Milk productivity of cows and the content of the metal-ligand forms of iron

T V Kazakova¹, O V Marshinskaia¹ and S V Notova¹,²
¹ Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, 29, 9 Yanvarya, st., Orenburg, Russian Federation
² Orenburg State University, 13, Pobedy av., Orenburg, Russian Federation

E-mail: vaisvais13@mail.ru

Abstract. The milk productivity of animals was evaluated and the content of the metal-ligand forms of iron was studied against the background of changes in the total content of manganese in the blood serum of dairy cows using the hybrid technique (HPLC-ICP-MS). For this purpose, groups were formed for comparing animals with low-normal values of manganese (Mn concentration up to the 25th percentile) and high-normal values (Mn concentration above the 75th percentile) according to the results of a previous study. The Mn content in the blood serum of all animals was within the physiological normal range. Analysis of the data obtained showed that high-normal levels of manganese in the blood were accompanied by a significant decrease in the indicators of milk productivity of cows. The metal-ligand forms of iron have undergone significant changes. Changes in metal ligand homeostasis can be considered early biomarkers for predicting the state of mineral metabolism to increase productivity.

1. Introduction
Chemical analysis of the spectrum of metal-ligand forms is a well-proven tool for studying the biological role and metabolism of trace elements in general [1]. The biological functions of each specific metal in vivo are mediated by its interaction with certain proteins during absorption, transport, and accumulation. For instance, it is known that a decrease in the copper-binding capacity of ceruloplasmin is associated with a higher toxicity of copper [2]. In that way, the patterns of copper binding to biological ligands can be used to predict copper-dependent pathology in ruminants and humans [3]. In this regard, the assessment of specific metal-binding species in blood serum is a valuable tool for assessing the nutritional or toxicological effects of a metal [4].

Despite the fact that the specific mechanisms of metal turnover in cattle have been studied, there are no data on the specific distribution of iron-binding species in the blood serum of cattle [5, 6]. There are only a few studies aimed at the speciation of metals [7]. The features of binding of iron in blood serum with high-molecular and low-molecular ligands in ruminants have not been studied.

For this purpose, the metal-ligand homeostasis was evaluated, the content of the metal-ligand forms of iron was studied against the background of changes in the gross content of manganese in the blood serum of dairy cows using the hybrid technique HPLC-ICP-MS.
2. Materials and methods

2.1. The object of the study. Holstein cows

Maintenance and experimental studies on animals were carried out in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (National Standard of the Russian Federation GOST R 53434-2009), as well as in accordance with the recommendations “The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)”. Efforts have been made in the studies to minimize animal suffering and reduce the number of samples used.

The experimental design was approved by the local ethical committee of the Federal Scientific Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences (No. 4 dated 02/05/2019).

2.2. Experiment scheme

The studies were carried out on a model of Holstein cows (n = 80) of the SPK PKZ Vologodskiy (Vologda region, Russia). Live weight of animals is 610-640 kg, age is 4-6 years.

Sampling and examination of milk samples. Samples of raw milk were taken individually from each cow three times a day at each milking, placed in sterile containers, cooled to 5 ° C and sent for analysis to the milk quality control laboratory of the Federal Scientific Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences (Russia, Orenburg).

The fat and protein content of milk was assessed using the FIL-IDF procedure on a MilkoScan ™ FT1 instrument (Foss Electric, DK-3400, Hillerød, Denmark).

2.3. Sampling and examination of blood serum samples

Blood samples were taken from each cow (the day after milk sampling) from the tail vein (by the veterinarian) into a vacuum tube, followed by separation of the blood serum. Sample preparation of serum samples was carried out by ashing biosubstrates using a microwave decomposition system MD-2000 (USA) with subsequent determination of chemical elements in the laboratory of ANO “Center for Biotic Medicine” (Moscow). Evaluation of manganese content and analysis of iron forms was carried out by separating blood serum into fractions with subsequent determination of the content of metals in each fraction by ICP-MS (on-line). ICP-MS NexION 300D was used as an element-specific detector to determine the forms of manganese binding in blood serum.

The earlier assessment of the elemental status by the chemical composition of blood serum allowed us to divide the animals into groups depending on the level of manganese accumulation. The Mn content in the blood serum of all animals was within the physiological normal range. Groups were formed for comparing animals with low-normal values of manganese (Mn concentration up to the 25th percentile) and high-normal values (Mn concentration above the 75th percentile) according to the results of a previous study. In the formed groups, the productivity indicators and the results of the speciation analysis were compared.

