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Original Scientific Article

CONTENT OF MACRO- AND MICROELEMENTS IN THE MILK OF CROATIAN COLDBLOOD MARES DURING LACTATION

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Summary: The concentrations of macro- and microelements in the milk of Croatian Coldblood mares were determined throughout the lactation phases by inductively coupled plasma-optical emission spectrometry. Element contents during days 10–180 of lactation were in the ranges (mg/kg): Ca 585–772, K 547–687, Na 131–165, Mg 56.8–71.0, Cu 0.085–0.14, Fe 0.013–0.41, Zn 1.86–2.15, Se 0.003–0.029. Variation trends for elements were found throughout the lactation stages. However, significantly higher Fe concentrations were found on days 10 and 40 of lactation than on day 60 ($p < 0.05$, both). There were no statistically significant differences between concentrations of other elements during lactation. Moderate correlations were found between the elements: Ca: K, Mg, Se; Mg: K, Cu; Se: Cu, Fe. The element concentrations in horse milk, with the exception of Ca and Cu, fell within the ranges previously reported for different breeds of dairy horses. Cu and Fe contents were lower, while the Zn content was similar to those obtained in different dairy horse breeds. Concentrations of elements obtained in horse milk were lower in comparison to cow and goat milk. Generally, levels of Ca and Mg were higher, Na and K were similar, but Cu, Fe and Zn were lower than those in human milk.

Key words: milk; lactation; horse; micro elements; macro elements; ICP-OES; Croatia

Introduction

Worldwide, hundreds of different breeds of horses are used for milk production. In the European Union, horses have been used for dairy production in Hungary, Austria, Bulgaria, Germany, Belarus and Ukraine (1, 2). The composition and quality of horse milk is attributed to the genetic, physiological, zoohygienic and feeding conditions, and varies among species with regard to the nutritional requirements of their newborns. Horse milk is suitable for human consumption due to the following similarities and differences compared to human milk: similar composition of major protein and immunoglobulins, high levels of polyunsaturated fatty acids, low nitrogen, low cholesterol contents, lower fat content, similar lactose content and higher vitamin C content (1, 3, 4). It has been estimated that more than 30 million people worldwide consume equine milk regularly, and that figure is showing significant annual increases (5).

Milk production in horses depends on the breed and is high during the first week of lactation and increases to a maximum between the first and

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third months (1, 6). Lactation in mares may be of different durations, though it usually lasts one year. The composition of horse milk is sufficient for the foal's demands in terms of nutritional requirements and providing the necessary elements (7). Element content variations were determined during the different lactation stages (5, 8). The elements represent different functions. Calcium and phosphorus have a fundamental role in the rapid skeletal development of the foal, while magnesium plays a part in bone mineralization (4, 9). Furthermore, Na^+ is an important cation in blood and extracellular fluid bathing cells and K^+ is a monovalent cation significant to the maintenance of fluid integrity within the cell.

The element content in horse milk has been studied in different countries (6, 8, 10-13). Differences were found in the element composition between different breeds of horses (4). The largest autochthonous horse breed in Croatia is the Croatian Coldblood (14, 15). Today, this breed is raised in many other parts of the Croatia, though the majority are found in Sisak-Moslavina County and Zagreb County. Throughout much of the year, they are kept in the open countryside. In the past, before the advent of mechanization, the Croatian Coldblood had great economic value as an agricultural labourer. Today, it is used for recreational and tourism purposes and in preserving both the cultural heritage and natural diversity in Croatia (16). In 2008, a total of 2778 Croatian Coldblood mares were registered in Croatia (15).

The aim of this study was to determine the macro- and micro mineral composition of Ca, K, Na, Mg, Cu, Fe, Zn, Mn and Se in the milk of nursing mares of the Croatian Coldblood breed throughout the lactation phases

Materials and methods

Sample collection

Six mares of the Croatian Coldblooded 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 were 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 bore foals 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. Milk samples (80–100 ml) were collected from February to October 2011 on days 10, 40, 60, 120 and 180 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.

