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Original Article

Specific of accumulation of manganese in organs and tissues of Hereford cattle

Especificidade da acumulação de manganês em órgãos e tecidos de bovinos Hereford

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Abstract

The elemental status of cattle is one of the important factors, which determine its growth, fertility, fetal development, meat and dairy production, etc. Therefore, the study of content of different elements in cattle organs and tissues and its correlation with cattle characteristics and diet is urgent task. It is also important to develop intravital and low-invasive methods to analyze element content in cattle to regulate its diet during lifetime. In the present work, we have studied the content and distribution of manganese in Hereford cattle from an ecologically clean zone of Western Siberia (Russia). 252 samples were taken from 31 bulls aged 15-18 months. They were collected from various livestock farms in the region and analyzed using atomic absorption spectrophotometry (organs and muscle tissue) and inductively coupled plasma atomic emission spectrometry (hair). The median values of manganese concentration obtained in natural moisture for hair, heart, kidneys, liver, lungs, muscles, spleen, testes, and brain were 25, 0.37, 1.0, 2.6, 0.4, 0.2, 0.4, 0.5, and 0.5 ppm. Accordingly, the concentration of manganese differs significantly in the organs and tissues of animals ($H = 188.6$, $df = 8$, $p < 0.0001$). Statistically significant associations of manganese were revealed in pairs: liver-testis, hair-testis, spleen-testis, and heart-brain. The classification of organs and tissues of animals according to the level of content and variability of manganese is carried out. The concentration of manganese in the body is not uniform, most of all it is deposited in the hair and excretory organs of the liver and kidneys. In other organs and muscle tissues, the distribution of manganese is more even and is in the range of 0.2-0.5 ppm. The resulting ranges can be used as a guideline for Hereford cattle bred in Western Siberia.

Keywords: manganese, trace metals, Hereford cattle, organs, muscle tissue, hair.

Resumo

O *status* elementar do gado é um dos fatores importantes que determinam seu crescimento, fertilidade, desenvolvimento fetal, produção de carne e laticínios, etc. Portanto, o estudo do conteúdo de diferentes elementos nos órgãos e tecidos do gado e sua correlação com as características do gado e dieta é tarefa urgente. Também é importante desenvolver métodos intravitais e pouco invasivos para analisar o conteúdo de elementos em bovinos para regular sua dieta durante a vida. No presente trabalho, estudamos o conteúdo e a distribuição de manganês em gado Hereford de uma zona ecologicamente limpa da Sibéria Ocidental (Rússia). Foram coletadas 252 amostras de 31 touros com idade entre 15 e 18 meses. Eles foram coletados em diversas fazendas pecuárias da região e analisados por espectrofotometria de absorção atômica (órgãos e tecido muscular) e espectrometria de emissão atômica com plasma indutivamente acoplado (cabelo). Os valores medianos da concentração de manganês obtidos na umidade natural para cabelo, coração, rins, fígado, pulmões, músculos, baço, testículos e cérebro foram 25, 0,37, 1,0, 2,6, 0,4, 0,2, 0,4, 0,5 e 0,5 ppm. Assim, a concentração de manganês difere significativamente nos órgãos e tecidos dos animais ($H = 188,6$, $df = 8$, $p < 0,0001$). Associações estatisticamente significativas de manganês foram reveladas em pares: fígado-testículo, cabelo-testículo, baço-testículo e coração-cérebro. É realizada a classificação dos órgãos e tecidos dos animais de acordo com o nível de conteúdo e variabilidade do manganês. A concentração de manganês no corpo não é uniforme, sendo principalmente depositada nos cabelos e nos órgãos excretores do fígado e dos rins. Em outros órgãos e tecidos musculares, a distribuição do manganês é mais uniforme e fica na faixa de 0,2-0,5 ppm. As faixas resultantes podem ser usadas como orientação para o gado Hereford criado na Sibéria Ocidental.

Palavras-chave: manganês, metais traço, gado Hereford, órgãos, tecido muscular, cabelo.

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1. Introduction

Manganese is the 10th most abundant element in the Earth's crust (Kuleshov, 2017). Manganese plays an important role in several physiological processes, being an integral part of many enzymes and an activator of other chemical elements (Van Saun, 2013). It is already known that this trace element is essential for the growth of calves, it is associated with bone formation, tissue development, and blood coagulation, with the functions of insulin, cholesterol synthesis, and as an activator of various enzymes. For example, Mn Superoxide Dismutase (MnSOD) is an antioxidant enzyme that is critical for reducing oxidative stress (Chen et al., 2019). Glycotransferase is involved in the formation of mucopolysaccharide (Zhang et al., 2014). Arginase is an Mn-dependent enzyme that regulates urea production in the liver and nitric oxide synthase in smooth muscle cells (Sarban et al., 2007). Other enzymes that use Mn as a cofactor or activator are involved in the metabolism of carbohydrates, amino acids and cholesterol, the formation of bone and cartilage, and wound healing (Freeland-Graves et al., 2005). These include pyruvate carboxylase, transferases, hydrolases, kinases, 3 lyases, oxidoreductases, isomerases, ligases, glutamine synthetase, and phosphoenolpyruvate decarboxylase (Aschner and Aschner, 2005). Also, Mn plays an important role in digestion, development, reproduction, immune function, and regulation of cellular activity.

According to its importance in metabolic processes in living organisms, Mn may be crucial element for cattle breeding. The manganese requirement for raising cattle is approximately 20 mg/kg ration (National Academies of Sciences, Engineering, and Medicine, 2016). In the same time, according to the review (Arthington and Ranches, 2021), deficiency of Mn and some other trace elements (Se, Cu, Zn, Co, and I) may often be observed even when cattle rely on forages, which meet the requirements due to the presence trace mineral antagonists (Fe, Mo, and S). Thus, Mn status of cattle should be monitored to avoid negative effects of its shortage (Arthington and Ranches, 2021). Many of the deficiencies seen with manganese deficiency appear to be related to decreased activity of glycosyltransferases, a group of enzymes that require manganese to function. Glycosyltransferases are involved in the synthesis of proteoglycans, which are critical for the development of bone matrix and play an important role in the integrity of cartilage. Manganese deficiency decreases mucopolysaccharides in cartilage, resulting in impaired skeletal development (Spears, 2011). It was show that Mn deficiency in feeding of heifers results in impairment in fetal growth and development (Hansen et al., 2006). It was shown that Mn supplementation in the form of combined mineral additive provided the increase milk production and fat content (Banadaky et al., 2021; Daniel et al., 2020).

