Praca oryginalna *Original paper*

Level of accumulation of selected heavy metals in horse tissue as a function of age

MARIUSZ RUDY, AGATA ZNAMIROWSKA, MAREK ZIN

Department of Biology and Agriculture, Rzeszów University, Faculty of Food Processing and Science of Commodities, ul. Ćwiklińskiej 2, 35-601 Rzeszów

Rudy M., Znamirowska A., Zin M.

Level of accumulation of selected heavy metals in horse tissue as a function of age

Summary

The level of accumulation of selected heavy metals (Pb, Cd, Hg, As) in horse tissues belonging to four age groups, each containing 20 horses, was studied in order to determine the relationship between age and level of meat contamination. The content of the above mentioned elements was analyzed in horses killed between 2002 and 2005 to determine any year-to-year increase. In order to determine the chemical composition of the meat a sample was taken from the longissimus dorsi muscle at the height of the last thoracal vertebrae. The study showed that meat contamination rose with the age of horses and additionally depended on the year of killing.

Keywords: horse meat, heavy metals

Amongst the numerous substances which are toxic to humans, are heavy metals such as lead, cadmium, mercury and arsenic. Even very low concentrations of these substances can disturb the metabolism, decrease its efficiency, and depress immunological and enzymatic processes, which results in various diseases and/or causes death.

The use of metals has increased along with the development of industry, particularly in such fields as metallurgy, mining, electrical technology and the chemical industry. This has created a danger not only for the people employed in these kinds of industries, but also for the people living in industrialized areas. Food, air and water have turned out to be the main sources of metals and metalloids. Mercury, cadmium and lead, are examples of toxic metals which cause acute and chronic poisonings, both in industry and in the surrounding environment.

The most typical toxicity mechanism of metals is based on altering protein synthesis and disturbing the production of ATP, which causes damage to cells, mainly the membranes of organelles (mitochondria, lysosomes and nuclei). Most metals react with sulfhydryl, carboxylic and phosphate groups of various biological ligands, and some metals have additionally carcinogenic properties.

Horses are long-living animals, and therefore their meat can accumulate excessive amounts of harmful substances (pesticides, heavy metals). Due to the high toxicity of the above mentioned chemicals, both Polish and international regulations determine their maximum allowed concentrations in food products. The content of these compounds and elements is analyzed in every monitoring program, particularly in highly contaminated regions.

This study is an example of such a monitoring program, hence the aim of it in this case was to measure the level of accumulation of selected heavy metals (Pb, Cd, Hg, As) in horse tissues belonging to different age groups in order to determine the relationship of age versus contamination. Additionally we looked at the concentrations of heavy metals in animals killed in different years (2002-2005) to detect possible temporal patterns.

Material and methods

The study was carried out in one meat factory in south-eastern Poland from 2002-2005. The study material included both foals (weighing 250-320 kg) and adult horses classified as quality category II of slaughter animals (largest export group, 75%) weighing 550-650 kg. Animals bought in a buying station were then transported to the slaughter house where they underwent pre-slaughter fasting. The horses were divided into four age groups of 20 individuals each: group I – foals, up to 2 years old, group II – 2-7 years old (young horses), group III – 7-14 years old (middle-aged horses), group IV – over 14 years (old horses). The age of the animals was estimated by looking at teeth and using purchasing documents. Slaughtering was performed in summer and autumn. Sex ratio was 1 : 1.

After transportation the horses were directed to a special waiting hall, where after rest and 24 hour fasting they

were slaughtered using the standard procedures of the meat industry. The carcasses were halved along the spinal cord and across the pelvic bone at the pubis, so that the medulla was uncovered. The tail was cut off from the right half-carcass during post-slaughtering treatment. Then the half-carcasses were cooled using an air current and kept at 0-4°C. After 48 hours the half-carcasses were dissected.

In order to determine the chemical composition of the meat a sample was taken from the longissimus dorsi muscle at the height of the last thoracal vertebrae. Meat samples were cleaned from outer fat, connective tissue and sinews. Water content was measured according to the PN-ISO 1442:2000 norm. Protein content was measured using the Kjeldahl method, where the measured nitrogen content was used to estimate the protein content using the PN-75/A-04018 norm. Fat content was measured using the Soxhlet method, according to the PN-ISO 1444:2000 norm and total ash content was measured according to the PN-ISO 936:2000 norm.

Material for measuring heavy metal contents (arsenic, cadmium, lead and mercury) was taken from the same longissimus dorsi muscles, whereas fragments of liver were taken from the caudate lobe of liver.

Lead and cadmium content was measured using atomic absorption spectrometry AAS with graphite furnace atomization (GFAAS) after pressure mineralization and using Zeeman background correction. Mercury content was measured using cold vapor atomic absorption spectrometry (CVAAS) after pressure mineralization, and arsenic content was measured according to the PN-59/A-04010 norm. Metal content in the measured tissues was expressed in mg/kg of fresh mass.

