Efficiency and safety of heavy metals in animal nutrition

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Abstract. The current ecological situation in large industrial cities is characterized by a negative impact on all elements of ecosystems, among which heavy metals are priority. The purpose of this study was to assess the effect of manganese load on the body of laboratory animals. The study was performed on female rats of mature age (3 months) weighing 200±10 g (n=18). The study evaluated the oral administration of manganese sulfate for 28 days using the behavioral and cognitive functions of laboratory animals using tests "Open field", "Light-dark transition test" and the installation "Water maze test". In animals, there was a decrease in interest in examining holes, an increase in acts of defecation and grooming, and a manifestation of passive fear in the new environment. The results of the "Water maze test" showed violations of spatial memory and the learning process in animals receiving manganese sulfate. It can be concluded that the salt of manganese sulfate has a negative effect on the central nervous system of animals.

1. Introduction
The current ecological situation in large industrial cities is characterized by a negative impact on all elements of ecosystems, among which heavy metals are priority. According to a number of scientists, pollution of the environment with heavy metals, given their prevalence and toxicity, is a great danger in comparison with other agents of industrial emissions. It is worth pointing out that metals play an important role in humans and animals, participating in the regulation of the antioxidant system, transmission of nerve impulses, gene expression and much more [1, 2]. However, their excessive exposure can have a toxic effect as a result of oxidative stress, disruption of the normal functioning of mitochondria, and disruption of the activity of many enzymes [3, 4]. One such metal is manganese.

On the one hand, manganese is a vital trace element that is involved in various enzymatic and cellular processes and is necessary for normal growth and development of the body [5]. However, on the other hand, manganese also has a toxic effect at high concentrations [6]. The toxicity is due to its widespread use in many industries, including mining, welding enterprises, production of steel, batteries, ceramics and glass, where workers are mainly exposed to manganese [10, 11]. In addition, it is reported that many food products, including baby food (infant formula), contain high doses of manganese, which have a negative impact on public health [14]. It has also been reported that people are at a higher risk of Mn intoxication when receiving general parenteral nutrition, especially if they additionally suffer from chronic liver failure [15].

According to epidemiological data, manganese may have a neurotoxic effect, especially among young populations [7–9]. It was reported that the central nervous system is the main target for manganese, which mainly accumulates in the basal ganglia, especially in the striatum, globus pallidus...
and black substance [12]. According to an analysis of the literature, chronic exposure to high doses of Mn leads to manganism, a disease accompanied by disorders resembling symptoms of Parkinson's disease [13].

In this regard, the aim of the study was to assess the effect of manganese load on the body of laboratory animals, namely on behavioral reactions.

2. Materials and methods

Experimental studies were conducted on laboratory animals in an experimental biological clinic on Wistar rats. The animals were kept on a standard diet in the form of granular feed, with free access to water and food, at a temperature of 22 ± 10 °C in plastic cages with litter of wood sawdust under artificial lighting (12-hour light day) and supply and exhaust ventilation.

The study on rats was aimed at identifying the psychophysiological and cognitive characteristics of animals with the phenomenon of "loaded metabolism". This study was performed on female adult rats (3 months) weighing 200 ± 10 g (n = 18).

Two groups were formed: control (n = 9) – oral administration of water for injection; experimental I (n = 9) – oral administration of manganese sulfate (MnSO₄) at a dose of 1433 mg/kg/day. The drug was administered using an intragastric probe for rats weighing 120–200 g (length – 7.5 cm; tip diameter – 2.25 mm (with a curved shape); size (G) – 18) for 28 days [18].

After 4 weeks of feeding, a study was conducted of the animal’s behavioral reactions, including the assessment of the emotional, motor and orientation research activity of animals using the tests "Open field", "Light-dark transition test" and the assessment of the cognitive functions of laboratory animals using the installation "Water maze test". The break between the tests was 1 week.

Processing of the obtained data was carried out using methods of variation statistics using the statistical package “StatSoft STATISTICA 10”. Storage of the research results and primary processing of the material was carried out in the original Microsoft Excel 2010 database. Verification of the compliance of the obtained data with the normal distribution law was carried out using the Kolmogorov consent criterion. The hypothesis that the data belong to the normal distribution was rejected in all cases with a probability of 95 %, which justified the use of nonparametric procedures for processing statistical aggregates (Mann-Whitney U-test). The data obtained are presented as the median (Me) and the 25th–75th quartile (Q₂₅–Q₇₅).

3. Results

At the end of the research phase, it was found that all laboratory animals survived until the end of the experiment period and did not show an obvious pathology signal (refusal of food or water).

Behavioral reactions were evaluated using the “Open field test”, which recorded horizontal locomotor activity, vertical locomotor activity, the number of peeks into field openings, the number of fecal boluses and grooming.

In the experimental group, there was a significant decrease in interest in inspection of holes 1.5 times (p <0.05) and an increase in the number of fecal boluses 3 times (p<0.01). The grooming indicator (short) was significantly reduced in the experiment 3 times (p<0.01).

To increase the reliability of the results obtained using the "Open field test", other installations were used, one of which was the “Light-dark transition test”, in which the time spent by the animal in the dark and light parts of the camera, as well as the number of peeks from the dark compartment to the light, were estimated.

A comparative analysis of the behavior of rats in this test revealed the following features. The animals of the control group remained within the space of the dark part of the chamber during the entire testing period, which is an environmentally characteristic form of protective behavior for rodents [16]. Significant periods of stay in the bright compartment were noted for the experimental group – the longest time spent in the bright compartment was reliably recorded.

Testing animals for 4 days showed that the ability to learn (when swimming in the pool) was weakened in the experimental group. The time for finding the platform in the experimental group was
significantly higher 1.3 times relative to the control on days 1, 2 and 3; 1.7 times on 4 days. Thus, the test results showed that the animals that were injected with manganese sulfate needed the greatest amount of time to find the platform, and, therefore, their path was longer than in the control group.

After several days of training, we tested the spatial memory by removing the platform from the target sector of the basin. It was noted that the animals of the experimental group remember the location of the platform worse and wander around the entire area of the pool, in contrast to the control group, the animals of which throughout the test almost did not swim outside the sector in which the platform was previously installed.

4. Discussion
General motor activity is a behavioral marker of excitation or inhibition of the central nervous system. Grooming and defecation are indicators of the autonomic nervous system excitement, and the number of holes examined reflects an orientational-research reaction [19]. Following from the above, a decrease in interest in examining holes, an increase in bowel movements and grooming indicates a manifestation of passive fear in the new environment. Single exits to the central part of the field indicate an insignificant manifestation of passive-defensive behavior [20].

For rodents, it is not typical to be in a lit compartment, therefore, their prolonged presence in this part of the chamber is considered as a violation of behavior. The interval of time spent in the dark compartment correlates with the level of anxiety, while the number of exits and the time of examination of the lit compartment are indicators of risk aversion and research activity. Thus, with a low level of anxiety, the number of transitions between the dark and light compartments of the camera in the experimental groups increases. A comparative analysis of the behavior of rats in this test revealed a relatively low level of anxiety in the animals of the experimental group, which also indicates the anxiolytic effect of salts of heavy metals.

The results of the “Water maze test” showed impaired spatial memory and learning in animals treated with manganese sulfate.

5. Conclusion
Summing up the results of behavioral testing, we can conclude that the manganese sulfate salt has a negative effect on the central nervous system of animals.

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