Despite the importance of manganese, in high concentrations, this metal can have harmful effects on biological systems (Crossgrove and Zheng, 2004). To prevent intoxication in organisms, there are mechanisms of cell homeostasis of manganese. The complexity of this process lies in the presence of several types of manganese carriers, which are involved in the specific transport of manganese or the general transport of divalent metal ions. These include Nramp H1-manganese transporters (Kehres et al., 2000; Portnoy at al., 2000; Yang et al., 2005) ATP-binding cassette manganese permeases (Horsburgh et al., 2002; McAllister et al., 2004; Zaharik et al., 2004), manganese transporting P-type ATPases (Antebi and Fink, 1992), Catalysts for the diffusion of cations (Montanini, et al., 2007; Rosch, et al., 2009), and inorganic phosphate transporters with high affinity to the Mn - HPO4 complexes (Jensen et al., 2003). Of the total magnesium pool in the body, 65-70% is found in bone, 15% in muscles, 15% in other soft tissues, and 1% in the extracellular fluid (National Academies of Sciences, Engineering, and Medicine, 2016). Despite potential negative effect of Mn excess, in cattle breeding practice, Mn toxicity is rare, since intestinal absorption rate of Mn is low, while liver has great capacity to excrete Mn with bile (Arthington and Ranches, 2021; Haskovic et al., 2021; Spears, 2011).

In our study, we consider the patterns of distribution and interaction of manganese in various organs and muscle tissues of beef cattle of the Hereford breed in an uncontaminated zone of Western Siberia. The goal of the study is of high interest both due to the importance of the data on Mn distribution in cattle from various geographic locations and grown with forage with different contamination levels for understanding general patterns (Orjales et al., 2018; Silva et al., 2022) and because the identification of such patterns may be useful for the development of intravital and low-invasive methods to analyze element status of cattle. For example, in the article by Narozhnykh (2023), the method was proposed for predicting iron level in the muscle tissue of Hereford cattle using results of blood analysis results. Therefore, similar methods may be developed for other crucial elements, including Mn.

2. Materials and Methods

2.1. Animals and experimental design

Total number of samples was 252 from muscles (n=31), hairs (n=30), heart (n=30), liver (n=30), kidneys (n=30), lungs (n=30), testes (n=30), spleens (n=31), and brain (n=10). Samples were taken from 31 Hereford bulls aged 16-18 months. The live weight of bulls at the time of slaughter averaged 543.8 kg. Samples of hair weighing 10 mg from each animal were taken from the withers region. The diaphragmatic muscles were taken as muscle tissue samples. All animals were clinically healthy at the time of slaughter. Slaughter was carried out by the rules detailed by the European Commissions' regulations CE 853/2004, 854/2004, and 1099/2009. The animals were raised on the farms of the Novosibirsk region at a distance of 100 km from the megalopolis in an ecologically clean area. The manganese content in study area in soil, water, and animal feed is presented in Table 1.

Table 1. The concentration of manganese in soil, water and feed samples, mean ± standard error, ppm.

Soil	**774 ± 86**	**Roughage**	**31.5 ± 4.5**
Water	0.75 ± 0.2	Wheat	25.9 ± 2.4

2.2. Sample analysis

Samples of organs and tissues from animals were taken immediately after slaughter, then they were frozen and stored at a temperature of –18 ° C. Study of the chemical composition of organs and muscle tissue was carried out by atomic absorption spectrometry with flame and electrothermal atomization on a Shimadzu AA-7000 spectrometer. Sample preparation of internal organs and muscle tissue of animals for atomic absorption analysis occurred in the following order. On beginning, tissues were cleaned of connective tissue and fat and homogenized. Then resulting mass was dried in an oven at a temperature of 60-70°C for about 12 hours until constant weight. From obtained dry residue, were taken 3 g, which were ashed in a muffle furnace at a temperature of 500-550°C. After 10-15 hours, mineralization ended, the ash acquired have grey or white color. After cooling of the samples at room temperature, ash residue was dissolved in 3 ml of 50% hydrochloric acid and then evaporated to a dry residue on an electric stove. This residue was transferred to a volumetric flask and diluted with 25 ml of distilled water. In the resulting ready-made solution, the concentration of manganese was determined.

Manganese in hair was determined by inductively coupled plasma atomic emission spectrometry using an iCAP-6500 spectrometer (Thermo Scientific, USA). For analysis, we took a hair sample weighing 100 mg from each specimen. To cleanse the hair from contamination, the sample was placed in a flask with bidistilled water, and then the sample was stirred for 1 minute with a mixer at a rotation speed of 1000 rpm. Then water was changed up to 10 times, repeating this procedure. Subsequently, hair was washed with OCh 49-5 acetone for 2 minutes, after which the remaining solution was washed off 3 times with deionized water and dried at room temperature. Then sample was dissolved in 2 ml of ultrapure 27-5 nitric acid and placed in a standard autoclave in a MARS-5 microwave oven (CEM), the autoclave was sealed, and dissolution was carried out gradually over 40 minutes, increasing the temperature to 180°C. The resulting solution was transferred quantitatively into a volumetric flask. The solutions were analyzed after 10- and 100-fold dilution of calibration solutions prepared based on multielement standards (Tsygankova et al., 2017).

2.3. Statistical analyses

Statistical analysis was performed using R programming language, version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria) (Racine, 2012). The Shapiro-Wilk test was used to assess the correspondence of the data distribution to the normal one. In the graphical presentation of the study results, logging (log (1 + x), x is the initial variable) of the original data was used to reduce the variability between organs. The Reference Interval was determined using the robust method, the Confidence Interval was calculated using the adjusted bootstrap percentile method (Efron and Tibshirani, 1994), which corrects for possible bias and asymmetry in the baseline distribution. The associations between the authorities for the concentration of heavy metals were determined by Kendall's methods, as it is fairly robust to emissions. To identify differences between organs in the concentration of manganese, the Kruskal-Wallis test was used (Kruskal and Wallis, 1952). And performs Dunn's test (Dunn, 1964) of multiple comparisons using rank sums and adjusts the p-value for multiple comparisons using the Holm (1979).

3. Results

According to results of the Shapiro-Wilk test, it was found that baseline data differs from normal (Figure 1). Table 2 shows data on the content of manganese in organs and tissues of animals. The highest concentration of metal is found in hair, where its concentration is much higher. Among the internal organs, liver and kidneys deposit manganese more than others. The minimum content of element is noted in muscle tissue. Studied organs, according to degree of manganese accumulation, can be arranged in a ranging row: muscles <heart <spleen = lung <brain = testes <kidneys <liver <hair, in a ratio of 1: 1.9: 2: 2: 2.5: 2.5: 5: 13: 125. Concentration of manganese in heart, lungs, testes, and hair is highly variable, in contrast to its levels in brain, kidneys, and liver.