Results and discussion

Horse meat is a specific material, differing from other kinds of meat in many chemical and physical parameters. It contains a larger proportion of protein and little fat with large proportions of unsaturated fatty acids (4, 6).

The results of the study suggest that the chemical composition of meat depends on age (tab. 1). Meat from foals and young carcasses contains more water, and less protein, fat and ash, whereas in older animals the meat contains less water and more other chemicals. Additionally it must be mentioned that these results are close to the results of other studies (2, 17). Korzeniowski et al. (5) state that with increasing age the meat of horses contains less and less water and more fat and minerals.

In 2002 within the program entitled Monitoring of Soils, Plants, Agricultural and Gastronomic Products, pigs, cattle, chickens and game were studied in respect to heavy metal content in 100 sites throughout the country. The results of the study revealed some cases where milk, meat, liver and kidneys contained heightened amounts of lead, cadmium, mercury and arsenic, although these levels did not exceed the permitted weekly heavy metal intake (15).

The content of heavy metals in horse tissues in 2002--2005, in this study, is presented in tab 2. The lowest lead and cadmium content, both in meat and liver, was found in 2002, and it gradually increased in successive years. For example the Pb content in meat was 0.049 mg/kg in 2002, and had increased by 14% in 2003, by 73% in 2004 and by as much as 243% in 2005; whereas the lead content in liver increased (from the level of 0.128 mg/kg in 2002) by 19%, 40% and 126% respectively. It should be noted that in 2005 its content in meat exceeded the permitted level of 0.1 mg/kg (European Commission Regulation nr 466/2001). In the 1980s in studies by Żmudzki et

Tab. 1. Chemical composition of horse meat of various ages (%) (n = 20; $\bar{x} \pm s$)

Composition of muscle tissue	Groups								$\bar{x}$
	I		II		III		IV		
Water	76.30	5.60	73.10	7.20	71.20	4.80	67.70	7.20	71.15
Protein	19.50	2.30	21.40	4.00	23.10	2.90	25.20	3.10	22.30
Fat	2.70	0.40	3.10	0.70	3.70	0.60	4.80	0.50	3.50
Ash	0.80	0.20	1.20	0.40	1.40	0.30	1.70	0.50	1.20

Tab. 2. Heavy metals content in horse tissues in 2002-2005 (mg/kg) (n = 20)

Heavy metal	2002			2003			2004			2005		
	$\bar{x}$	x_{min}	x_{max}	$\bar{x}$	x_{min}	x_{max}	$\bar{x}$	x_{min}	x_{max}	$\bar{x}$	x_{min}	x_{max}
Meat: Pb	0.049	0.017	0.063	0.056	0.023	0.074	0.085	0.047	0.0175	0.168	0.081	0.298
Cd	0.037	0.017	0.058	0.084	0.049	0.098	0.161	0.030	0.1890	0.269	0.029	0.525
Hg	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Liver: Pb	0.128	0.062	0.231	0.148	0.068	0.258	0.179	0.085	0.321	0.289	0.075	1.204
Cd	1.323	0.543	1.987	1.514	0.658	2.480	4.594	0.857	6.652	7.658	0.892	20.457
Hg	0.012	0.001	0.019	0.013	0.006	0.025	0.043	0.005	0.069	0.075	0.008	0.132
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Explanation: nd – no detected

al. (18) the average level of lead was 0.086 mg/kg and it exceeded the norm only in 1982 (0.11 mg/kg), whereas in this monitoring study the level of lead in liver was five times higher.

Cadmium is more dangerous for human and animal health, being additionally carcinogenic. Lind et al. (9) found that a key factor in the bioaccumulation of this element is feeding animals with contaminated fodder. In this study the concentration of cadmium was 0.037 mg/kg in 2002 and increased in consecutive years by 127%, 335% and 627% respectively. The Cd content in muscles exceeded the allowed norm (0.2 mg/kg) in 2005. In liver its concentration exceeded the norm for this tissue (0.5 mg/kg) in all the studied years. In 2002 the concentration was 1.323 mg/kg and increased in consecutive years, being seven times higher in 2005 compared to 2002. In 2005 in one case the concentration of this element in liver was 40 times higher than the norm. This is why horse livers should not be eaten. High concentrations of cadmium in horse meat and liver have been confirmed in many studies (3, 11-14, 16). For instance Baldini et al. (1) found 0.041 mg/kg of cadmium in muscles and 2.10-2.46 mg/kg in horse liver.

