magnification).



Fug. 3. Histological structure of bed of directional hair in standard mink (*Mustela vison*) males aged 3 months (left), **7 and 12 months** (right up and right down). Scanning electron microscopy (Hitachi-S-520, Hitachi, AIC, Inc., Japan; ×2200 magnification).

We had established mineral composition of hair coat for each age group of standard minks (Table 2).

2. Concentration (μmol/l) **of macro- and microelements in hair coat of standard mink (*Mustela vison*) males of different age** (*M*±SEM, pedigree farm Saltykovskii, Balashikha District, Moscow Region)

Element	Age, months				
	1 (n = 25)	2 (n = 25[a]	3 (n = 25)[b]	7 (n = 25)[c]	12 (n = 25)[d]
Ca	8.5±0.8	7.8±0.8[#]	7.0±0.5[#],[#]	7.0±1.0[#],[#],[#]	4.5±0.5[#],*,**,**
Co	(2.7±0.4)×10^{-4}	(2.4±0.2)×10^{-4}[#]	(1.01±0.12)×10^{-4}***,***	(1.19±0.12)×10^{-4}[#],*,**	(0.92±0.05)×10^{-4}*,***,***,[#]
Cr	(7.9±0.6)×10^{-3}	(5.0±0.4)×10^{-3}***	(2.3±0.2)×10^{-3}***,***	(3.8±0.4)×10^{-3}**,***,*	(2.5±0.2)×10^{-3}**,[#],***,***
Cu	0.073±0.05	0.042±0.03***	0.023±0.002***,***	0.031±0.004*,***,***	0.020±0.002**,[#],***,***
Fe	0.41±0.04	0.30±0.02***	0.102±0.008***,***	0.104±0.008[#],***,***	0.148±0.009**,***,***,***
I	(7.2±0.5)×10^{-3}	(2.7±0.2)×10^{-3}***	(1.6±0.1)×10^{-3}***,***	(25.2±0.2)×10^{-3}**,[#],***	(7.1±0.6)×10^{-3}***,***,***,[#]
K	1.7±0.3	1.03±0.18*	0.87±0.05[#],**	0.74±0.13[#],**,[#]	0.44±0.08*,***,**,***
Mg	1.92±0.17	1.46±0.08*	0.77±0.03***,***	0.70±0.03[#],***,***	0.88±0.08*,[#],***,***
Mn	(17.0±1.0)×10^{-3}	(10.4±0.7)×10^{-3}***	(6.9±0.5)×10^{-3}***,***	(3.8±0.4)×10^{-3}***,***,***	(5.3±0.5)×10^{-3}*,***,***,*
Na	39±3	32±2*	28±3[#],**	9.1±0.4***,***,***	12±2[#],*,***,***
P	2.4±0.4	2.4±0.4[#]	2.3±0.4[#],[#]	0.97±0.17**,**,**	1.1±0.2[#],*,*,*
Se	(22.2±1.3)×10^{-4}	(46.0±5.0)×10^{-4}***	(24.0±3.0)×10^{-4}***,[#]	(20.0±3.0)×10^{-4}[#],[#],***	(20.1±1.3)×10^{-4}[#],[#],***,[#]
Zn	0.369±0.015	0.35±0.03[#]	0.369±0.015[#],[#]	0.46±0.03*,*,*	0.277±0.015***,***,**,**

N o t e . [a] — differences between 2 months and 1 month; [b] — between 3 months and 2 months, 3 months and 1 month; [c] — between 7 months and 3 months, 7 months and 2 months, 7 months and 1 month; [d]— between 12 months and 7 months, 12 months and 3 months, 12 months and 2 months, 12 months and 1 month.

*, **, *** Differences are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; [#] — unreliable differences.

Thus, levels of most macro- and microelements in hair were maximum in minks aged 1, 2, and 3 months, when primary and secondary hair coat is formed (see Fig. 1). Our histological study shows highly developed fat tissue in derma (see Fig. 1, light field), which, due to morphological immaturity of skin, serves as depot of required elements, including minerals. Hair follicles (bulbar partы) are immersed in fat tissue. In 1 month old animals, histostructure of bed cuticle and grain of directional hair, which has yet been formed during embryonal ontogenesis, differed from other age groups (see Fig. 2, 3) with no defects of scales. Starting from month 3, cuticle structure was slightly changing with ageing, with no defects in scale layer too (see Fig. 2, 3). Morphological and histological studies showed great changes in skin and hair coat by 3 months (see Fig.