Based on the data obtained, 95% reference intervals with 90% Confidence Interval were calculated for the manganese content in the studied organs and tissues (Table 3). Given high variability and low concentrations of manganese in lungs, muscle tissue, hair, and testes, confidence intervals start at zero.

According to the results of the Kruskal-Wallis test (H = 188.6, df = 8, p <0.0001), Mn concentration significantly differs in the organs and tissues of animals. Significant pairwise comparisons are presented in Table 4. Of all combinations of pairs, significant differences were observed in 20, and in 16 pairs, no statistically significant differences were found. Concentration of manganese in the hair was different in all organs except the liver. There was also a statistically significant difference between the kidneys, liver and other organs and muscle tissue.

Significant associations were found between level of manganese in organs and tissues of Hereford cattle

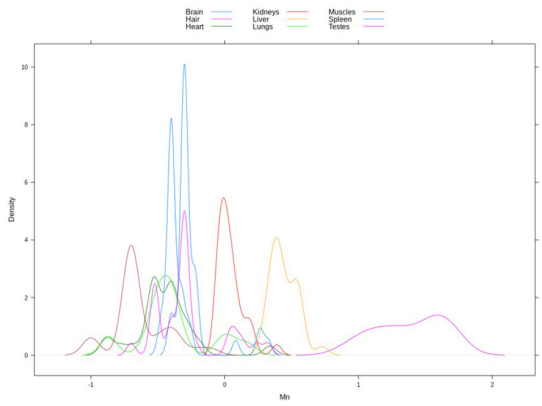

Figure 1. Distribution of manganese levels in organs and tissues of animals.

Table 2. The content of manganese in organs and tissues of Hereford cattle, ppm.

Organ/Tissue	Av	Min	Max	Q1	Q3	IQR
Hair	25	7.8	54.0	14.3	41.2	26.9
Heart	0.37	0.13	2.18	0.3	0.42	0.12
Kidneys	1.0	0.9	2.45	0.92	1.2	0.28
Liver	2.6	2.0	5.34	2.36	3.41	1.05
Lungs	0.4	0.13	1.56	0.3	0.48	0.18
Muscles	0.2	0.1	0.8	0.2	0.32	0.12
Spleen	0.4	0.33	2.12	0.4	0.48	0.08
Testes	0.5	0.2	2.1	0.37	0.53	0.16
Brain	0.5	0.4	0.6	0.5	0.51	0.01

Table 3. Reference intervals with 90% confidence intervals (CI) for the content of heavy metals in the spleen of Herefod cattle (mg, kg).

Organ/Tissue	95% Reference Interval	Lower 90% Confidence Interval	Upper 90% Confidence Interval
Hair	0 - 59.7	0	49.6 - 67.5
Heart	0.072 - 0.614	0 - 0.140	0.539 - 0.697
Kidneys	0.74 - 1.33	0.65 - 0.79	1.23 - 1.43
Liver	1.15 - 4.27	0.29 - 1.57	3.79 - 5.10
Lungs	0 - 0.745	0 - 0.108	0.633 - 0.984
Muscles	0 - 0.556	0 - 0.045	0.480 - 0.631
Spleen	0.281 - 0.516	0.251 - 0.313	0.461 - 0.544
Testes	0 - 0.875	0 - 0.151	0.713 - 1.102

Table 4. Pairwise comparison of manganese concentration in organs and cattle tissues (Z statistic; adjusted p-value for multiple comparisons Holm).

Organ/Tissue	Brain	Hair	Heart	Kidneys	Liver	Lungs	Muscles	Spleen
Hair	-4.259 0.0002*							
Heart	2.113 8.6003	0.2075 <0.0001*						
Kidneys	-1.592	37.904	-5.135					
	0.5563	0.0016*	<0.0001*					
Liver	-31.940	1.548	-72.874	-22.924				
	0.0119*	0.5472	<0.0001*	0.1422				
Lungs	1.518.281	80.345	-0.8656	44.261	66.642			
	0.4513	<0.0001*	0.7734	0.0001*	<0.0001*			
Muscles	31.981	104.089	14.167	68.592	90.952	23.766		
	0.0124*	<0.0001*	0.4697	<0.0001*	<0.0001*	0.1310		
Spleen	0.7943	70.879	-18.706	3.4175	56.818	-10.368	-34.417	
	0.3377	<0.0001*	0.6405	0.0060*	<0.0001*	0.7496	0.0058*	
Testes	0.432750	64.901	-23.239	28.562	50.879	-15.195	-38.878	-0.5039
	0.3326	<0.0001*	0.1409	0.0343	<0.0001*	0.5145	0.0011*	0.6144

* p-value.

(Figure 2). The statistically significant ones were between liver testis, hair testis, spleen testis, and heart-brain. First three of them are negative, and only the last is positive. This means, most likely, that negative relationships may indicate a protective reaction of the body in the distribution of manganese from more important reproductive organs to organs that perform the functions of excretion and filtration.

The similarity of organs and tissues of cattle in terms of manganese concentration is shown on dendrogram (Figure 3). Dendrogram shows three main clusters, of which cluster 1 consists only of hair. It differs to a greater extent from other organs and tissues in terms of manganese content. The second cluster includes kidneys and liver. In these organs, heavy metals accumulate most intensively, in contrast to other organs that are part of the third cluster.

Based on data obtained, it can be seen that concentration of manganese in the body is heterogeneous; most of all, it is deposited in hair and excretory organs of liver and kidneys. In other organs and muscle tissues, distribution of manganese is fairly even and is in the range of 0.2-0.5 ppm.

4. Discussion

Mn is an essential trace mineral required for normal tissue development and activity (Arthington and Ranches, 2021; Carvalho et al., 2010; Górski and Saba, 2015; Spears, 2011; Utami and Pangestu, 2017; Van Saun, 2013). In our research, we studied the content of manganese in the organs and tissues of Hereford cattle in the conditions of Western Siberia in an ecologically safe breeding zone.

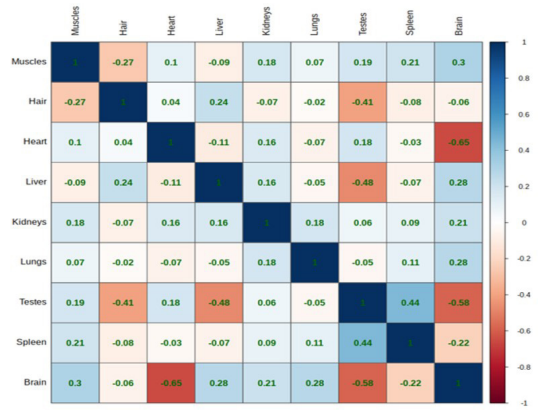

Figure 2. Correlation of manganese level between organs and goby tissues.