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

Reagents and standards

Analytical reagent grade HNO_3 and H_2O_2 were purchased from Kemika, Croatia. Ultra-pure water (18 MΩxcm) was generated by the purification system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia). 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. In the calibration process, stock standard solutions with the concentrations of Ca, K, Na, Mg, Cu, Fe, Zn and Se (Perkin Elmer, USA) of 1 g/L prepared with diluted HNO_3 (0.5%) were used.

Sample preparation

Milk samples were weighed (2 g) in a PFA digestion vessel and 1 ml of H_2O_2 and 6 ml HNO_3 were added. Acidic digestion of samples were performed by microwave oven Multiwave 3000 (Anton Paar, Ostfildern, Germany) using a two-step digestion program: step I power 800 W, ramped 15 min, 800 W for 15 min; step II power 0 W for 15 min.

Digested samples were diluted to the final volume of 50 ml with ultra-pure water. All samples were run in batches that included blanks, a standard calibration curve and two spiked specimens. The limits of detection were calculated according to three times the standard deviation of ten blank samples (mg/kg): Ca 0.01, Na 0.01, K 0.025, Mg 0.02, Cu 0.01, Fe 0.005, Zn 0.005 and Se 0.001.

Skim milk powder (BCR-063, IRMM, Belgium) was used as certified reference materials for checking the quality of results. The reference material was treated and analysed under the same conditions as the samples. The results showed

good accuracy with certified reference materials and the recovery results for elements were (%): Ca 98.3, K 98.5, Na 96.1, Mg 93.7, Cu 97.7, Fe 94.6 and Zn 98.9. To calculate the recovery percentage for Se, five milk samples were spiked with known amounts of elements. The quality of data showed good accuracy with a recovery rate of 96.9%.

Analysis of elements

An inductively coupled plasma optical emission spectrometer (ICP-OES) with axial and radial viewing plasma configuration Model Optima 8000 (Perkin Elmer, Waltham, Massachusetts, USA) operating at a 40 MHz free-running ratio-frequency and equipped with S 10 autosampler was utilized. The instrumental operating conditions used are shown in Table 1.

Statistical analysis

Statistical analysis was calculated using the Statistica 6.1 software (StatSoft® Inc., Tulsa, USA). Concentrations were expressed as mean ± standard deviation, minimum and maximum values. One-way analysis of variance was used to test for differences in element levels in milk samples. Differences between 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 ($r > 0.7$).

Results and discussion

Descriptive statistics of the concentrations of Ca, Na, K, Mg, Cu, Fe, Zn and Se in horse milk are presented in Table 2. For all elements studied, except Zn, the highest mean values were determined at early lactation on day 10 postpartum. Variation trends for all elements were found throughout the lactation stages. Significantly higher Fe concentrations were found on days 10 and 40 of lactation than those measured on day 60 ($p < 0.05$, both). However, there were no statistically significant differences during the lactation phases between concentrations of other elements.

The correlations between the measured macro- and microelements in horse milk were investigated, and moderate and significant positive correlations were found between: Ca and K (r=0.35, $p<0.05$), Ca and Mg (r=0.61, $p<0.001$), Ca and Se (r=0.52, $p<0.01$), Mg and K (r=0.64, $p<0.001$), Mg and Cu (r=0.43, $p<0.01$), Se and Cu (r=0.41, $p<0.05$), Se and Fe (r=0.64, $p<0.001$). In previous reports, moderate to strong positive correlations were found among macroelements, except between Na and Mg in horse milk (5).

Element concentrations in horse milk in different lactation stages obtained in different countries are presented in Table 3. The results obtained in the present study, with the exception of lower Ca and Cu values, fell within the ranges previously reported for different breeds of dairy horses (5, 7, 12, 17-19, 32) (mg/kg): Ca, 544.2–1220; K, 413.1–928.6; Na, 120–320; Mg, 43.8–139.7; Cu, 0.19–1.06; Fe, 0.34–1.58; Zn, 0.21–2.95.

