Original scientific paper - Izvorni znanstveni rad UDK: 637.112.2

Differences of the essential mineral element levels in the milk of Croatian Coldblood horse and Littoral-Dinaric donkey

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Received - Prispjelo: 18.11.2013.
Accepted - Prihvaćeno: 20.01.2014.

Abstract

The concentrations of essential elements in the milk of Croatian Coldblood mares and Littoral-Dinaric donkey were measured. The mean element contents in horse milk were (mg/kg): Ca 687.1, Mg 72.1, K 637.7, Na 143.5, Cu 0.126, Fe 0.235, Se 0.01, Zn 2.06, Mn<0.01. In donkey milk, the mean element contents were (mg/kg): Ca 815.4, Mg 80.8, K 887.7, Na 217.6, Cu 0.027, Fe 0.196, Zn 2.36, Mn 0.016. In horse milk, moderate correlations were found between the elements: Ca-K, Mg-Se, Mg-K, Mg-Cu, Se-Cu, Se-Fe, Mn-K. Strong correlations were found among elements in donkey milk: Ca-Na, Ca-K, Ca-Cu, K-Na, K-Cu, Cu-Mn. Significant differences between Na, K and Cu concentrations were obtained between the two species. Other elements concentrations were similar in both species.

Key words: milk, horse, donkey, essential elements, Croatia

Introduction

There are more than three hundred different breeds of horses all over the world and any breed can be developed into a milking herd. Horses have been used for dairy purposes in countries of European Union: Hungary, Austria, Bulgaria, Germany, Belarus and Ukraine (Park et al., 2007). The composition and quality of horse milk is attributed to the genetic, physiological, zoohygienic and feeding conditions. There are differences in the nutritional value in horse, human and cow milk. The basic similarities between horse and human milk is the composition of major protein components and immunoglobulins and therefore horse milk is used for the prevention and treatment of bronchitis and allergies, stimulation of immunity after chemotherapy and radiation, for metabolic, gastrointestinal and liver problems after surgery and for dermatological diseases as neurodermatitis and psoriasis. Generally, essential element is required for the various physiological functions of the organism: Ca and P for skeletal development, Mg for bone mineralization, Na as a cation in blood and extracellular fluid, K for the maintenance of fluid integrity within the cell (Sheng and Fang, 2009).

The Croatian Coldblood is the largest autochthonous horse breed in Croatia mainly raised in Sisak-Moslavina and Zagreb County (Čačić et al., 2006; Čačić, 2009). Today these horses are kept in the open countryside during year and use for meat production, tourism, cultural and folklore events. In Croatia production of horse milk is not economically profitable and most of production is related to meat production (Baban et al., 2011). Studies of essential element content in horse milk has been mainly related to different horse breed from Italy, Hungary

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and New Zealand (Schryver et al. 1986; Doreau et al., 1990; Csapó-Kiss et al., 1995; Martuzzi et al., 1997; Grace et al., 1999; Summer et al., 2004). Variations in the major element compositions between different breeds of horses are evident (Sheng and Fang, 2009).

Over the past decade, donkey milk has become attractive as substitute for the human infants with allergic reactions to cow milk and in the treatment of complicated cases of multiple food intolerance (Iacono et al., 1992; Carroccio et al., 2000; Monti et al., 2007). The composition of proteins and lipids in donkey milk are similar to human milk and it contains low amounts of casein and the potential allergen component β-lactoglobulin, and high concentrations of lysozyme, essential to protect the digestive system of children from various infections (Ivanković et al., 2009). Also, the content of β-lactoglobulin in donkey milk is equivalent or lower than in horse milk (Doreau and Martin-Rosset, 2002; Malacarne et al., 2002). There are a few literature data regarding the essential elements in donkey milk (Salimei et al., 2005; Fantuz et al., 2009, 2012).