1). Because of intensive growth of the secondary hair coat, shedding of the primary coat and the beginning of winter hair coat formation, derma becomes thicken, depth of follicles of the secondary and winter hair coats changes (see Fig. 1). The main dermal part is reticulate structures where hair coat formation processes actively occur. Herewith, fat tissue decreases in the skin with observed dense network of blood vessels transporting nutrition substance, including minerals, to cells, cellular components of derma become numerous. These morpho-physiological changes are accompanied by a decrease in the number of all elements (except zinc) in hair coat (differences are statistically insignificant for sodium and phosphorus in 2- and 3-month aged animals).

In minks aged 7 months, hair coat was matured and skin was transferred to rest state. Depth of epidermis, derma, size of hair follicles and their depth during the period of termination of growth and maturation of winter hair coat decreased, whereas number of hairs per bundle increased reaching the maximum at complete maturity of winter hair coat. At that, depth of derma became minimal, it had well developed papillary layer (see Fig. 1).

The mineral state changed with morphophysiolgical formation of hair coat, i.e. chrome, cuprum, iodine, and zinc increased and manganese, sodium, and phosphorus decreased, with insignificant changes for other elements. Physiological maturation in minks terminates at age of 12 months, which coincides with spring shedding. At that, the number of hair follicle per bundle and width of the secondary and primary follicles increase, and derma becomes loose and thick (see Fig. 1). Change of dermal function during spring shedding was accompanied by changing mineral state of hair: Mg, I, Fe, and Mn concentrations grew, Zn, Ca, Cr, Co, K, and Cu significantly decreased, and other elements changed insignificantly (see Table 2). Correlation analysis revealed positive and negative high and medium correlations between the mineral composition of blood and hair coat (r_1) for eight elements (Na, P, Ca, Mg, I, Cr, Se, and Co). Mineral composition of the diets and hair in minks was interrelated whatever the age. At that, for 13 studied micro- and macroelements correlations (r_2) were close and medium (positive and negative):

	K	Na	I	Ca	Mg	Cu	Zn	Co	Mn	Fe	Cr	Se	P
r_1	0.19^a	-0.88^c	-0.55^b	0.67^b	0.93^c	0.21^a	0.20^a	0.92^c	-0.11^a	-0.17^a	-0.58^b	0.78^c	0.67^b
r_2	$0.83^в$	0.85^c	0.74^c	0.97^c	0.98^c	0.77^c	0.83^c	0.83^c	0.72^c	0.92^c	0.72^c	0.62^b	0.76^c

N o t e. Correlation coefficients (r) are provided for blood and hair coat concentrations (r_1) and for hair coat and diet concentrations (r_2). At $r < 0.3$ and $r > -0.3$ correlations are weak (a), at $0.3 < r < 0.69$ and $-0.69 < r < -0.3$ they are medium (b), and at $r > 0.69$ and $r < -0.69$ they are strong (c). Correlation coefficients marked by b and c letters are statistically significant ($p < 0.05$; $p < 0.01$ or $p < 0.001$).

It is known that distribution of mineral elements over organs and tissues of animals is strictly determined and depends on their age and type, and deviation of these indicators from the normal values evidences on disturbance of the mineral metabolism [7, 16, 39]. Usually, defects of hair coat (dull hair, changed pigmentation, hair breakage, loss, etc.), keratosis, dermatitis, wetting evidence on disturbances of mineral metabolism [6, 16-18]. Our research had shown the importance of control over the mineral balance of the mink's hair coat and diets.

Therefore, our findings provide certain levels of K, Ca, Mg, Na, P, Co, Cu, Cr, Fe, I, Mn, Se, and Z in hair coat and whole blood, which are characteristic of each age of typical minks. Content of all macro- and micro elements in whole blood is maximum in 1 and 3 month aged animals, while minimum in 7- and 12-month aged animals. One-month old animals show the highest levels of elements in hair coat, with minimum in 7-month aged animals. There are correlations between blood and hair mineral compositions for Na, I, Ca, Mg, Co, Cr, Se and P. In minks mineral compositions of hair and diets correlate for

all 13 studied micro- and macroelements. We believe that this could be an indicator to assess feed mineral balance to provide physiological needs of animals during different age-related periods.

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