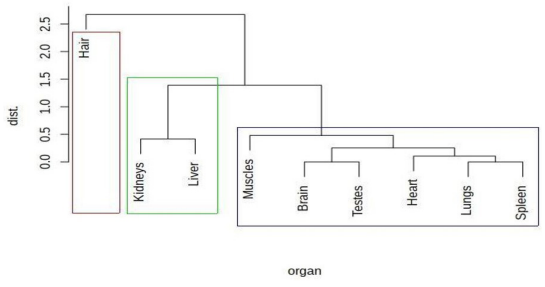

Figure 3. Dendrogram of organ and tissue similarity in terms of manganese levels.

The reference values we presented are slightly lower than those suggested by Puls (1994). Revealed parentage may be due to different trends in animal productivity and animal husbandry. Interesting data are cited by Georgievsky et al. (1979) on the content of manganese in organs of mammals. The data we obtained differs from that presented in his work. Thus, the concentration of manganese in the hair and spleen in Hereford cattle was higher, and in the kidneys, somewhat lower than in mammals as a whole (Georgievsky et al., 1979).

Manganese is widely distributed in the body, and it is usually more abundant in mitochondrial-rich tissues. Higher Mn concentrations are often associated with pigmented tissue (Hidiroglou, 1979). The manganese content in hair in our study was different from previously published data. So, authors (Miroshnikov et al., 2017) studying the content of manganese in hair of cows and heifers of Hereford breed, median value in these groups was 30 ppm manganese. In another study, he also determined manganese in Holstein lactating cows, and concentration of manganese was significantly lower than 4.5 ppm median value in relationship (Miroshnikov et al., 2020). Perhaps one of factors that have significant impact on such significant differences in manganese content is breed and direction of productivity of animals. Polish Holstein-Fresian breeds raised on organic and conventional farms had a manganese content of 22-31.5 ppm (Gabryszuk et al., 2010). Earlier studies have noted great variability in manganese accumulation in livestock hair. The authors (Findrik et al., 1969) found a low level of manganese of 1.47 ppm in the hair of dairy cows raised in various regions of Croatia. The authors Rasbech (1968) studied cow hair in Germany with different manganese content in the diet. Animals fed an average of 148 mg Mn/day had in hair manganese concentration of 3.35 ppm, while heifers fed a 10-fold increase in hair manganese diet had 10.3 ppm Mn. On the other hand, authors (Fonseca and Lang, 1976) reported that concentration of Mn in hair sampled from dairy cows in areas of Costa Rica was 91, 73, and 18 ppm. From the above, it can be assumed that the manganese content in hair of cattle has high variability, which can be associated with direction of productivity, breed, region, and diet. Similar conclusions were obtained in the work (Dermauw et al., 2014), which presents the results on data on trace element concentrations in all tissues in zebu (*B. indicus*) cattle in Ethiopia. We found that the concentration of manganese in muscle tissue of Hereford cattle was 0.2 ppm, which is 2-13 times lower than in other organs and 125 times lower than in hair. In cattle of beef bred Charolais, Hereford, Simmental at age of 24-25 months, bred near a city in northwestern Poland, the content of manganese in muscle tissue was found to be more than 3 times higher than in our study and amounted to 0.62, 0.72, 0.76 ppm, respectively (Pilarczyk, 2014). Authors (Cabrera et al., 2010) showed that the concentration of manganese could vary significantly depending on the type of muscle in cattle. Therefore, in tenderloin, the eye of rump, striploin, eye round, tri-tip, rib-eye roll and three rib plate

flanks on meat cuts of Bradford and Hereford breed bulls bred in Uruguay, the next concentration of manganese was revealed 0.11, 0.09, 0.08, 0.47, 0.11, 0.10, 0.04 and 0.11, 0.12, 0.08, 0.16, 0.10, 0.07, 0.05 ppm, respectively. In all muscles of Bradfords and Herefords, level of manganese was about 1.5 or more times lower than in our study, except for eye round, where level of manganese was almost 2.5 times higher in the Bradford. Another study examined influence of breed, muscle type and muscle interactions on level of manganese in muscle tissue of beef steers, Polish Red, White-Backed Polish Black-and-White, Polish Holstein-Friesian and Simmental at 6 months of age. A significant influence of the breed and interaction of breed muscle type factor on concentration of manganese in muscle tissue of livestock was revealed, and variability of manganese was quite high depending on the breed and type of muscles - 0.11-0.59 ppm (Domaradzki, et al. 2016). Manganese levels in Holstein-Friesian, Galician Blonde bulls and their crosses at age of 10 months. grown in northern Spain was in the moderate range of 0.09-0.12 ppm (Pereira et al., 2018). In a study by author (Ramos et al., 2012), influence of age and breed on level of manganese accumulation in muscle tissue was shown. According to their data, age did not significantly affect level of manganese in muscle tissue. However, breed differences were found, as the Hereford cattle had higher levels of manganese than the Bradford cattle. In general, manganese levels in bulls of both breeds were in the range of 0.07-0.14 ppm, depending on age and muscle type. Similarly, relatively low levels of manganese 0.03-0.11 ppm were found in different anatomical location meat cuts (Gerber et al., 2009). In the work of the authors (Alonso et al., 2004) the concentration of manganese in meat from cows aged 3-16 years, raised in northern Spain, was similar to our data and amounted to 0.19 ppm. In another study, also conducted on beef cows raised in Spain, manganese levels varied between 0.08-0.29 ppm depending on muscle type (Alonso et al., 2002). These data indicate that the variability of the level of manganese in cattle meat is influenced by the type of muscle, breed and direction of productivity.

It was found that the concentration of manganese in the liver and kidneys was significantly higher than in other organs and muscle tissues. These organs play a key role in the metabolism of trace elements (Alonso et al., 2004; Rahil-Khazen et al., 2002; Taylor, 1996). The concentration of manganese in the kidneys and liver in our study is similar to the data of the author (Georgievsky et al., 1979). According to the classification proposed by the author (Puls, 1994), the manganese level in our study corresponded to the marginal for the kidneys (0.93-1.2 ppm) and liver (1.5-3 ppm). However, in the author's work (Falandysz, 1993; Falandysz and Lorenc-Biala, 1989) manganese content in liver and kidney disease in cattle was lower than in our study. And animals from Sweden and Finland had higher Mn levels than livestock from Western Siberia (Jorhem et al., 1989; Nuurtamo et al., 1980). In northern Spain, several studies were carried out, where the level of manganese in the kidneys and liver of cattle was studied. Therefore, in the study of the authors (Alonso et al., 2004) the concentration of manganese in liver and kidney was lower and in (Miranda et al., 2006). Copper, zinc, iron, and manganese accumulation in cattle from Asturias (northern Spain) is higher than in our study. Authors Erdogan et al. (2004) studied the manganese content in the liver and kidneys of cattle in different ecological zones of Turkey and assessed the influence of the season of the year on this indicator. According to their data, the influence of these factors is insignificant, and the concentration of metals in the liver was higher. In kidneys, level of manganese was similar to the data obtained by the authors of the article. In work of authors Kottferová and Koréneková (1995) animals were studied that for two years were in a contaminated area near a metallurgical plant and a conditionally safe area. According to results of the study, there was no significant influence of ecologically different zones on the level of manganese in the kidneys and liver of animals. In some cases, differences in the concentration of manganese in muscle tissue were observed; however, they may be due to random factors and a small volume of the population. Author's work (Sawhney and Kehar, 1961) Revealed seasonal fluctuations in the Mn concentration in the liver and kidneys of cattle. Livestock. Based on above sources and data of author of article, it can be determined that level of manganese in the kidneys and liver is in the range from 0.7-2.0 and 1.0-3.5 ppm.