In Croatia, mostly in the Mediterranean region, donkeys were used in the past as working animals. Today however, donkeys are used also for riding, tourism, oniotherapy and in recent years for meat and milk production (Ivanković et al., 2009). The total donkey population in Croatia has been estimated at four thousand. Among the three breeds of donkeys recognized and defined in Croatia, i.e. the Istrian, Northern-Adriatic and Littoral-Dinaric donkey breeds, the Littoral-Dinaric donkey is the most common, accounting for approximately 80 % of all donkeys in Croatia (Ivanković et al., 2000).

The aim of this study was to determine the macro- and micro mineral composition (Ca, K, Na, Mg, Cu, Fe, Zn, Mn and Se) in the milk from nursing mares of the Croatian Coldblood breed and in Littoral-Dinaric donkeys and differences in element concentration between the two domestic equine species and the interrelationships between the elements.

Materials and methods

Sample collection

Seven mares of the Croatian Coldblooded breed reared at horse farms in Lonjsko Polje Nature Park (Central Croatia), aged from 5 to 11 years and weighed between 650 and 750 kg were used in study. Mares were kept under similar conditions of barn and at pasture from spring to autumn. Winter feeding consisted of hay (3 kg), concentrate (2 kg) and straw (*ad libitum*). In total 35 milk samples (80-100 mL) were collected on the 10^{th}, 40^{th}, 60^{th}, 120^{th} and 180^{th} days of lactation by hand milking from a single mammary gland in the presence of the foal that had been prevented from suckling.

Seven donkeys of the Littoral-Dinaric breed from a farm in Central Croatia, aged from 2 to 8 years, weighed between 80 and 115 kg were used in study. Nutrition was solely based on grazing and hay addition. Total 28 milk samples (50 mL) were collected during lactation period on the 90^{th}, 150^{th}, 210^{th} and 270^{th} day. Donkeys were milked manually once in the morning period (at 8:00 a.m.). During the milking process, 2 hours prior to milking and immediately after milking, foals were physically separated from their mother, but left within visual contact.

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

Reagents and standards

Reagents HNO_3 (65 %, v/v) and H_2O_2 (30 %, v/v) were analytical grade (Kemika, Croatia). Ultra-pure water (18 MΩxcm) was generated by purification system NIRO VV UV UF 20 (Nirosta d.o.o. Water Technologies, Osijek, Croatia). Calibrations were prepared with Ca, K, Na, Mg, Cu, Fe, Se and Zn standard solutions of 1000 mg/L (Perkin Elmer, USA). The stock solution was diluted in HNO_3 (0.5%).

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 digestion program in two step: 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 with ultra-pure water to volume of 50 mL.

Analytical batches contained blank sample and two spiked samples. The limits of detection were calculated according to three times the standard deviati-

Table 1. Operating conditions for ICP-OES

Condition	Elements	
	Ca, K, Na, Mg	Cu, Fe, Zn, Se, Mn
Parameter	Intensity	Intensity
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

on of ten blank samples and were (mg/kg): Ca 0.01, Na 0.01, K 0.025, Mg 0.02, Cu 0.01, Fe 0.005, Se 0.001, Zn 0.005 and Mn 0.01.

Skim milk powder (BCR-063, IRMM, Belgium) was used as certified reference materials for recovery analysis. Results showed good accuracy with the following recovery rates for elements (%): Ca 98.3, Na 96.1, K 98.5, Mg 93.7, Cu 97.7, Fe 94.6 and Zn 98.9. To calculate the recovery percentage for Se and Mn, five milk samples were spiked with known amounts of elements. The quality of data showed good accuracy with a recovery rate (%): Se 96.9; Mn 94.8.

Analysis of mineral elements

Inductively coupled plasma optical emission spectrometer (ICP-OES) Model Optima 8000 (Perkin Elmer, Waltham, Massachusetts, USA) was used for element determination. Operating conditions for ICP-OES are presented in Table 1.

