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Concentrations of microelements Al, Co, Cr, Li, Mo, Ni, Sb and Sr in the milk of Croatian Coldblood mares

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Summary

Microelements concentrations of Al, Co, Cr, Li, Mo, Ni, Sb and Sr were determined in the milk of Croatian Coldblood mares by inductively coupled plasma-optical emission spectrometry. The total mean element contents in mares milk were (μg/kg): Al<10, Co<10, Cr 26.7, Li 4.35, Mo 11.0, Ni<10, Sb 70.1 and Sr 467. Variation of Li, Sb and Sr concentrations were found throughout the lactation stages (days 10-180). Correlations were found among elements in mares milk. In general, very limited data are available on the microelements content of Al, Cr, Co, Li, Mo, Ni, Sb and Sr in mares milk. To our knowledge this is the first report of the content of these microelements in milk of mares in Croatia.

Key words: microelements, milk, horse, ICP-OES, Croatia

Introduction

The use of mares milk for human nutrition has been increasing over the past decade. Horse breeding for milk production was expanded in Central Asia (the Caucasus region, Kazakhstan, Uzbekistan, Kyrgyzstan, Tajikistan, Turkmenistan), in some parts of Russia near Kazakhstan (Kalmukia, Bashkiria), Mongolia, Siberia, North China and Tibet. To a lesser extent mares milk is produced in Eastern (Ukraine, Belarus) and Central Europe (Hungary, Austria, Bulgaria, Germany) (Park et al., 2007; Sheng and Fang, 2009).

Regarding nutritional values, differences have been found between horse, human and cow milk: the polyunsaturated fatty acid content in mares milk is higher, though nitrogen and cholesterol were lower in comparison to human and cow milk, the protein and mineral salt content in horse and human milk is lower than cow milk, the fat content in mares milk is noticeably less than in human and cow milk, the lactose content in mares milk is similar to that of human milk and higher than cow milk, the vitamin C content in mares milk is much richer than in human and cow milk, and the energy supply of mares milk is lower than human and cow milk (Solaroli et al., 1993; Malacarne et al., 2002; Sheng and Fang, 2009). Accordingly, regarding the high levels of polyunsaturated fatty acids and low nitrogen and cholesterol contents in comparison to human and cow, and the similar composition of major protein components and immunoglobulins to human milk, mares milk is very suitable for use in human nutrition (Doreau and Martin-Rosset, 2002; Massimo et al., 2002; Sheng and Fang, 2009). Fresh and fermented mares milk contain a favourable immunoglobulin composition and are traditionally used in the prevention and treatment of long and serious illness (tuberculosis), metabolic, gastrointestinal and liver problems after surgery, in the treatment of bronchitis and allergies, and are considered to stimulate immunity after chemotherapy and radia-

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tion therapy. In some countries (Poland, France), the use of mares milk as a substitution for breast milk is being explored, due to its similarity to human milk (Martuzzi et al., 2006). The healing properties of mares milk has been proven with dermatological diseases (neurodermatitis, psoriasis), and an application has been found in the making of cosmetic preparations. Due to its specific chemical and nutritional characteristics, mares milk is receiving greater attention in nutrition and has been proposed as a substitute for cow's milk in children with allergies, as well as a replacement for breast milk in premature infants (Caroprese et al., 2007). The milk composition can vary within a single milking, and the largest changes were observed in the amount of fat, such that the fat content at the end of milking can be 10 to 20 times higher than at the beginning (Salamon et al., 2009).

The element content in mares milk has been studied in different countries, and variations in the major element compositions between different breeds of dairy horses has been determined (Schryver et al. 1986; Doreau et al., 1990; Csapó-Kiss et al., 1995, 2009; Martuzzi et al., 1997; Grace et al., 1999; Summer et al., 2004; Sheng and Fang, 2009). To date, however, there have been only few studies on the microelements content in mares milk.