The circulation of mercury in nature enables not only its acquisition into food via water, but also its accumulation in living organisms. More than half of all the mercury in the muscles and liver of pigs, beef, and horse meat as well as yolk and egg whites is methyl mercury. In this study (tab. 2) the mercury content in horse muscles did not exceed 0.001 mg/kg in any of the studied years (acceptable level: 0.02 mg/kg). In livers the mercury concentration was lowest in 2002 (0.012 mg/kg) and gradually increased in consecutive years. In 2005 it was over five times higher than in 2002 and reached 0.075 mg/kg, exceeding the acceptable level of 0.05 mg/kg. It must also be noted that in 2005 the differences between individual mercury levels were high. The lowest figure was 0.008 mg/kg, and the largest – 0.132 mg/kg, which is 16 times more. For comparison, Żmudzki et al. (18), found 0.012 mg/kg of mercury, and the acceptable concentration was exceeded in 1.2% of samples, whereas in horse meat they found very small amounts of mercury, between 0.002 and 0.004 mg/kg of Hg.

In this study no arsenic was detected, either in horse meat or in livers. Low concentrations of As in horse tissues are confirmed by other studies (7, 8), where the concentration level of this element was on the level of a thousandth mg/kg and in livers its was a hundredth mg/kg.

The concentration of heavy metals in horse tissues is presented in tab. 3. In no age groups was arsenic detected, either in horse meat or in livers. Additionally, the mercury concentration in muscles was lower than 0.001 mg/kg in all age groups. Litwińczuk et al. (10) also found low concentrations of mercury in horse meat (0.0003-0.0141 mg/kg), regardless of age and usage type. On the other hand, lead and cadmium contents in muscles and liver and mercury content in liver increased with the age of the animal. The lowest concentration of lead was found in horses under 2 years old – 0.037 mg/kg. In group II the content was already 0.053 mg/kg, for middle-aged horses it was 2.5 times higher than for the youngest group, and for the oldest horses it was five times higher than for the youngest. In the oldest horses' category the concentration of this element was nearly twice as high as the acceptable limit of 0.1 mg/kg, and for one individual the value was three times higher than the limit. Moreover, the limit was exceeded in the muscles of one individual from age group III. As far as liver is concerned, lead concentration was nearly four times higher than in muscles in all age groups. In the middle-aged horses group the Pb concentration in liver (0.475 mg/kg) was nearly as high as the limit (0.5 mg/kg), and for the oldest horses group it exceeded the limit (0.725 mg/kg). It must be borne in mind that in individual cases of livers, the limit was exceeded both in groups III and IV. There is very high correlation between the age of horses and lead concentration, both for muscles ($r = 0.87$, $P < 0.05$), and liver ($r = 0.91$, $P < 0.05$). Such a relationship was also found by Znamirowska (17).

Tab. 3. Heavy metals content in horse tissues of various ages (mg/kg) (n = 20)

Heavy metal	Groups												r
	I			II			III			IV			
	$\bar{x}$	x_{min}	x_{max}	$\bar{x}$	x_{min}	x_{max}	$\bar{x}$	x_{min}	x_{max}	$\bar{x}$	x_{min}	x_{max}	
Meat: Pb	0.037	0.017	0.065	0.053	0.025	0.071	0.089	0.041	0.125	0.197	0.087	0.298	0.87
Cd	0.042	0.017	0.140	0.123	0.057	0.720	0.207	0.101	0.293	0.312	0.143	0.525	0.91
Hg	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	–
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–
Liver: Pb	0.145	0.062	0.210	0.253	0.099	0.459	0.475	0.145	0.705	0.725	0.202	1.204	0.91
Cd	1.125	0.543	3.523	3.275	0.860	7.548	7.254	1.112	12.853	13.273	3.561	20.457	0.83
Hg	0.003	0.001	0.007	0.017	0.001	0.023	0.034	0.001	0.043	0.061	0.010	0.132	0.53
As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	–

Explanation: as in tab. 2.

Horse tissues contain 25-50 times more cadmium compared to other species. The permitted content of this element in horse tissue, according to the European Union Regulation no. 466/2001, is 0.20 mg/kg. This means that in this study (tab. 3) the limit was exceeded for age groups III and IV, and, in individual cases, also in younger horses. The lowest Cd content in muscles was found in group I (0.042 mg/kg), and the highest in the group of oldest horses (0.312 mg/kg). However the concentration of Cd in the liver exceeds the permitted amount of this element (0.5 mg/kg) in all analyzed horse age groups: by twice the amount in group I; by 6.5 times in group II; by 14 times in group III and as much as 26 times in group IV. A very high correlation was noted between the age of the horse and the accumulation of cadmium in the muscles ($r = 0.91$), as well as between the age of the horse and the contents of this element in the liver ($r = 0.83$). The rise of cadmium content in horse tissues along with age is confirmed by the results of studies by other researchers (13, 17, 18).

Conclusions

The analysis carried out in the previous chapter allows us to formulate the following conclusions:

1. The water content in horse meat decreases along with the age of the horse, and the protein, fat and ash content increases.