Statistical analysis

Statistical analysis was performed 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 elements levels in milk samples. In addition, differences between the element concentrations in milk between two species 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 ($r>0.7$).

Results and discussion

The concentration of essential elements (Ca, Na, K, Mg, Cu, Fe, Zn, Se and Mn) in horse and donkey milk are summarized in Table 2. The mean element contents (mg/kg) in horse milk were: Ca, 687.1; K, 637.7; Na, 143.5; Mg, 72.1; Cu, 0.126; Fe, 0.235; Se, 0.01; Zn, 2.06, Mn<0.01. Concentrations of all elements, with the exception of lower Ca, Cu and Mn levels, fell within the ranges reported in previous studies for horses (mg/kg): Ca, 787-1220; K, 250-701; Na, 127-198; Mg, 29-77; Cu, 0.23-1.06; Fe, 0.27-1.47; Zn, 0.89-2.95, Mn, 0.044-0.054 (Csapo-Kiss et al., 1994, 1995, 2009; Martuzzi et al., 1998, 2004; Grace et al., 1999; Summer et al., 2004). In the present study, the concentrations of Mn in milk of horse was found at trace levels, below 10 μg/kg. The concentration of selenium was also low and in range 0.01-0.113 mg/kg. However in previous studies (Martuzzi et

Table 2. Essential Element concentrations in milk of Croatian Coldblooded horses and Littoral-Dinaric donkeys

Element	Statistics	Croatian Coldblooded horse	Littoral-Dinaric donkeys
		(n=35)	(n=28)
Ca	Mean±SD	687.1±215.6	815.4±150.3
	Min-max	331.0-1246	600.2-1060
K	Mean±SD	637.7±132.7[a]	887.7±132.1[a]
	Min-max	366.2-859.7	666.7-1043
Na	Mean±SD	143.5±39.5[a]	217.6±16.6[a]
	Min-max	15.9-260.8	185.5-240.1
Mg	Mean±SD	72.1±22.4	80.8±10.7
	Min-max	30.9-134.5	57.9-90.9
Cu	Mean±SD	0.126±0.047[a]	0.027±0.029[a]
	Min-max	0.019-0.246	0.007-0.092
Fe	Mean±SD	0.235±0.23	0.196±0.131
	Min-max	0.066.6-1.12	0.069-0.431
Zn	Mean±SD	2.06±0.875	2.36±0.203
	Min-max	0.834-4.86	2.03-2.59
Se	Mean±SD	0.010±0.018	0.009±0.005
	Min-max	0.001-0.113	0.002-0.016
Mn	Mean±SD	<0.01	0.016±0.005
	Min-max		0.011-0.027

Significant differences: [a]$p<0.001$

al., 1998, 2004; Summer et al., 2004) there are no data for Se content in horse or donkey milk.

The correlations between the measured macro and micro elements in horse and donkey milk are presented in Table 3. Correlations between the investigated elements in horse milk were moderate. Moderate and significant positive correlations were found between Ca and K, Mg and Se. Magnesium were moderate and positively correlated with K and Cu. Also, moderate positive correlations were observed between Se and Cu and Fe. However, Mn was moderate and negative correlated with K. Moderate to strong positive correlations have previously been found among macro elements, except between Na and Mg in horse milk (Summer et al., 2004).

Very limited data are available on the element content of donkey milk in general and to our knowledge no data have been reported on macro and micro elements in Croatia. The mean values of element content in donkey milk determined in this study were (mg/kg): Ca, 815.4; Mg, 80.8; K, 887.7; Na, 217.6; Cu, 0.027; Fe, 0.196; Zn, 2.36; Mn, 0.016. The results obtained showed higher Ca and K concentrations and lower Cu and Fe levels in comparison to the ranges reported previously for different breeds of donkey (mg/kg): Ca, 330-1140; Mg, 40-83; K, 240-747; Na, 100-268; Cu, 0.08-0.30; Fe, 0.43-2.64; Zn, 1.23-3.19; Mn, trace (Salimei et al., 2004; Fantuz et al., 2009; Fantuz et al., 2012; Salimei and Fantuz, 2012).

