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Correlation between age and levels of heavy metals in mares' colostrum and milk

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SUMMARY

The content of heavy metals in the mammary gland secretions of mares should be tested not only because of the possibility of human consumption of mare's milk, but also because these elements influence the development of foals in the suckling period and later in adolescence. The aim of the research was to assess the dependence of the concentration of heavy metals in mammary gland secretions on the age of the mare. The study was carried out on 16 Thoroughbred mares. Mares aged 4 to 19 years were divided into 2 research groups: Group Y – young mares ≤ 10 years of age (n = 8) and Group O – mares older than 10 years of age (n = 8) The experimental material consisted of colostrum collected up to 12 hours after delivery and milk collected on the 3rd day after foaling. The concentrations of metals (Zn, Cu, Ni, Mn, Fe, Pb, and Cd) were determined by atomic absorption spectrometry (Unicam 929 AAS spectrometer). All elements (Ni, Zn, Cu, Mn, Fe, and Cd) analysed in the colostrum showed higher concentrations in young mares. Highly significant differences were noted for Ni and Cd and significant differences for Fe. The statistical analysis showed highly significant differences for milk Cu content between older and young mares; highly significant differences for Cu and Cd and significant differences for Zn between the milk and colostrum of older mares; and highly significant differences for Cu and significant differences for Cd between the milk and colostrum of younger mares. Lead (Pb) was not detected in the samples.

KEY WORDS: Mares, colostrum, milk, heavy metals

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INTRODUCTION

Heavy metals are a problem that has received widespread attention around the world. The importance of the toxic effects of these minerals lies in their non-biodegradability and low concentrations (Ismail et al., 2014). In terms of nutrition, metals in milk and dairy products can be grouped into essential elements (iron, copper, manganese, and zinc), toxic essential metals (nickel and chromium) or toxic metals (lead and cadmium). The essential elements iron, copper, manganese and zinc are of nutritional importance in infant development, but the presence of Pb and Cd, even in low concentrations, leads to metabolic disorders with extremely serious consequences (Khan et al., 2008). Dairy animals absorb metals while grazing on the pasture and when fed contaminated concentrate feeds. In cows, however, transfer of minerals to milk is highly variable (Mass et al., 2011). Maternal milk may sometimes contain chemical contaminants which could have adverse effects on nursing young. Persistent hazardous environmental chemicals are key sources of heavy metal contamination of colostrum and milk. Increasing environmental pollution necessitates assessment of heavy metal exposure. Exposure of suckling mammals to toxic heavy metals through milk may constitute a hazard, especially when the initial burden is already raised due to placental transfer. Infants are much more sensitive to the effects of environmental pollutants, including heavy metals, due to the immaturity of some organs and systems and hence the reduced efficiency of mechanisms of toxin excretion from the body (Krzywy et al., 2011). There are also significant differences between the youngest population group and adults in terms of metabolism and biotransformation of xenobiotics already accumulating in the body (Nieć et al., 2013). Exposure to heavy metals in the neonatal period often causes long-term health effects which are not observed until adolescence or adulthood. They mainly manifest themselves in impaired metabolic processes, as well as disturbances in the functioning of the nervous and hematopoietic systems. The toxicity and carcinogenicity of heavy metals are dose-dependent. High-dose exposure leads to severe responses in animals, increasing the risk of DNA damage and neuropsychiatric disorders (Gorini et al., 2014). The toxic mechanism of various heavy metals functions in similar pathways, usually via generation of reactive oxygen species (ROS), enzyme inactivation, and suppression of antioxidant defence. Some of them, however, cause toxicities in a specific pattern and bind selectively to specific macromolecules. The adverse health effects of heavy metals on children are much higher than in adults. Studies have found that heavy metal exposure is associated with low immune function in children. Exposure to heavy metals, particularly Pb, Cd, As, and Hg, not only reduced lymphocyte numbers and suppressed adaptive immune responses in children, but also altered the innate immune response, impairing the body's ability to fight pathogens. Epidemiological evidence suggests that heavy metal exposure alters cytokine levels and is associated with the development of inflammatory responses in children. Pb, As, and Hg exposure was associated with vaccination failure and decreased antibody titres, as well as increased risk of immune-related diseases in children due to altered specific immunoglobulin levels (Zheng et al., 2023). There are differences between young and adult mammals regarding the toxicokinetic aspects and clinical manifestations of heavy metal intoxication. Toxic Cd intake causes microcytotic hypochromic anaemia in young rats at lower exposure levels and after shorter exposure periods than in adult animals. Cd absorption is increased by co-administration of milk. After long exposure periods, toxic Cd concentrations accumulate in the kidney cortex, a process that begins very early in life. Hg++