After studying science literature it was noted, that many authors argue that in publish not enough information on the content of manganese in testes, lungs, spleen, heart, and brain in cattle. Perhaps this is because these by-products, as a rule, are consumed in a small amount of food, and some are not consumed at all. Therefore, manganese content in them is normalized. However, author Puls (1994) in his monograph provides data on concentration of this metal in the spleen and brain of cattle. In authors' study, the level of manganese was more than 2 times, and in the spleen, it was 6 times lower. In the handbook of authors Georgievsky et al. (1979) give a lower range of manganese concentrations in heart, spleen, and brain, which range from 0, 2 to 0.4 ppm. This data is similar to ours. Author (Sawhney and Kehar, 1961) Studied the influence of the season on the content of manganese in brain and testes. According to his data, seasonality does not significantly affect level of manganese in these organs. Still, in the liver, kidneys and bone marrow, it gradually increases from June during the hot, dry season to November, with abundant grazing after the rainy season. However, concentration of manganese in our work was 2 times lower in the testes and 4 times lower in the brain than that of the author Sawhney and Kehar (1961). In another study, it was shown that, depending on the year of study, the concentration of manganese in the heart of livestock could differ significantly (Kottferová and Koréneková, 1995) calves fed a low and high manganese diet had higher organ manganese accumulation (Howes and Dyer, 1971). Compared to our data, the study by Howes and Dyer (1971) shows 2 to 5 times less metal concentration in the spleen, lungs, and brain, which may indicate the accumulation of manganese in the organs of livestock during ontogenesis.

Difference in organs by the concentration of manganese in organs and tissues of livestock has been noted in several studies (Alonso et al., 2002, 2004; Falandysz, 1993; Falandysz and Lorenc-Biala, 1989; Howes and Dyer, 1971; Puls 1994;

Rahil-Khazen et al., 2002; Taylor, 1996). In the same time, recent work demonstrated that manganese distribution in dairy cow organisms depends on element status and Mn redistribution may occur when general Mn supply and digestion change (Notova et al., 2023). Thus, the results of the works similar to the present one and meta-analysis of works obtained by the example of different locations may be used for understanding general patterns on Mn distribution depending on elemental status of cattle and control its supply.

Results of our study confirm and clearly show the difference between the organs (Table 2, Figure 3). Variability of manganese in the brain and liver was relatively low, in hair and muscle tissue, it was moderate, and in the heart, lungs, spleen, and testes — high. In terms of the degree of accumulation and variability of the level of manganese, hair stands out from other organs (Figure 3). This reflects the function of excreting excess metals in animals (Lakshmi Priya and Geetha, 2010). In the same time, despite hair of cows is widely used for the analysis of element profile of cattle (Miroshnikov et al., 2015), the results of the present work demonstrated that Mn content in hair cannot be reliable parameter allowing to estimate its content in other organ and tissues and should not be used for the development models and approaches used to regulate Mn supply.

5. Conclusion

The variability of the level of manganese in the organs and tissues of animals largely depends on the breeding zone, age and breed. Liver and kidneys are the main target organs for manganese accumulation. The spleen, lungs, heart, testes, brain, and muscles are highly similar in manganese accumulation. Hair contains 10 to 125 times more manganese than the organs and tissues of cattle. In muscle tissue, minimum value of manganese relative to other organs is recorded. Significant negative correlations were revealed between the level of manganese in the testes and liver, hair, and spleen, which possibly indicates the existence of biochemical mechanisms for protecting the reproductive system from an excess of this metal. The data obtained on the content of manganese in organs and tissues of Hereford cattle can be used as a preliminary norm in the conditions of Western Siberia.

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References

ALONSO, M.L., BENEDITO, J., MIRANDA, M., CASTILLO, C., HERNANDEZ, J. and SHORE, R.F., 2002. Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution. *Archives of Environmental Contamination and Toxicology*, vol. 42, no. 2, pp. 165-172. http://doi.org/10.1007/s00244-001-0012-7. PMid:11815807.

ALONSO, M.L., MONTAÑA, F.P., MIRANDA, M., CASTILLO, C., HERNÁNDEZ, J. and BENEDITO, J.L., 2004. Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *Biometals*, vol. 17, no. 4, pp. 389-397. http://doi.org/10.1023/B:BIOM.0000029434.89679.a2. PMid:15259359.

ANTEBI, A. and FINK, G.R., 1992. The yeast Ca (2 þ)-ATPase homologue, PMR1, is required for normal Golgi function and localizes in a novel Golgi-like distribution. *Molecular Biology of the Cell*, vol. 3, no. 6, pp. 633-654. http://doi.org/10.1091/mbc.3.6.633. PMid:1379856.

ARTHINGTON, J.D. and RANCHES, J., 2021. Trace mineral nutrition of grazing beef cattle. *Animals (Basel)*, vol. 11, no. 10, pp. 2767. http://doi.org/10.3390/ani11102767. PMid:34679788.

ASCHNER, J.L. and ASCHNER, M., 2005. Nutritional aspects of manganese homeostasis. *Molecular Aspects of Medicine*, vol. 26, no. 4-5, pp. 353-362. http://doi.org/10.1016/j.mam.2005.07.003. PMid:16099026.

BANADAKY, M.D., RAJAEI-SHARIFABADI, H., HAFIZI, M., HASHEMI, S.A., KALANAKY, S., FAKHARZADEH, S., SHAHBEDINI, S.P., REZAYAZDI, K. and NAZARA, M.H., 2021. Lactation responses of Holstein dairy cows to supplementation with a combination of trace minerals produced using the advanced chelate compounds technology. *Tropical Animal Health and Production*, vol. 53, no. 1, pp. 55. http://doi.org/10.1007/s11250-020-02539-5. PMid:33389213.