The correlations between elements in donkey milk shown strong and positive correlation of Ca to Na and Ca and strong negative to K. Potassium was strongly negative correlated with Na and Cu. Copper was strongly positively correlated with Mn. All the remaining investigated correlations between

Table 3. Correlation coefficients between macro minerals i trace elements in mares and donkey milk

	Ca	K	Na	Mg	Cu	Fe	Zn	Se
K	0,35[c]							
	-0,92[b]							
Na	0,92[b]	-0,83[c]						
Mg	0,61[a]	0,64[a]	NS					
Cu	0,79[c]	-0,77[c]	NS	0,43[b]				
Fe	NS	NS	NS	NS	NS			
Zn	NS	NS	NS	NS	NS	NS		
Se	0,52[b]	NS	NS	NS	0,41[c]	0,64[a]	NS	
Mn	NS	-0,40[c]	NS	NS	0,92[b]	NS	NS	NS

Nonunderline coefficient - horse; underline coefficient - donkey
Significance is: [a]$p<0.001$; [b]$p<0.01$; [c]$p<0.05$; NS, not significant

elements in donkey milk were not significant. However, positive and significant correlations between macro elements in donkey milk, except between Ca and Na, have recently been determined (Fantuz et al., 2012).

The mean Ca content of donkey milk was similar to recently reported data in donkey milk in Italy (Fantuz et al., 2012). Also, in the present study, a comparison of the mean Ca content showed higher but not significant values in donkey than in horse milk. In previous studies, it was found that the Ca, Na and K concentrations are similar in horse and donkey milk (Salimei et al., 2005; Fantuz et al., 2009, 2012; Salimei and Fantuz, 2012). In the present study, significantly higher levels of Na and K were found in donkey milk in comparison to horse milk ($p<0.001$, both). On the other hand, significantly lower Cu content were measured in donkey milk than in milk of Croatian Coldblooded horses ($p<0.001$). The Cu and Fe contents in donkey milk showed high variability, ranging from 0.007-0.092 and 0.069 to 0.431 mg/kg. The mean of these elements were 5-10 times lower than those observed in previous studies in donkey (Fantuz et al., 2009; Salimei and Fantuz, 2012) and horse milk (Martuzzi et al., 1998; Csapo-Kiss et al., 1994, 1995; Csapo et al., 2009).

The Mg ranges observed in donkey milk in the present study were similar to previously reported ranges (Salimei et al., 2004; Fantuz et al., 2009, 2012) and were also similar to mean values in milk of Croatian Coldblooded horses.

Conclusions

The concentrations of mineral elements in horse milk, with the exception of Ca, Cu and Mn, fell within the ranges previously reported for different breeds of horses. Copper, Fe and Mn contents were lower, while the Zn content was similar to those obtained in different horse breeds. In donkey milk, the obtained Ca and K concentrations were higher, while Cu and Fe content were lower in comparison to previously reported levels for different donkey breeds. It is important to emphasize that the sampling of milk of both equine species was conducted at different stages of lactation. A comparison of the element concentrations in the milk of the two species showed higher levels of Na and K in donkey milk than in horse milk, while the Cu content was higher in horse than in donkey milk. For other elements, concentrations were similar in the milk of both equine species.