accumulation in the brains of suckling rats is about 10 times as high as in grown animals. Milk increases the bioavailability of Hg^{++}. In suckling rats Hg is bound to ligands in the erythrocytes to a greater extent than in adult rats. Exposure to Hg during lactation increases oxidative stress and can contribute to the pathogenesis of health problems. This is particularly dangerous during neuronal development (Al-Saleh et al. 2013). Methyl-Hg concentrations in milk can be up to 5% of those in maternal plasma, which is a serious hazard for breastfed children of exposed mothers. Pb absorption is increased in the neonatal period. Toxic Pb concentrations can lead to Pb encephalopathy. Anaemia and neurological disorders have been observed in children after exposure to very low levels of Pb. Heavy metal concentrations were observed in the same order of magnitude in commercial infant formulas and in breast milk. When infant formulas are reconstituted with contaminated tap water, however, Pb and Cd concentrations can be much higher. This applies equally to formulas for children and milk replacers for animals (Schümann 1990).

The content of heavy metals in the secretions of the mare's mammary gland should be tested not only because of the possibility of human consumption of mare's milk, but also because these elements influence the development of foals in the neonatal period and later in adolescence.

The aim of the study was to assess the dependence of heavy metal concentrations in the mammary gland secretions on the age of the mare.

MATERIALS AND METHODS

Animals

The study was carried out on 16 Thoroughbred mares. All mares were kept in the same stable in individual boxes (size 2.15 × 3.50 m) on permanent straw bedding at the Krasne Stud farm (Masovian Voivodeship). All animals were clinically healthy throughout the experimental period. Mares were fed ad libitum on hay (*Lolium* 40%, *Trifolium* L.20%) with the addition of oat in the amount of 1.5 kg/mare per day (according to Institute of Physiology and Animal Nutrition standards, 1997). Animals had access to automatic water drinkers (water flow ~10 l/min). Mares aged 4 to 19 were divided into 2 research groups:

Group Y - young mares ≤ 10 years of age (n = 8)

Group O - older mares >10 years of age (n = 8)

Experimental procedures

The experimental material consisted of mammary gland secretions. Colostrum was collected up to 12 hours after delivery, and milk was collected on the 3rd day after foaling. A 10 ml sample of secretions was collected each time. The material was collected during a period of one month (February 2020) and stored at −24°C until analysis.

Determining metal concentrations

Samples of mare mammary gland secretions were mineralized with 10 ml of a 1:3 mixture of perchloric acid (70% $HClO4$) and nitric acid (65% $HNO3$) for about 24 h, at room temperature. The samples were then subjected to thermal mineralization with the Velp 20/26 mineralizer, gradually increasing the temperature from 100°C to 180°C for 6–7 h. The resulting clear liquid was then diluted to 10 ml with deionized water. The concentrations of metals (Zn, Cu, Ni, Mn, Fe, Pb, and Cd) were determined by atomic absorption spectrometry (Unicam 929 AAS spectrometer) (Agemian et al., 1980). The results were read on a standard curve using atomic absorption standards based on those developed at the Central Office of Measures in Warsaw. Results are shown in milligrams per kilogram of mare mammary gland secretion.

Statistical analysis

The SAS statistical analysis software package (SAS 2014) was used for statistical analysis for the levels of nickel (Ni), zinc (Zn), copper (Cu), manganese (Mn), iron (Fe) and cadmium (Cd). Basic statistics (mean, median, minimum and maximum, and number of observations) were estimated. Then the normality of the distribution was tested for all features, and a significant deviation from the normal distribution was found in each of them. Therefore, a non-parametric one-way analysis of variance (NPAR1WAY procedure of SAS) with the Wilcoxon–Mann–Whitney test was used to analyse the significance of the influence of the age group (young and older mares) and type of material (colostrum, milk). The level of lead (Pb) was not tested because it was not present in the colostrum or milk samples.