The largest indigenous horse breed in Croatia is the Croatian Coldblood, which takes up proportion of 32.59 % in the total number of horses in Croatia with a total number of 6.304 (HCK, 2011). These horses are raised in many parts of the Croatia, but the majority are found in Sisak-Moslavina County and Zagreb County (Čačić et al., 2006; Čačić, 2009). Horses are kept in the open countryside most of the year. In the past, the Croatian Coldblood had great economic value as an agricultural labourer, but today it has become a significant breed in preserving protected landscapes, cultural heritage and natural diversity, and is used for recreational and tourism purposes (Čačić, 2009). Currently in Croatia there are 11 private stud farms. Production of mares milk is still not reachable, while the biggest part takes horse meat production (Baban et al., 2011).

The aim of this study was to determine the concentrations of Al, Co, Cr, Li, Mo, Ni, Sb and Sr in the milk of the Croatian Coldblood horse throughout the lactation phases and to determine the interrelationships between these microelements.

Materials and methods

Sample collection

In total, 24 mares of the Croatian Coldblood breed reared at horse farms in Lonjsko Polje Nature Park (Central Croatia) were included in this study. Mares were aged from 5 to 11 years and weighed between 650 and 750 kg. Mares were kept under similar conditions of snow barn and at pasture from spring to autumn, with supplemental feeding of 3 kg oats per day when necessary. Winter feeding was 3 kg hay, 2 kg concentrate and *ad libitum* straw daily. Mares were foaling from late January to early May. Animals were kept in the stalls of owners, while during summer some remained in the nature park, where milk samples were collected. The milk samples (80-100 mL) were collected from February to October in 2011 on the 10th , 40th ,60th , 120th and 180th day of lactation. Milk was collected by hand milking from a single mammary gland, in the presence of the foal that had been prevented from suckling.

Following collection, samples were placed into clean, acid-washed polyethylene bottles, labelled and stored at -18 °C until analysis.

Reagents and standards

All reagents were of analytical reagent grade, HNO_3 and H_2O_2 (Kemika, Croatia). Ultra high purity water processed through a purification system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia) was used for all dilutions. Plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9, v/v) and by subsequent rinsing with double deionised water and drying prior to use. Calibrations were prepared with Al, Co, Cr, Li, Mo, Ni, Sb and Sr standard solutions of 1 g/L (Perkin Elmer, USA). The stock solution was diluted in HNO_3 (0.5 %).

Sample preparation

Samples (2 g) were accurately weighed in a PFA digestion vessel, before the addition of 1 mL of H_2O_2 and 6 ml HNO_3 (65 % v/v). A blank digest was carried out in the same way. A high-pressure laboratory microwave oven (Multiwave 3000, Anton Paar, Germany) was used to perform the acid digestion of samples. The digestion program began at a power of 800 W then ramped for 15 min, after which samples were held at 800 W for 15 min. The second step began at a power of 0 W and held for 15 min. Each digested sample was diluted to a final volume of 50 mL with double deionised water.

Digested samples were diluted to a final volume of 50 mL with double deionised water. All samples were run in batches that included blanks, a standard calibration curve and two spiked specimens. Detection limits were determined as the concentration corresponding to three times the standard deviation of ten blanks. The limits of detection were determined (μg/kg): Al 10, Co 10, Cr 20, Li 5, Mo 3, Ni 10, Sb 10 and Sr 10. The recovery percentage for Al, Co, Cr, Li, Mo, Ni, Sb and Sr in spiked milk samples shown good accuracy with a recovery rate (%) between 93.5 and 97.8.

Elements analysis

An inductively coupled plasma optical emission spectrometer (ICP-OES) with axial and radial viewing plasma configuration Model Optima 8000 (Perkin-Elmer, USA) operating at a 40 MHz free-running ratio-frequency and provided with an S 10 autosampler (Perkin-Elmer) was utilized. The nebulization system was equipped with a chemical-resistant concentric glass nebulizer coupled to a glass cyclonic spray chamber. A torch with an alumina-made injector was used. The polychromator, equipped with an Echelle grating, had a spectral range of 160-900 nm and a resolution of 0.009 nm at 200 nm. The UV-sensitive dual backside illuminated Charge-Coupled Device (CDD) array detector was used. The CDD-array detector collects both the analyte spectra and nearby background spectra, allowing for simultaneous background correction and providing improved precision and analytical speed. The instrumental operating conditions used are shown in Table 1.