Razlike u koncentracijama esencijalnih elemenata u mlijeku kobila hrvatskih hladnokrvnjaka i primorsko-dinarskih magarica

Sažetak

Određene su koncentracije esencijalnih elemenata u mlijeku kobila hrvatskih hladnokrvnjaka i primorsko-dinarskih magarica. Srednje vrijednosti koncentracija elemenata u mlijeku kobila su (mg/kg): Ca 687,1; Mg 72,1; K 637,7; Na 143,5; Cu 0,126; Fe 0,235; Se 0,01; Zn 2,06; Mn<0,01. U mlijeku magarica određene su srednje koncentracije elemenata (mg/kg): Ca 815,4; Mg 80,8; K 887,7; Na 217,6; Cu 0,027; Fe 0,196; Zn 2,36; Mn 0,016. U mlijeku kobila utvrđena je srednja korelacija između elemenata: Ca-K, Mg-Se, Mg-K, Mg-Cu, Se-Cu, Se-Fe, Mn-K. U mlijeku magarica utvrđena je jaka korelacija između elemenata: Ca-Na, Ca-K, Ca-Cu, K-Na, K-Cu, Cu-Mn. Statistički značajne razlike u koncentracijama Na, K i Cu utvrđene su u mlijeku tih dviju vrsta. Koncentracije drugih elemenata su slične u obje vrste.

Ključne riječi: mlijeko, konji, magarci, esencijalni elementi, Hrvatska

References

1. Baban, M., Sakač, M., Korabi, N., Antunović, B., Mijić, P., Ivanković, A., Ramljak, J. (2011): Analysis of horse breeding and equestrian sports in the Republic of Croatia. *Biotechnology in Animal Husbandry 27* (3), 415-429.
2. Carroccio, A., Cavataio, F., Montaldo, G., D'amico, D., Alabrese, L., Iacono, G. (2000): Intolerance to hydrolyzed cow's milk proteins in infants: clinical characteristics and dietary treatment. *Clinical and Experimental Allergy 30*, 1597-1603.
3. Csapó-Kiss, Zs., Stefler, J., Martin, T.G., Makray, S., Csapó, J. (1994): Composition of horse colostrum and milk III. Micro- and macro elements and vitamin content. *Acta Alimentaria 23*, 177-192.
4. Csapó-Kiss, Zs., Stefler, J., Martin, T.G., Makray, S., Csapó, J. (1995): Composition of mares' colostrum and milk. Protein content, amino acid composition and contents of macro- and micro-elements. *International Dairy Journal 5*, 403-415.
5. Csapó-Kiss, Zs., Stefler, J., Martin, T.G., Makray, S., Csapó, J. (2009): Composition of mares' colostrum and milk II. Protein content, amino acid composition and contents of macro- and micro-elements. *Acta Univ. Sapientiae, Alimentaria 2*, 133-148.
6. Čačić, M., Kolarić, S., Korabi, N., Mlađenović, M., Tadić, D., Pavan, J., Baban, M., Ivanković, A. (2006): Sistematizacija uzgoja izvorne pasmine konja hrvatski posavac. *Stočarstvo 60 (1)*, 25-29.
7. Čačić, M. (2009): The authentic horsebreed Croatian Coldblood. *Stočarstvo 63*, 135-149.
8. Doreau, M., Boulot, S., Barlet, J.-P., Patureau-Mirand, P. (1990): Yield and composition of milk from lactating mares: effect of lactation stage and individual differences. *Journal of Dairy Research 57*, 449-454.
9. Doreau, M., Martin-Rosset, W. (2002): Horse. In H. Roginski, J. W. Fuquay, and P. F. Fox, (Eds.), *Encyclopedia of Dairy Science*. Academic Press New York, p. 630-637.
10. Fantuz, F., Maglieri, C., Lebboroni, G., Salimei, E. (2009): Ca, Mg, Zn, Fe, Cu and Mn content of ass's milk. *Italian Journal of Animal Science 8 (Suppl. 2)*, 703-705.
11. Fantuz, F., Ferraro, S., Todini, L., Piloni, R., Mariani, P., Salimei, E. (2012): Donkey milk concentration of calcium, phosphorus, potassium, sodium and magnesium. *International Dairy Journal 24*, 143-145.
12. Grace, N.D., Pearce, S.G., Firth, E.C., Fennessy P.F. (1999): Concentrations of macro- and micro-elements in the milk of pasture-fed Thoroughbred mares. *Australian Veterinary Journal 77*, 177-180.
13. Iacono, G., Carroccio, A., Cavataio F., Montalto, G., Soresi, M., Balsamo, V. (1992): Use of ass's milk in multiple food allergy. *Journal of Pediatric Gastroenterology and Nutrition 14*, 177-181.
14. Ivanković, A., Caput, P., Mioč, B., Pavić, V. (2000): Fenotipske značajke magaraca u Hrvatskoj. *Agriculturae Conspectus Scientificus 65*, 99-105.
15. Ivanković, A., Ramljak, J., Štulina, I., Antunac, N., Bašić, I., Kelava, N., Konjačić, M. (2009): Characteristics of the lactation, chemical composition and milk hygiene quality of the Littoral-Dinaric ass. *Mljekarstvo 59 (2)*, 107-113.
16. Malacarne, M., Martuzzi, F., Summer, A., Mariani, P. (2002): Protein and fat composition of horse milk: some nutritional remarks with reference to human and cow's milk. *International Dairy Journal 12*, 869-877.
17. Martuzzi, F., Catalano, A.L., Summer, A., Mariani, P. (1997): Calcium, phosphorus and magnesium in the milk of nursing mares from Italian Saddle Horse breed and their variations during lactation. *Annali Facolta di Medicina Veterinaria, Universita di Parma 17*, 205-212.
18. Martuzzi, F., Summer, A., Catalano, A. L., Mariani, P. (1998): Macro- and micro-mineral elements of the milk and sialic acid bound to casein and to whey proteins in nursing mares of Bardigiano horse breed. *Annali Facolta di Medicina Veterinaria, Universita di Parma 18*, 57-64.
19. Martuzzi, F., Summer, A., Farmaggio, P., Mariani, P. (2004): Milk in Italian Saddle and Haflinger nursing mares: physico-chemical characteristics, nitrogen composition and mineral elements at the and of lactation. *Italian Journal of Animal Science 3*, 293-299.
20. Monti, G., Bertino, E., Muratore, M.C., Coscia, A., Cresi, F., Silvestro, L. (2007): Efficiency of donkey's milk in treating highly problematic cow's milk allergic children: An vivo and in vitro study. *Pediatric Allergy and Immunology 18*, 258-264.