RESULTS AND DISCUSSION:

None of samples had Pb concentrations above the limit of quantification (Table 1 and 2), so lead concentration was omitted from the analyses. For all other elements, all concentrations were above the limit of quantification. In the group of younger mares, the levels of the elements were ranked as follows: Fe>Ni>Zn>Cu>Mn>Cd in milk and Fe>Zn>Ni>Cu>Mn>Cd in colostrum. In both age groups the analysis showed higher concentrations of nickel (Ni), manganese (Mn), iron (Fe) and cadmium (Cd) in milk samples compared to colostrum. On the other hand, the contents of zinc (Zn) and copper (Cu) were higher in colostrum than in milk. Lead (Pb) was not detected in any of the samples. The statistical analysis showed highly significant differences between the milk and colostrum of younger mares for Cu content, and significant differences for Cd content (Table 1).

Table 1.
Content of heavy metals (mg/kg) in the mammary gland secretions of young mares

Dependent variable	Type of secretion	Median	Min	Max	S*	p-Value
Ni	**Milk**	1.459	1.191	8.002	66.0	0.8785
	colostrum	2.654	0.803	3.369		
Zn	**Milk**	6.576	5.713	7.025	84.0	0.1049
	colostrum	11.170	4.219	15.810		
Cu	**Milk**	0.559	0.507	0.616	100.0	*0.0002*
	colostrum	1.188	0.982	1.455		
Mn	**Milk**	0.340	0.114	0.884	52.0	0.1049
	colostrum	0.168	0.072	0.355		
Fe	**Milk**	7.184	5.934	49.100	78.0	0.3282
	colostrum	10.114	6.490	14.390		
Cd	**Milk**	0.074	0.066	0.086	47.5	*0.0303*
	colostrum	0.064	0.060	0.077		

*S – Wilcoxon–Mann–Whitney test value

In the group of older mares, the concentrations of the elements were ranked as follows: Fe> Zn> Ni>Cu>Mn>Cd in milk and Zn> Fe> Cu>Ni>Mn>Cd in colostrum. The statistical analysis showed highly significant differences between the milk and colostrum of older mares for Cu and Cd and significant differences for Zn (Table 2).

Table 2

Content of heavy metals (mg/kg) in the mammary gland secretions of older mares

Dependent variable	Type of secretion	Median	Min	Max	S*	p-Value
Ni	**Milk**	1.299	0.504	2.993	56.0	0.2345
	Colostrum	0.787	0.280	1.768		
Zn	**Milk**	6.4535	5.052	7.030	90.0	*0.0207*
	Colostrum	8.035	5.987	9.939		
Cu	**Milk**	0.723	0.674	1.060	97.0	*0.0011*
	Colostrum	1.101	0.970	1.247		
Mn	**Milk**	0.139	0.060	0.284	61.0	0.5054
	Colostrum	0.107	0.064	0.203		
Fe	**Milk**	7.146	5.402	15.500	52.0	0.1049
	Colostrum	6.079	3.709	8.514		
Cd	**Milk**	0.065	0.029	0.079	41.5	*0.0031*
	Colostrum	0.026	0.010	0.040		

*S – Wilcoxon–Mann–Whitney test value

Ni, Zn, Mn, Fe and Cd content were higher in the milk samples from young mares, while Cu content was higher in the milk of older mares. The statistical analysis showed highly significant differences for Cu content in milk from older vs young mares (Table 3).

Table 3

The content of heavy metals (mg/kg) in the milk of older and young mares

Dependent variable	Age group	Median	Min	Max	S*	p-Value
Ni	**young mares**	1.459	1.191	8.002	55.0	0.1949
	older mares	2.654	0.803	3.369		
Zn	**young mares**	6.576	5.713	7.025	62.0	0.5737
	older mares	11.170	4.219	15.810		
Cu	**young mares**	0.558	0.507	0.616	100.0	*0.0002*
	older mares	1.188	0.982	1.455		
Mn	**young mares**	0.340	0.114	0.884	52.0	0.1049
	older mares	0.168	0.072	0.355		
Fe	**young mares**	7.183	5.934	49.100	60.0	0.4418
	older mares	10.114	6.490	14.390		
Cd	**young mares**	0.074	0.066	0.086	50.0	0.0611
	older mares	0.064	0.060	0.077		

*S – Wilcoxon–Mann–Whitney test value

Concentrations of all elements (Ni, Zn, Cu, Mn, Fe, Cd) analysed in colostrum were higher in young mares. We found highly significant differences for Ni and Cd and significant differences for Fe (Table 4).