CABRERA, M.C., RAMOS, A., SAADOUN, A. and BRITO, G., 2010. Selenium, copper, zinc, iron and manganese content of seven meat cuts from Hereford and Braford steers fed pasture in Uruguay. *Meat Science*, vol. 84, no. 3, pp. 518-528. http://doi.org/10.1016/j.meatsci.2009.10.007. PMid:20374819.

CARVALHO, P.R., GONCALVES PITA, M.K., LOUREIRO, J.E., TANAKA, H.R. and SOARES RIBEIRO, J.C., 2010. Manganese deficiency in bovines: connection between manganese metalloenzyme dependent in gestation and congenital defects in newborn calves. *Pakistan Journal of Nutrition*, vol. 9, no. 5, pp. 488-503. http://doi.org/10.3923/pjn.2010.488.503.

CHEN, P., BORNHORST, J. and ASCHNER, M., 2019. Manganese metabolism in humans. *Frontiers in Bioscience*, vol. 23, no. 9, pp. 1655-1679. http://doi.org/10.2741/4665. PMid:29293455.

CROSSGROVE, J. and ZHENG, W., 2004. Manganese toxicity upon overexposure. *NMR in Biomedicine*, vol. 17, no. 8, pp. 544-553. http://doi.org/10.1002/nbm.931. PMid:15617053.

DANIEL, J.B., KVIDERA, S.K. and MARTÍN-TERESO, J., 2020. Total-tract digestibility and milk productivity of dairy cows as affected by trace mineral sources. *Journal of Dairy Science*, vol. 103, no. 10, pp. 9081-9089. http://doi.org/10.3168/jds.2020-18754. PMid:32828500.

DERMAUW, V., LOPÉZ ALONSO, M., DUCHATEAU, L., DU LAING, G., TOLOSA, T., DIERENFELD, E., CLAUSS, M. and JANSSENS, G.P., 2014. Trace element distribution in selected edible tissues of zebu (bos indicus). Cattle slaughtered at Jimma, SW Ethiopia. *PLoS One*, vol. 9, no. 1, pp. e85300. http://doi.org/10.1371/journal.pone.0085300. PMid:24465529.

DOMARADZKI, P., FLOREK, M., STASZOWSKA, A. and LITWIŃCZUK, Z., 2016. Evaluation of the mineral concentration in beef from Polish native cattle. *Biological Trace Element Research*, vol. 171, no. 2, pp. 328-332. http://doi.org/10.1007/s12011-015-0549-3. PMid:26498196.

DUNN, O.J., 1964. Multiple comparisons using rank sums. *Technometrics*, vol. 6, no. 3, pp. 241-252. http://doi.org/10.1080/00401706.1964.10490181.

EFRON, B. and TIBSHIRANI, R.J., 1994. *An Introduction to the Bootstrap*. Boca Raton: CRC Press. 456 p. http://doi.org/10.1201/9780429246593.

ERDOGAN, S., CELIK, S. and ERDOGAN, Z., 2004. Seasonal and locational effects on serum, milk, liver and kidney chromium, manganese, copper, zinc, and iron concentrations of dairy cows. *Biological Trace Element Research*, vol. 98, no. 1, pp. 51-61. http://doi.org/10.1385/BTER:98:1:51. PMid:15051900.

FALANDYSZ, J. and LORENC-BIALA, H., 1989. Metals in muscle, liver and kidneys of slaughtered animals from the northern part of Poland. *Bromatologia i Chemia Toksykologiczna*, vol. 22, pp. 19-22.

FALANDYSZ, J., 1993. Some toxic and essential trace metals in cattle from the northern part of Poland. *The Science of the Total Environment*, vol. 136, no. 1-2, pp. 177-191. http://doi.org/10.1016/0048-9697(93)90306-Q. PMid:8211108.

FINDRIK, M., SKRGATIC, J., KALIVODA, M., ROSET, P. and VINOVRSKI, Z., 1969. Istrazivanje prometa mangana u goveda. *Veterinarski Arhiv*, vol. 39, pp. 134-140.

FONSECA, H. and LANG, C., 1976. Conrenido de manganeso en los forrajes del valle de orosi y su effecto sobre le concentracion en el oelo v la reproduccion en vacas lecheras. In: S.M. FREEMAN, ed. *Nuclear techniques in animal production and health*. Vienna: International Atomic Energy Agency, pp. 171-177.

FREELAND-GRAVES, J., BOSE, T. and KARBASSIAN, A., 2005. 25Mn manganese metallotherapeutics. In: M. GIELEN and E. TIEKINK, eds. *Metallotherapeutic drugs and metal-based diagnostic agents: the use of metals in medicine*. Hoboken: Wiley, pp. 159-178. http://doi.org/10.1002/0470864052.ch9.

GABRYSZUK, M., SLONIEWSKI, K., METERA, E. and SAKOWSKI, T., 2010. Content of mineral elements in milk and hair of cows from organic farms. *Journal of Elementology*, vol. 15, no. 2, pp. 259-267. http://doi.org/10.5601/jelem.2010.15.2.259-267.

GEORGIEVSKY, V.I., ANNENKOV, B.N. and SAMOKHIN, V.T., 1979. *Mineral animal nutrition*. Kolos: Moscow, pp. 366-397.

GERBER, N., BROGIOLI, R., HATTENDORF, B., SCHEEDER, M.R., WENK, C. and GÜNTHER, D., 2009. Variability of selected trace elements of different meat cuts determined by ICP-MS and DRC-ICPMS. *Animal*, vol. 3, no. 1, pp. 166-172. http://doi.org/10.1017/S1751731108003212. PMid:22444183.

GÓRSKI, K. and SABA, L., 2015. Assessment of manganese levels in the soil and feeds, and in the bodies of milk cows from central-eastern Poland administered a mineral compound feed. *Acta Scientiae Veterinariae*, vol. 43, pp. 1288.

HANSEN, S.L., SPEARS, J.W., LLOYD, K.E. and WHISNANT, C.S., 2006. Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. *Journal of Dairy Science*, vol. 89, no. 11, pp. 4305-4311. http://doi.org/10.3168/jds.S0022-0302(06)72477-8. PMid:17033018.

HASKOVIC, E., MUHIC-SARAC, T., LUKIC, M., MARJANOVIC, M., ZERO, S. and ISLAMAGIC, E., 2021. Determination of heavy metals in liver and skeletal muscles of pigs and calves: experience from Bosnia and Herzegovina. *Bulgarian Journal of Agricultural Science*, vol. 27, no. 3, pp. 593-599.

HIDIROGLOU, M., 1979. Manganese in ruminant nutrition. *Canadian Journal of Animal Science*, vol. 59, no. 2, pp. 217-236. http://doi.org/10.4141/cjas79-028.