2.4. Statistical processing

The data obtained were processed using the methods of variation statistics using the statistical package “StatSoft STATISTICA 10”. Storage of the research results and primary processing of the material were carried out in the original database “Microsoft Excel 2010”. The verification of the compliance of the obtained data with the normal distribution law was carried out using the Kolmogorov agreement criterion. The hypothesis that the data belongs 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 (the Mann-Whitney U-test). The data obtained are presented as the median (Me) and the 25-75 quartile (Q25-Q75).
3. Results
Data analysis showed that animals with high-normal values had a significant decrease (p<0.05) in productivity indicators. The average daily milk yield decreased by 1.5 %, the percentage of fat and protein by 1.2 % (table 1).

Table 1. Indicators of milk productivity of black-and-white cows depending on the level of serum manganese, Me (q25–q75).

| Indicators           | I group (Low-normal values of Mn) | II group (High-normal Mn values) |
|----------------------|----------------------------------|----------------------------------|
| The average daily milk yield, kg | 47.8 (46.2–48.9) | 36.93 (34.58–39.75) $^a$ |
| Yield of fat, kg/day  | 4.2 (4.19–4.35)     | 3.12 (3.06–3.84) $^a$         |
| Yield of protein, kg/day | 3.71 (3.66–3.98)  | 2.76 (2.50–2.18) $^a$       |

$^a$ – at p<0.05 when comparing group I with group II.

In groups with different levels of manganese, changes in the iron content in the fractions were observed (figure 1).

![Figure 1. Specification analysis of the absolute content of iron forms in the blood serum of cattle depending on the level of manganese accumulation in the blood, µg/ml. * – significant difference between groups, at p <0.05.](image)

In the group with a higher level of manganese in the blood serum, a statistically significant increase in the level of iron in the a2Macro-globulin fraction (tetramer) was found by 3 times (p <0.05). There was a tendency to increase the iron content in the serum fractions of a2Macro-globulin (monomer), transferrin, iron citrates, "free" iron and the total content on average more than 3 times.

When considering the distribution of iron by fractions, its redistribution was noted. A significant change was characteristic of the "free" iron fraction – in the group with a higher level of manganese, the percentage of "free" iron decreased by 7 %, a tendency towards a decrease in transferrin by 17 % was established, against this background, the share of a2Macro-globulin monomer increased by 23 %.

4. Discussion
Analysis of the data obtained shows that high-normal levels of manganese in the blood were accompanied by a decrease in the indicators of milk production of cows. It is possible that an increase in the level of manganese in the blood above the median is indirectly related to the productivity of animals. In fact, we are dealing with a more complex phenomenon, expressed in the violation of the
metabolism of chemical elements in the body of cows. In the study, a change was noted in the redistribution of an important essential element – iron.

Iron is the most abundant transition metal in the body. Iron is present mainly in the oxidation state of iron (Fe$^{2+}$) or iron (Fe$^{3+}$) in biological systems. Due to its critical role in cellular metabolism, iron makes up a significant part of eukaryotic metalloma [8].

Iron is well suited for redox reactions due to its ability to act as an electron donor and an acceptor. In eukaryotic cells, iron is a cofactor for a wide range of metalloproteins involved in energy metabolism, oxygen binding, biosynthesis and DNA repair, synthesis of biopolymers, cofactors and vitamins, drug metabolism, antioxidant function, and many others. However, free iron can lead to the formation of reactive oxygen species such as a hydroxyl radical, which in turn can damage proteins, lipids, membranes, and DNA. Thus, cells must maintain a delicate balance between iron deficiency and iron overload, which includes coordinated control at the transcriptional, posttranscriptional, and posttranslational levels [9].

The data obtained indicate that, despite the absence of a significant difference in the total levels of manganese in the blood serum, the metal-ligand forms of iron undergo significant changes.

It should be noted that manganese can affect iron homeostasis. One study showed that a shift in the Fe (II) / (III) to Fe (II) ratio was noted in the brains of rats exposed to Mn [10, 11, 12].

In that way, the use of speciation analysis made it possible to reveal significant changes in the distribution of essential metal for individual forms in blood serum, which indicates a change in their metabolism.

5. Conclusion
The observed changes in metal-ligand homeostasis, disturbances in the redistribution of iron between ligands with high and low molecular weight against the background of a high-normal level of manganese in the blood serum can be considered as early biomarkers for predicting the state of mineral metabolism.

The findings suggest a potential role for metal metabolism, although the exact mechanisms require further detailed study. Highly-normal Mn values were accompanied by a decrease in productivity.

Thus, the use of speciation analysis indicates its high potential for use in laboratory diagnostics of metabolic disorders in the body and will make it possible to predict and correct the exchange of elements at early stages to increase productivity.

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