Table 4
Content of heavy metals (mg/kg) in the colostrum of older and young mares

Dependent variable	Age group	Median	Min	Max	S*	p-Value
Ni	**young mares**	2.654	0.803	3.369	42.0	*0.0047*
	older mares	1.459	1.191	8.002		
Zn	**young mares**	11.170	4.219	15.810	54.0	0.1605
	older mares	6.576	5.713	7.025		
Cu	**young mares**	1.188	0.982	1.455	54.0	0.1605
	older mares	0.558	0.507	0.616		
Mn	**young mares**	0.168	0.072	0.355	54.0	0.1605
	older mares	0.340	0.114	0.884		
Fe	**young mares**	10.114	6.490	14.390	44.0	*0.0104*
	older mares	7.183	5.934	49.100		
Cd	**young mares**	0.064	0.060	0.077	36.0	*0.0002*
	older mares	0.074	0.066	0.086		

*S – Wilcoxon–Mann–Whitney test value

Kazhanova investigated the seasonal content of heavy metals in milk from horses in Kazakhstan and found that the Cd concentration varied from 0.01 to 0.02 mg/L, and the Pb concentration from 0.03 to 0.15 mg/L. The present study showed higher Cd concentrations, of 0.066 and 0.029 mg/kg in young and old mares, respectively, while Pb was not detected in any of the test samples. The study cited showed that Cd and Pb concentrations were lower in summer than in autumn (Kazhanova et al., 2021). The discrepant data may be due to differences in environmental pollution with these metals in the regions where the horses were raised. Grace et al. observed changes in the concentrations of elements on consecutive days of lactation in thoroughbred mares. The concentrations of Mg, Na, K, S, Cu, Fe and Zn were higher in the colostrum than in milk (Grace et al., 1999). Our findings are partially in agreement with those cited. We observed higher concentrations of Zn and Cu in the colostrum than in the milk, while Fe content was higher in the milk of both older and younger mares.

Heavy metals enter the mammary gland of various mammalian species. Most available results pertain to cow milk, due to its common use for human consumption (Pietrzak-Fiećko et al., 2013; Dobrzański et al., 2009; Patra et al., 2008; Simsek et al., 2000; Domagała and Kisza 1996). Domagała and Kisza (1996) analysed raw cow milk collected from various farms in the Lesser Poland voivodeship for heavy metals (Pb, Zn, and Cu) and found Pb content of 0.19– 0.24 mg/kg, Cu 0.6–0.78 mg/kg and Zn 6.1–7.4 mg/kg. In our study Pb was not detected in mare milk, while the other elements analysed were present at similar levels to those reported by Domagała and Kisza (1996). According to WHO standards, the highest permissible levels of these elements in raw cow milk are 0.15 mg/kg for Pb, 0.5 mg/kg for Cu, and 5 mg/kg for Zn. Domagała and Kisza

(1996) reported that the content of these metals in milk exceeded WHO standards by 0.04–0.09 mg/kg in the case of Pb, by 0.1–0.28 mg/kg for Cu, and by 1.1–0.24 mg/kg for Zn.

Simsek (2000) analysed 75 samples of cow milk collected from rural and industrial areas for the content of arsenic (As), lead (Pb), zinc (Zn), copper (Cu), and iron (Fe). Higher contamination of heavy metals was reported for samples from industrial areas compared to rural areas. The results showed a high correlation between environmental pollution and the environment where the milk was collected. The present study was conducted in a rural area, which may explain the absence of Pb in the samples. Reports of environmental pollution affecting heavy metal content in mare milk are rare. The presence of lead in human milk does not necessarily result from the mother's direct exposure during pregnancy or lactation. This heavy metal has a tendency to deposit in bones, where it remains for life, as evidenced by the higher concentration of lead in the milk of women aged over 30 (Chao et al. 2014).