Table 3 clearly shows that the content of Ca, K, Na and Mg decreased throughout the lactation period in mares of different breeds in Hungary (Hungarian Draught, Haflinger, Breton, & Boulonnais mares; 17, 32) and in Italy (Haflinger mares; 5, 7, 18, 19). Also, variation and an irregular decrease of elements throughout the lactation period was determined in Thoroughbred mares from USA and New Zealand and in Italian Saddle mares from Italy (9, 12, 18). The present study also determined the trend of variation of content of Ca, K, Na and Mg during lactation. In contrast to the significant decline in the concentration of these elements towards the end of lactation in previous studies, in this study no significant decreases in concentrations were identified at the end of lactation. According to previous available data, this can be explained as a consequence of the differences between horse breeds.

Cu and Fe contents determined in the present study were 1.8–4.8 and 4.2–6.7 times lower than values measured in mares form Hungary (7, 17). On the other hand, Zn values measured by these authors were similar to those in the present study. It was previously determined that the concentrations of Cu, Zn and Fe in the milk of late gestating and lactating mares were not influenced by supplementation with higher dietary trace element levels (20).

In previous reports, there are no data regarding the Se content in horse milk. The results obtained in the present study ranged from 0.003–0.029 mg/kg. The mean Se content was similar to concentrations measured in goat (0.0129 mg/kg) and human (0.0141 and 0.0152 mg/kg) milk but lower than in bovine milk (0.0215–0.4 mg/

kg) (2, 21-24). Element concentrations in cow, goat and human milk from different countries are reported in Table 4. Studies have shown that Ca concentrations in horse milk were approximately three times higher than in human milk (251 mg/kg) and about two times lower than in cow and goat milk (21, 23, 24, 26-28). Mg concentrations ranged from 56.8 to 71.0 mg/kg, i.e. they were 2.5-3.5 times higher than the values 24–40 mg/L reported for human milk (2, 21), but 1.5-2 times lower than concentrations measured in cow and goat milk (23, 24, 26-29). Na and K contents were similar to those in human milk (2, 21), though they were approximately three times lower than the values reported in cow and goat milk (23, 24, 26-29). Furthermore, concentrations of Cu, Fe and Zn determined in horse milk in the present study were lower than those in cow, goat and human milk.

Table 1: Working conditions for ICP-OES.

Element / Parameter	Ca, Na, Mg, K	Fe, Cu, Zn, Se
Plasma viewing mode	Radial	Axial
Read time	1-5 s	1-5 s
Measurement replicates	3	3
RF incident power	1000 W	1300 W
Plasma argon flow rate	8 L/min	15 L/min
Nebulizer argon flow rate	0.85 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 elements in milk of Croatian Coldblooded mares

		Days postpartum				
Element (mg/kg)	Statistics	10 (*n*=6)	40 (*n*=6)	60 (*n*=6)	120 (*n*=6)	180 (*n*=6)
Ca	Mean±SD Min-max	772 ± 265 543-1246	598 ± 178 331-820	608 ± 165 426.4-910.8	585 ± 195 387.2-885.1	674 ± 144 557-942
K	Mean±SD Min-max	687 ± 145 531-840	547 ± 167 366-860	561 ± 108 429-676	634 ± 93.6 529-807	677 ± 135 510-800
Na	Mean±SD Min-max	165 ± 54.2 106-261	138 ± 33.7 94.9-168	138 ± 13.4 125-157	131 ± 73.5 15.9-245	138 ± 12.6 123-151
Mg	Mean±SD Min-max	71.0 ± 17.9 50.5-92.6	56.8 ± 19.8 30.9-85.2	63.8 ± 11.7 46.1-80.2	68.6 ± 17.7 47.5-96.6	69.8 ± 17.4 49.9-92.7
Cu	Mean±SD Min-max	0.14 ± 0.079 0.021-0.25	0.11 ± 0.048 0.040-0.17	0.12 ± 0.032 0.077-0.16	0.13 ± 0.032 0.076-0.17	0.085 ± 0.042 0.019-0.14
Fe	Mean±SD Min-max	0.41 ± 0.35 0.082-1.12	0.33 ± 0.31 0.084-1.02	0.13 ± 0.047 0.066-0.21	0.15 ± 0.082 0.082-0.285	0.18 ± 0.16 0.072-0.51
Zn	Mean±SD Min-max	1.86 ± 0.47 1.44-2.44	2.01 ± 0.19 1.64-2.23	1.99 ± 1.13 0.83-3.27	2.11 ± 1.44 0.85-4.85	2.15 ± 0.98 1.05-4.39
Se	Mean±SD Min-max	0.029 ± 0.041 0.003-0.11	0.004±0.002 0.002-0.008	0.0027±0.003 0.002-0.008	0.0027±0.004 0.001-0.011	0.007±0.004 0.006-0.011