Data analysis

Statistical analysis was performed using the Statistica 6.1 software package (StatSoft[Ò] Inc., Tulsa, USA). One-way analysis of variance was used to test for differences in element levels in milk samples. Differences between the element concentrations between lactate stages were analysed using the t-test. Results were considered significant at $p<0.05$. Association between variables was examined by calculating simple linear correlations. Significant correlations were declared weak ($r<0.3$), moderate (r from 0.3 to 0.7) or strong ($r>0.7$).

Table 1. Operating conditions for ICP-OES

Condition / Element	Co, Ni	Al, Cr, Li, Mo, Sb, Sr
Parameter	Intensity	Intensity
Plasma viewing mode	Axial	Axial
Read time	1-5 s	1-5 s
Measurement replicates	3	3
RF incident power	1400 W	1300 W
Plasma argon flow rate	8 L/min	15 L/min
Nebulizer argon flow rate	0.3 L/min	0.55 L/min
Auxiliary argon flow rate	0.2 L/min	0.2 L/min
Sample uptake rate	1.5 mL/min	1.5 mL/min
Inner diameter of the torch injector	2.0 mm	2.0 mm
Nebulizer type	Concentric glass (Meinhard)	Concentric glass (Meinhard)
Spray chamber type	Glass cyclonic spray chamber	Glass cyclonic spray chamber

Table 2. Concentrations of microelements (mean±SD, min-max, mg/kg) in milk of Croatian Coldblood mares

Days postpartum	Cr	Li	Mo	Sb	Sr
	Element (mean±SD, min-max) (g/kg)				
10	27.2±3.39	5.49±3.80	12.3±7.75	65.4±21.7	662±319
	23.5-32.5	1.29-10.2	1.41-20.3	33.3-93.2	202-1103
40	25.8±6.71	4.17±2.65	15.5±4.46	64.1±7.74	394±325
	20.9-37.4	0.01-7.23	11.6-23.1	54.9-76.0	181-959
60	27.1±3.98	2.79±2.39	12.6±2.99	77.8±11.9	411±43.9
	23.3-33.2	0.01-5.89	9.41-16.5	62.6-92.1	345-449
120	23.9±6.19	4.83±3.43	7.72±5.13	73.5±6.73	400±144
	16.9-30.8	0.01-8.23	0.95-14.5	66.1-81.9	263-603
180	29.4±7.02	4.50 ±3.44	6.85±4.37	69.5±18.1	470±186
	20.8-37.7	0.01-7.84	1.32-13.3	47.9-98.3	173-688
Total	26.7±5.51	4.35±3.05	11.0±5.79	70.1±14.2	467±234
	16.9-37.7	0.01-10.42	0.95-23.1	33.3-98.3	173-1103

Results and discussion

Among about 20 elements that are considered nutritionally essential, some trace elements such as Li, Cr, Mn, Co, Ni, Cu, Zn, Se and Mo are essential micronutrients that need to be consumed in adequate amounts to maintain normal physiological function (Goldhaber, 2003). Milk and dairy products can represent an important contribution to the daily intake of some of these elements. The literature offers few data describing the content of microelements Cu, Fe and Mn in mares milk (Csapò-Kiss et al., 1994, 1995; Martuzzi et al., 1998). To our knowledge, this is the first report on these microelements content in the milk of horse breeds in Croatia. The mean concentrations and range of Cr, Li, Mo, Sb and Sr in mares milk are presented in Table 2. Concentrations of Al, Co, Mn and Ni were below the limit of detection for these elements (<10 μg/kg).

Variations of Cr, Li, Mo, Sb and Sr concentrations throughout the lactation stages (days: 10, 40, 60, 120, 180) were slight but not significant. In previous studies, variation and an irregular decrease for macroelements Ca, K, Na and Mg throughout the lactation period in mares of different breeds in Hungary and Italy were determined (Csapò-Kiss et al., 1994, 1995; Martuzzi et al., 1998, 2004; Summer et al., 2004).