HOLM, S., 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, vol. 6, pp. 65-70.

HORSBURGH, M.J., WHARTON, S.J., COX, A.G., INGHAM, E., PEACOCK, S. and FOSTER, S.J., 2002. MntR modulates expression of the PerR regulon and superoxide resistance in Staphylococcus aureus through control of manganese uptake. *Molecular Microbiology*, vol. 44, no. 5, pp. 1269-1286. http://doi.org/10.1046/j.1365-2958.2002.02944.x. PMid:12028379.

HOWES, A.D. and DYER, I.A., 1971. Diet and supplemental mineral effects on manganese metabolism in newborn calves. *Journal of Animal Science*, vol. 32, no. 1, pp. 141-145. http://doi.org/10.2527/jas1971.321141x. PMid:5546873.

JENSEN, L.T., AJUA-ALEMANJI, M. and CULOTTA, V.C., 2003. The *Saccharomyces cerevisiae* high affinity phosphate transporter encoded by PHO84 also functions in manganese homeostasis. *The Journal of Biological Chemistry*, vol. 278, no. 43, pp. 42036-42040. http://doi.org/10.1074/jbc.M307413200. PMid:12923174.

JORHEM, L., SUNDSTRÖM, B., ASTRAND, C. and HAEGGLUND, G., 1989. The levels of zinc, copper, manganese, selenium, chromium, nickel, cobalt and aluminum in the meat, liver and kidney of Swedish pigs and cattle. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, vol. 188, no. 1, pp. 39-44. http://doi.org/10.1007/BF01027620. PMid:2711754.

KEHRES, D.G., ZAHARIK, M.L., FINLAY, B.B. and MAGUIRE, M.E., 2000. The NRAMP proteins of Salmonella typhimurium and Escherichia coli are selective manganese transporters involved in the response to reactive oxygen. *Molecular Microbiology*, vol. 36, no. 5, pp. 1085-1100. http://doi.org/10.1046/j.1365-2958.2000.01922.x. PMid:10844693.

KOTTFEROVÁ, J. and KORÉNEKOVÁ, B., 1995. The effect of emissions on heavy metals concentrations in cattle from the area of an industrial plant in Slovakia. *Archives of Environmental Contamination and Toxicology*, vol. 29, no. 3, pp. 400-405. http://doi.org/10.1007/BF00212507. PMid:7487159.

KRUSKAL, W.H. and WALLIS, A., 1952. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, vol. 47, no. 260, pp. 583-621. http://doi.org/10.1080/01621459.1952.10483441.

KULESHOV, V., 2017. *Isotope geochemistry: the origin and formation of manganese rocks and ores*. USA: Elsevier.

LAKSHMI PRIYA, M.D. and GEETHA, A., 2010. Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. *Biological Trace Element Research*, vol. 142, no. 2, pp. 148-158. http://doi.org/10.1007/s12011-010-8766-2. PMid:20625937.

MCALLISTER, L.J., TSENG, H.J., OGUNNIYI, A.D., JENNINGS, M.P., MCEWAN, A.G. and PATON, J.C., 2004. Molecular analysis of the PSA permease complex of *Streptococcus pneumoniae*. *Molecular Microbiology*, vol. 53, no. 3, pp. 889-901. http://doi.org/10.1111/j.1365-2958.2004.04164.x. PMid:15255900.

MIRANDA, M., ALONSO, M.L. and BENEDITO, J.L., 2006. Copper, zinc, iron, and manganese accumulation in cattle from Asturias (Northern Spain). *Biological Trace Element Research*, vol. 109, no. 2, pp. 135-143. http://doi.org/10.1385/BTER:109:2:135. PMid:16444003.

MIROSHNIKOV, S., KHARLAMOV, A., ZAVYALOV, O., FROLOV, A., BOLODURINA, I., ARAPOVA, O. and DUSKAEV, G., 2015. Method of sampling beef cattle hair for assessment of elemental profile. *Pakistan Journal of Nutrition*, vol. 14, no. 9, pp. 632-636. http://doi.org/10.3923/pjn.2015.632.636.

MIROSHNIKOV, S.A., SKALNY, A.V., ZAVYALOV, O.A., FROLOV, A.N. and GRABEKLIS, A.R., 2020. The reference values of hair content of trace elements in dairy cows of holstein breed. *Biological Trace Element Research*, vol. 194, no. 1, pp. 145-151. http://doi.org/10.1007/s12011-019-01768-6. PMid:31175633.

MIROSHNIKOV, S.A., ZAVYALOV, O.A., FROLOV, A.N., BOLODURINA, I.P., KALASHNIKOV, V.V., GRABEKLIS, A.R., TINKOV, A.A. and SKALNY, A.V., 2017. The reference intervals of hair trace element content in hereford cows and heifers (bos taurus). *Biological Trace Element Research*, vol. 180, no. 1, pp. 56-62. http://doi.org/10.1007/s12011-017-0991-5. PMid:28315118.

MONTANINI, B., BLAUDEZ, D., JEANDROZ, S., SANDERS, D. and CHALOT, M., 2007. Phylogenetic and functional analysis of

the Cation Diffusion Facilitator (CDF) family. *BMC Genomics*, vol. 8, no. 1, pp. 107. http://doi.org/10.1186/1471-2164-8-107. PMid:17448255.

NAROZHNYKH, K., 2023. Prediction models of iron level in beef muscle tissue toward ecological well-being. *Global Journal Environmental Science and Management*, vol. 9, no. 4, pp. 833-850. http://doi.org/10.22034/gjesm.2023.04.12.

NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE - NASEM, 2016. *Nutrient requirements of beef cattle*. 8th ed. Washington: The National Academies Press. http://doi.org/10.17226/19014.

NOTOVA, S.V., LEBEDEV, S.V., MARSHINSKAIA, O.V., KAZAKOVA, T.V. and AJSUVAKOVA, O.P., 2023. Speciation analysis of manganese against the background of its different content in the blood serum of dairy cows. *Biometals*, vol. 36, no. 1, pp. 35-48. http://doi.org/10.1007/s10534-022-00456-8. PMid:36282443.

NUURTAMO, M., VARO, P., SAARI, E. and KOIVISTOINEN, P., 1980. Mineral element composition of Finnish foods. *Acta Agriculturae Scandinavica*, suppl. 22, pp. 57-70.

ORJALES, I., HERRERO-LATORRE, C., MIRANDA, M., REY-CRESPO, F., RODRÍGUEZ-BERMÚDEZ, R. and LÓPEZ-ALONSO, M., 2018. Evaluation of trace element status of organic dairy cattle. *Animal*, vol. 12, no. 6, pp. 1296-1305. http://doi.org/10.1017/S1751731117002890. PMid:29103395.