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

Elements	Brazil (30) (mg/L)	Greece (29) (mg/kg)	Iceland (24) (mg/kg)	Italy (22) (mg/kg)	Spain (21) (mg/L)	Spain (26, 28) (mg/kg)	Sweden (31) (mg/L)	Tenerife (23) (mg/kg)	Source: 2007 (2) (mg/kg)	Source: 2009 (27) (mg/kg)
Ca		G 1320	C 1140-1260	C 1263	H 251	G 1586 (26) G 1940 (28) C 1135.8 (26) C 1936 (28)		G 1340	H 330 G 1340	C 1220
K		G 1520		C 1096				G 1240	H 550 G 1810	C 1520
Na		G 594	C 399-401	C 441	H 164			G 510	H 150 G 410	C 580
Mg		G 158.7	C 96.0-99.8	C 118	H 24	G 129.2 (26) G 178.2 (28) C 94.0 (26) C 150.1 (28)		G 120	H 40 G 160	C 120
Cu	H 0.54	G 0.80	C 0.041-0.046		H 0.311	G 0.42 (26) C 0.14 (26)	H 0.12	G 0.18	H 0.60 G 0.50	C 0.6
Fe	H 1.72	G 0.60	C 0.20-0.27	C 0.3	H 0.388	G 1.5 (26) C 0.9 (26)	H 0.29	G 0.70	H 2.0 G 0.70	C 0.8
Zn	H 6.97	G 3.7	C 3.89-4.33	C 3.6	H 3.8	G 5.28 (26) G 4.46 (28) C 4.63 (26) C 4.03 (28)	H 0.46	G 3.20	H 3.8 G 5.6	C 5.3
Se			C 0.0215-0.0263	C 0.4	H 0.0141			G 0.0129	H 0.0152 G 0.013	C 0.0096

C – cow milk; G – goat milk; H – human milk

*The number in parentheses refers to the reference

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VSEBNOST MIKRO- IN MAKROELEMENTOV V MLEKU HRVAŠKIH HLADNOKRVNIH KOBIL V ČASU LAKTACIJE

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Povzetek: Vsebnost makro- in mikroelementov v mleku hrvaških hladnokrvnih kobil v različnih fazah laktacije je bila ugotavljana s pomočjo indukcijske plazmonske optične emisijske spektrometrije. Vsebnost elementov med 10. in 180. dnevom laktacije je bila v mejah (mg/kg): Ca 585–772, K 547–687, Na 131–165, Mg 56,8–71,0, Cu 0,085–0,14, Fe 0,013–0,41, Zn 1,86–2,15 ter Se 0,003–0,029. Koncentracije vseh elementov so se v času laktacije spreminjale, vendar pa so bile razlike statistično značilne samo pri železu, ki ga je bilo 10. in 40. dan statistično značilno več kot 60. dan laktacije ($p < 0,05$). Korelacijska analiza je pokazala zmerno korelacijo med elementi Ca, K, Mg, Se, Mg, K, Cu, Se, Cu in Fe. Razen za Ca in Cu so bile koncentracije vseh ostalih elementov znotraj meja, o katerih so že prej poročali za različne pasme konj. Koncentracije vseh elementov v konjskem mleku so bile nižje, kot so običajno v kravjem ali kozjem mleku. V primerjavi s človeškim mlekom pa so bile koncentracije Ca in Mg višje, Na in K podobne, koncentracije Cu, Fe in Zn pa nižje v konjskem kot v človeškem mleku

Ključne besede: mleko; laktacija; konj; mikroelementi; makroelementi; ICP-OES; Hrvaška