The correlations among the elements are presented in Table 3. Significant correlations were determined between the elements: negative moderate Cr - Li, positive moderate Cr - Sb, weak negative Li - Mo, Sb - Sr, Mo - Sr, Sb - Sr; positive weak Cr - Sr, Mo - Sb.

There are no data available for the microelements content of Al, Cr, Co, Li, Mo, Ni, Sb and Sr in mares milk, although microelements levels measured were compared to element levels of other species. Concentrations of microelements in cow, goat and human milk in different countries are presented in Table 4.

Table 3. Correlation coefficients between elements in mares milk

	Cr	Li	Mo	Sb
Li	-0.34[a]			
Mo	NS	-0.26[b]		
Sb	0.45[a]	-0.23[a]	0.14[a]	
Sr	0.11[a]	-0.13[a]	-0.01[a]	-0.06[a]

Significance: ap<0.001; b p<0.01; NS, not significant

Table 4. Concentrations of essential elements in cow, goat and human milk from different countries

Elements	Brasil	Croatia	France	Greece	Italy*	Poland	Spain
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/L)	(g/L)
Al			C 0.696[a]		G 0.850	C 1.18	H 20[a]
					G 1.35		C 26[a]
Co	C 0.010[a]		C 0.004[b]				
			C 0.001[d]				
Cr	C 0.032[a]		C 0.120[b]		G 0.03	C 0.04	H 0.8[a]
	C 0.079[b]		C 0.02[d]		G 0.039		C 1.1[a]
							C 4.03[b]
Li			C 0.003[b]				
			C 0.006[d]				
Mn	C 0.10[a]	C 0.026-0.055	C 0.029[b]	H 0.0048[a]	G 0.111	C 0.08	H 11.4[a]
	C 0.081[b]		C 0.09[d]	G 0.0653[b]	G 0.145		C 53[a]
							C 29.1[b]
Mo	C 0.045[a]		C 0.046[b]		G 0.023	C 0.04	
			C 0.039[d]		G 0.015		
Ni	C 0.073[b]		C 0.055[b]		G 0.015	C 0.04	H 10.1[a]
			C 0.07[d]		G 0.023		C 9.5[a]
Sb			C 0.0006[a]			C 0.10	
Sr	C 1.2[a]		C 0.293[c]				H 88[a]
							C 675[a]

C - cow milk; G - goat milk; H - human milk
*results per dry weight
Brasil: [a]Nardi et al. (2009), [b]Soares et al. (2010); Croatia: Sikirić et al. (2003); France: [a]Millour et al. (2011), [b]Noël et al. (2012), [c]Millour et al. (2012), [d]Leblanc et al. (2005); Greece: [a]Leotsinidis et al. (2005), [b]Kondyli et al. (2007); Italy: Coni et al. (1996); Poland: Dobrzanski et al. (2005); Spain: [a]Martino et al. (2001), [b]Sola-Larrańaga and Navarro-Blasco (2009)

Concentrations of Al, Co, Mn and Ni were determined below the limit of detection, which is similar to levels found in human milk (Martino et al., 2001; Leotsinidis et al., 2005) and lower than those in cow (Martino et al., 2001; Dobrzanski et al., 2005; Sola-Larrańaga and Navarro-Blasco, 2009; Millour et al., 2012; Noël et al., 2012) and goat milk (Coni et al., 1996; Kondyli et al., 2007). Aluminium is widely used as a household foil for packaging and storing different types of food products and may enter the food chain. In the human body, Al ions could inhibit different metabolic processes caused by competition reactions between Al and other ions such as Ca, Mg and Fe (Macdonald and Martin, 1988). In recent reports, Al has been associated with several skeletal and neurological disorders (Ranau et al., 2001).

Cobalt is necessary as it forms part of vitamin B12, though exposure to high levels of Co can result in lung and heart effects and dermatitis (Agency for Toxic Substances and Disease Registry, 2004). Manganese is associated with bone development and amino acid, lipid, and carbohydrate metabolism, and it is found in different enzymes and is transported in the body by transferrin, macroglobulins and albumin (Rabin et al., 1993). In excess levels it is toxic and can cause a Parkinson-type syndrome (Aschner, 2000). Nickel can cause respiratory problems and is carcinogenic (Agency for Toxic Substances and Disease Registry, 2004).