21. Salimei, E., Fantuz, F., Coppola, R., Chiofalo, B., Polidori, P., & Varisco, G. (2004). Composition and characteristics of ass's milk. *Animal Research 53*, 67-78.
22. Park, Y.W., Juárez, M., Ramos, M., Haenlein, G.F.W. (2007): Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research 68*, 88-113.
23. Salimei, E., Fantuz, F., Varisco, G., Maglieri, C., Polidori, M. (2005): Different fibre sources in dairy ass's diet: effects on milk yield and composition. Italian J*ournal of Animal Science 4 (Suppl. 2)*, 430-432.
24. Salimei, E., Fantuz, F. (2012): Equid milk for human consumption. *International Dairy Journal 24*, 130-142.
25. Schryver, H.F., Oftedal, O.T., Williams, J., Soder-holm, L.V., Hintz, H.F. (1986): Lactation in the horse: the mineral composition of mare milk. *Journal of Nutrition 116*, 2142-2147.
26. Sheng, Q., Fang, X. (2009): Bioactive Components in Mare milk. In. Y.W. Park (Eds.), *Bioactive Components in Milk and Dairy Products*. Wiley-Blackwell, Ames, Iowa, USA, p. 195-213.
27. Summer, A., Sabbioni, A., Formaggioni, P., Mariani, P. (2004): Trend in ash and mineral element content of milk from Haflinger nursing mares throughout six lactation months. *Livestock Prodroduction Science 88*, 55-62.