PEREIRA, V., CARBAJALES, P., LOPEZ-ALONSO, M. and MIRANDA, M., 2018. Trace element concentrations in beef cattle related to the breed aptitude. *Biological Trace Element Research*, vol. 186, no. 1, pp. 135-142. http://doi.org/10.1007/s12011-018-1276-3. PMid:29478230.

PILARCZYK, R., 2014. Concentrations of toxic and nutritional essential elements in meat from different beef breeds reared under intensive production systems. *Biological Trace Element Research*, vol. 158, no. 1, pp. 36-44. http://doi.org/10.1007/s12011-014-9913-y. PMid:24526320.

PORTNOY, M.E., LIU, X.F. and CULOTTA, V.C., 2000. Saccharomyces cerevisiae expresses three functionally distinct homologues of the NRAMP family of metal transporters. *Molecular and Cellular Biology*, vol. 20, no. 21, pp. 7893-7902. http://doi.org/10.1128/MCB.20.21.7893-7902.2000. PMid:11027260.

PULS, R., 1994. *Mineral levels in animal health*. 2nd ed. Clearbrook: Sherpa International.

RACINE, J.S., 2012. RStudio: a platform-independent IDE for R and Sweave. *Journal of Applied Econometrics*, vol. 27, no. 1, pp. 167-172. http://doi.org/10.1002/jae.1278.

RAHIL-KHAZEN, R., BOLANN, B.J. and ULVIK, R.J., 2002. Correlations of trace element levels within and between different normal autopsy tissues analyzed by Inductively coupled plasma atomic emission spectrometry (ICP-AES). *Biometals*, vol. 15, no. 1, pp. 87-98. http://doi.org/10.1023/A:1013197120350. PMid:11860027.

RAMOS, A., CABRERA, M.C. and SAADOUN, A., 2012. Bioaccessibility of Se, Cu, Zn, Mn and Fe, and heme iron content in unaged and aged meat of Hereford and Braford steers fed pasture. *Meat Science*, vol. 91, no. 2, pp. 116-124. http://doi.org/10.1016/j.meatsci.2012.01.001. PMid:22296840.

RASBECH, N.O., 1968. Mangankonzentrationen in Ovarien von Farsen gefiittert mitvariierenden Manganmangen. *Reproduction in Domestic Animals*, vol. 3, no. 2, pp. 57-62. http://doi.org/10.1111/j.1439-0531.1968.tb00048.x.

ROSCH, J.W., GAO, G., RIDOUT, G., WANG, Y.D. and TUOMANEN, E.I., 2009. Role of the manganese efflux system *mntE* for signalling and pathogenesis in *Streptococcus pneumonia*. *Molecular Microbiology*, vol. 72, no. 1, pp. 12-25. http://doi.org/10.1111/j.1365-2958.2009.06638.x. PMid:19226324.

SPEARS, J.W., 2011. Importance of manganese in cattle and poultry. *Salt Institute Newsletter*, 1-6.

SARBAN, S., ISIKAN, U., KOCABEY, Y. and KOCYIGIT, A., 2007. Relationship between synovial fluid and plasma manganese, arginase, and nitric oxide in patients with rheumatoid arthritis. *Biological Trace Element Research*, vol. 115, no. 2, pp. 97-106. http://doi.org/10.1007/BF02686022. PMid:17435254.

SAWHNEY, P.C. and KEHAR, N.D., 1961. Variations in the manganese content of blood and tissues of cattle. *Annals of Biochemistry and Experimental Medicine*, vol. 21, pp. 125-128. PMid:13746944.

SILVA, F.L., OLIVEIRA-JÚNIOR, E.S., SILVA, M.H.M., LÓPEZ-ALONSO, M. and PIERANGELI, M.A.P., 2022. Trace elements in beef cattle: a review of the scientific approach from one health perspective. *Animals (Basel)*, vol. 12, no. 17, pp. 2254. http://doi.org/10.3390/ani12172254. PMid:36077974.

TAYLOR, A., 1996. Detection and monitoring of disorders of essential trace elements. *Annals of Clinical Biochemistry*, vol. 33, no. 6, pp. 486-510. http://doi.org/10.1177/000456329603300603. PMid:8937580.

TSYGANKOVA, A.R., KUPTSOV, A.V., NAROZHNYKH, K.N., SAPRYKIN, A.I., KONOVALOVA, T.V., SEBEZHKO, O.I., KOROTKEVICH, O., PETUKHOV, V.L. and OSADCHUK, L., 2017. Analysis of trace elements in the hair of farm animals by atomic emission spectrometry with Dc Arc excitation sources. *Journal of Pharmaceutical Sciences and Research*, vol. 9, no. 5, pp. 601-605.

UTAMI, M.T. and PANGESTU, E., 2017. Status mineral mangan pada sapi potong di daerah aliran sungai jratunseluna (Mineral manganese status of beef cattle at Jratunseluna Watershed). *Agromedia*, vol. 35, no. 1, pp. 42-49.

VAN SAUN, R.J., 2013. Nutritional requirements. In: C. CEBRA, D.E. ANDERSON, A. TIBARY, R.J. VAN SAUN and L.W. JOHNSON, eds. *Llama and alpaca care: medicine, surgery, reproduction, nutrition, and herd health*. USA: Elsevier Inc., pp. 59-80.

YANG, M., JENSEN, L.T., GARDNER, A.J. and CULOTTA, V.C., 2005. Manganese toxicity and *Saccharomyces cerevisiae* Mam3p, a member of the ACDP (ancient conserved domain protein) family. *The Biochemical Journal*, vol. 386, no. Pt 3, pp. 479-487. http://doi.org/10.1042/BJ20041582. PMid:15498024.

ZAHARIK, M.L., CULLEN, V.L., FUNG, A.M., LIBBY, S.J., KUJAT CHOY, S.L., COBURN, B., KEHRES, D.G., MAGUIRE, M.E., FANG, F.C. and FINLAY, B.B., 2004. The *Salmonella enterica* serovar typhimurium divalent cation transport systems MntH and SitABCD are essential for virulence in an Nramp1G169 murine typhoid model. *Infection and Immunity*, vol. 72, no. 9, pp. 5522-5525. http://doi.org/10.1128/IAI.72.9.5522-5525.2004. PMid:15322058.

ZHANG, H.H., ZHOU, N., ZHANG, T.T., BAO, K., XU, C., SONG, X.C. and LI, G.Y., 2014. Effects of different dietary manganese levels on growth performance and N balance of growing mink (neovision vision). *Biological Trace Element Research*, vol. 160, no. 2, pp. 206-211. http://doi.org/10.1007/s12011-014-0008-6. PMid:24958021.