2. The smallest content of lead and cadmium in the meat and liver was measured in 2002. However, in subsequent years the level of these elements increased.

3. The accumulation of lead and cadmium in the muscles and liver, as well as mercury in the liver, increased in each age group by an average of 70% in the muscles and 100% in the case of the concentration of these elements in the liver.

4. The contamination of horse meat by the aforementioned elements is clearly dependent on the age of the animal and the year in which the study was carried out.

References

1. *Baldini M., Stacchini P., Cubadda F., Miniero R., Parodi P., Facelii P.*: Cadmium in organs and tissues of horses slaughtered in Italy. Food Additives Contam. 2000, 17, 679-687.
2. *Borowski J., Rotkiewicz W., Tomczyński R.*: Zmiany w mięsie końskim w zależności od sposobu żywienia i zastosowanej obróbki cieplnej. Acta Acad. Agric. Tech. Olst. 1994, 26, 55-62.
3. *Falandysz J., Lorenc-Biała H., Centkowska D.*: Metale w mięśniach, wątrobie i nerkach zwierząt rzeźnych z regionu Polski północnej. Rocz. PZH. 1989, 40, 279-283.
4. *Jankowska B., Korzeniowski W., Kwiatkowska A.*: Fatty acid composition of horse tissues depending on their position in the carcass. Pol. J. Food Nutr. Sci. 1996, 46, 41-49.
5. *Korzeniowski W., Jankowska B., Kwiatkowska A.*: Jakość końskiej tkanki mięśniowej i tłuszczowej. Medycyna Wet. 1994, 50, 3-5.
6. *Korzeniowski W., Kwiatkowska A., Jankowska B.*: Ocena kulinarnego mięsa końskiego. Warto polubić koninę. Przegl. Gastronom. 1999, 8, 8-9.
7. *Krupa J., Zin M., Szmulik A.*: Ocena zawartości niektórych metali ciężkich w tkankach bydła i koni z regionu południowo-wschodniej Polski. Prace Tow. Naukowego w Rzeszowie 1994, 2, 35-43.
8. *Krupa J., Zin M., Szmulik A.*: Pozostałości pestycydów i niektórych metali ciężkich w tkankach koni z południowo-wschodniej Polski. Prace Tow. Naukowego w Rzeszowie 1995, 3, 87-95.
9. *Lind Y., Engman J., Jorhem L., Glynn A. W.*: Cadmium Absorption in Mice: Effects of Broiling on Bioavailability of Cadmium in Foods of Animal Origin. J. Toxicol. Environ. Health A 2001, 62, 269-280.
10. *Litwińczuk A., Kaproń H., Kaproń M., Tymochowicz U.*: Zawartość metali ciężkich (Pb, Cd, Hg) w mięsie koni z regionu południowo-wschodniej Polski. Zesz. Nauk. Przegl. Hod. 2000, 50, 333-338.
11. *Lücker E.*: Direct solid sampling ETAAS determination of cadmium in equine muscle. J. Anal. At. Spectrom. 1999, 14, 583-587.
12. *Lücker E., Kónig H., Gabriel W., Rosopulo A.*: Analytical quality control by solid sampling GFAAS in the production of animal tissue reference material: bovine liver, teeth, bone, muscle, blood and horse kidney. Fresenius J. Anal. Chem. 1992, 342, 941-949.
13. *Lücker E., Weyermann F.*: Cadium contamination of consumer-relevant muscle-tissue in horses. Fleischwirtschaft 1998, 78, 251-254.
14. *Salisbury D. C., Chan W.*: Multielement concentrations in liver and kidney tissues from five species of Canadian sławghter animals. J. Assoc. Off. Anal. Chem. 1991, 74, 587-591.
15. *Szkoda J., Żmudzki J.*: Ołów, kadm, rtęć i arsen w żywności pochodzenia zwierzęcego, ocena ryzyka. Roczn. PZH 2003, 54, 84-85.
16. *Weyermann F., Lücker E.*: Cadmiumbelastung verbraucherrelevanter Muskulatur beim Pferd. Fleischwirtschaft 1998, 78, 251-254.
17. *Znamirowska A.*: Wartość rzeźna, jakość mięsa i tłuszczu chłodzonego i mrożonego oraz poziom akumulacji związków toksycznych w zależności od wieku koni. Praca hab., Wyd. Uniwersytetu Rzeszowskiego, Rzeszów 2005.
18. *Żmudzki J., Juszkiewicz T., Szkoda J.*: Kadm w tkankach koni w Polsce. Medycyna Wet. 1991, 47, 162-164.

Author's address: dr eng. Mariusz Rudy, Department of Biology and Agriculture, Faculty of Food Processing and Science of Commodities, Rzeszów University, ul. Ćwiklińskiej 2, 35-601 Rzeszów; e-mail: mrudy@univ.rzeszow.pl