The mean Cr content determined in mares milk was similar to concentrations measured in cow and goat (Coni et al., 1996; Nardi et al., 2009; Soares et al., 2010) but lower than in human milk (Martino et al., 2001). Chromium (III) is an essential element that helps the body to use sugar, protein, and fat, though chromium (VI) is carcinogenic (Institute of Medicine, 2002). Excessive amounts of chromium (III) may cause adverse health effects (Agency for Toxic Substances and Disease Registry, 2004).

In the present study, Sr and Li concentrations measured were similar to concentrations determined in previous studies in cow milk (Martino et al., 2001; Millour et al., 2012; Noël et al., 2012). Due to the chemical similarity to Ca, Sr may be absorbed in body as Ca. Stable forms of Sr might be beneficial and were found to aid bone growth, increase bone density, and lessen vertebral, peripheral, and hip fractures (Meunier et al., 2004). Lithium is not considered an essential mineral for vital function as no symptoms of its deficiency in humans have been reported. The biochemical mechanisms of the action of Li appear to be multifactorial and are intercorrelated with the functions of several enzymes, hormones and vitamins, as well as with growth and transforming factors (Schrauzer, 2002).

The contents of Sb in cow milk (Millour et al., 2012) and Sr in human milk (Martino et al., 2001) were 116 and 5.3-times lower than in mares milk in the present study. Antimony is used in industry on a massive scale and many of its compounds are toxic. The most serious effect of acute antimony poisoning is cardiotoxicity and the resulting myocarditis (Foster et al., 2005). Prolonged skin contact by Sb may cause dermatitis or damage the kidneys and the liver, causing violent and frequent vomiting, leading to death in a few days (Sundar and Chakravarty, 2010).

Conclusions

There are few literature reports offering data on the microelements contents in mares milk. To our knowledge, this is the first report on the content of these microelements in the milk of horse breeds in Croatia. Concentrations of elements Al, Co, Mn and Ni were below the limit of detection of 10 μg/kg, which is similar to the content in human milk measured in previous studies. Slight but not significant variation of Cr, Li, Mo, Sb and Sr concentrations in milk were determined throughout the examined lactation stages. Also, significant correlations between elements were determined: Cr - Li, Cr - Sb, Cr - Sr, Li - Mo, Sb - Sr, Mo - Sr, Mo - Sb. The mean Cr, Sr and Li content determined in mares milk were similar to concentrations measured in cow milk. Antimony and Sr levels found were higher than in cow and human milk. Further investigations of the same and other microelements in mares milk throughout the lactation stages should be performed for a detailed overview of the microelements composition.

Koncentracije mikroelemenata Al, Co, Cr, Li, Mo, Ni, Sb i Sr u mlijeku kobila hrvatskog hladnokrvnjaka

Koncentracije mikroelemenata Al, Co, Cr, Li, Mo, Ni, Sb i Sr određene su u mlijeku kobila hrvatskog hladnokrvnjaka tehnikom induktivno spregnute plazme s optičkom emisijom. Ukupna srednja vrijednost elemenata u mlijeku kobila je (mg/kg): Al<10, Co<10, Cr 26,7, Li 4.35, Mo 11,0, Ni<10, Sb 70,1 i Sr 467. Varijacije koncentracija Li, Sb i Sr utvrđene su u svim fazama laktacije (10.-180. dan). Utvrđene su korelacije između elemenata. Općenito, dostupan je vrlo ograničeni broj podataka o koncentracijama mikroelemenata Al, Cr, Co, Li, Mo, Ni, Sb i Sr u mlijeku kobila. Prema našim saznanjima ovo je prvi izvještaj o sadržaju tih mikroelemenata u mlijeku kobila u Hrvatskoj.

Ključne riječi: mikroelementi, mlijeko, konji, ICP-OES, Hrvatska

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