Studies have been undertaken to assess the effect of age on the accumulation of heavy metals in the hair of horses. An analysis of hair from the mane revealed different levels of cadmium and mercury in animals of different ages. Iwase et al. (2020) analysed 30 mares, of which 17 were 3–20 years old and 13 were foals up to the age of 2 years. In the younger horses, the content of mercury in the hair ranged from 0.015 ug/g to 0.031 ug/g, depending on where the samples were collected, while the range in older horses was 0.009 ug/g to 0.016 ug/g. The authors also reported differences in cadmium content, which ranged from 0.049 ug/g to 0.143 ug/g in young horses and from 0.033 ug/g to 0.062 ug/g in older horses. The authors pointed out that differences in metal content may be caused by the non-stabilized oestrous cycle in young horses and higher levels of sex hormones, especially oestrogens. Similar results were found in the present study, in which young horses had higher content of heavy metals in their milk and colostrum, which may be linked to levels of sex hormones. This hypothesis may be supported by Gorlach et al. (1996), who analysed cadmium levels in tissues of wild roe deer. The highest cadmium content was found in the tissues of roe deer at the age of 2–4 years, by which they have reached sexual maturity, so that sex hormone levels may be higher.

Malmsten analysed kidneys from female wild boar from Sweden and found higher Mg and Mn levels in juveniles than in older wild boars; this age effect had not, to our knowledge, been described before (Malmsten et al., 2021). We observed a similar relationship for Mn in our own research. In the same study, adult boars had significantly higher average concentrations of Cd, while the opposite was observed in the present study. The type of tissue that has been tested may be of critical importance.

An analysis of lead content in human breast milk did not support this hypothesis. Shawahna (2021) found no differences in the content of this element in the milk of women before and after the age of 25. This may be explained by the stable hormonal balance between the ages of 20 and 25 years. In the present study, higher content of heavy metals was observed in milk compared to colostrum. Similar results were obtained by Szukalska et al. (2021), who analysed heavy metal content in human colostrum and milk and reported higher content in milk. Turan et al. (2001) also reported higher cadmium, nickel and lead content in milk samples compared to colostrum. Research on mare's milk rarely shows content of heavy metals exceeding acceptable limits. In an analysis by Pietrzak-Fiećko et al. (2013), only some samples exceeded the lead standard. In that study, 14 samples from mares from the Warmia and Mazury region were analysed. The mean level of lead was 0.0180 mg/kg of milk, and the highest content was 0.0340 mg/kg. In 29% of samples the acceptable limit for lead content was exceeded by 0.02 mg/kg. This is in contrast to our results, as lead was not detected in any of the samples. It should be noted that there are reports indicating that heavy metal content in milk has been decreasing in recent years (Bakuła et al., 2013). A study conducted in Romania assessed the impact of the environment on the penetration of heavy metals into cows' milk. Cu, Pb, Zn, and Cd were detected in all soil, water, forage, and milk samples, suggesting possible pollution with these elements. The mean content of the metals, in decreasing order, was Zn > Pb > Cu > Cd in the case of soil and Zn > Cu > Pb > Cd in the case of water, forage, and milk (Miclean at al. 2019). In the present study, we observed Fe > Ni > Zn > Cu > Mn > Cd in the milk of young mares and Fe > Zn > Ni > Cu > Mn > Cd in the milk of older mares. In the study cited, the alert threshold was exceeded in more than two-thirds of the soil samples for Pb and Cd and one-third of the soil samples for Cu and Zn, while the intervention threshold was exceeded in about half of the samples for Pb and Cd and in one sample for Cu. The authors generally observed high variability in the content of all metals, probably due to the presence of legacy pollution sources related to mining and ore processing, especially poorly managed mine tailings that favour the dispersion of metal-rich dust in the surrounding area. These data confirmed that soil is the main metal pollution vector of vegetation, which is passed on to food. Animals ingest metals mainly with water and food (Miclean at al. 2019).

CONCLUSION

Heavy metal contamination of the body and mammary gland secretions of horses is very rarely reported in the literature, as mare's milk is not used for human consumption as often as cow, sheep or goat milk. An interesting point which should be analysed is the influence of steroid hormones on the accumulation of heavy metals in tissues and secretions. This should be taken into consideration not only because of the threat to human health in the case of consumption of mare milk, but also because contaminated milk poses a threat for young foals. To prevent accumulation of heavy metals, special attention must be paid to the quality of feed and water, which should meet all applicable standards.

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