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Minor and potentially toxic trace elements in milk and blood serum of dairy donkeys

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ABSTRACT

The aim of this trial was to study the concentration of Ti, V, As, Rb, Sr, Mo, Cd, Cs, and Pb in donkey milk and blood serum. One hundred twelve individual milk and blood serum samples were collected from 16 lactating donkeys (Martina-Franca-derived population; 6 to 12 yr old; 3 to 7 parities; average live weight 205.4 kg; 32 to 58 d after foaling at the beginning of the trial) during a 3-mo-long experiment. The samples were analyzed for the aforementioned elements by inductively coupled plasma-mass spectrometry. Feedstuff and drinking water were also analyzed for the investigated elements. Data were processed by ANOVA for repeated measures. Average milk concentrations (±SD) of Ti, Rb, Sr, Mo, Cs, and Pb were 77.3 (±7.7), 339.1 (±82.1), 881.7 (±270.4), 4.5 (±1.6), 0.49 (±0.09), and 3.2 (±2.7) μg/L, respectively. More than 80% of samples were below the limit of detection for V, As, and Cd in milk and for Cd, and Pb in blood serum. The lower bound calculated for milk V, As, and Cd was 0.03 μg/L for the 3 elements, the upper bound was calculated at 0.23, 0.10, and 0.31 μg/L and the maximum value was observed at 0.54, 0.15, and 0.51 μg/L, respectively. The average milk concentrations of Ti, Rb, Sr, Mo, and Cs were 600, 458, 346, 16, and 294%, respectively, than those of blood serum. Yet, Cs concentrations were in the same order of magnitude in milk and serum. Moderate to strong positive and significant correlation coefficients were observed between milk and blood serum concentrations for Ti, Rb, Sr, and Cs. The effect of the stage of lactation was significant for all the investigated elements in milk and blood serum, but most of the elements showed only small changes or inconsistent trends, and only the concentrations of Rb and Sr showed decreasing trends both in milk and blood serum. The relationship between milk and blood serum element concentrations indicates that the mammary gland plays a role in determining the milk concentrations of Mo, Ti, Rb, Sr, Mo, and Cs. In the current experimental conditions, in agreement with the low levels in drinking water and feedstuff, donkey milk concentration of potentially toxic elements was very low and did not raise health concerns for human consumption.

Key words: dairy donkey, donkey milk, occasionally beneficial element, potentially toxic element

INTRODUCTION

Scientific interest in donkeys as a dairy species has increased because donkey milk can be considered a functional food for sensitive consumers such as infants and elderly people (Salimei, 2011). In particular, donkey milk can be considered a valid alternative to the available hypoallergenic formula for infants suffering from cow milk protein allergy, as reviewed by Salimei and Fantuz (2013). Knowledge of donkey milk production and composition has improved greatly in recent years: donkey milk shows similarities with human milk with regard to CP, lactose, and ash content, whereas the fat content is lower in donkey milk (Salimei and Fantuz, 2013). Detailed information on the nitrogen and fat fraction are now available (Salimei and Fantuz, 2013) but less attention has been paid to donkey milk macro mineral (Fantuz et al., 2009, 2012; Martini et al., 2014) and trace element content (Fantuz et al., 2013; Potorti et al., 2013; Bilandzic et al., 2014), despite the importance of dietary minerals in human nutrition. Milk contains several well-known nutritionally essential macro minerals and trace elements at different concentrations, but it also contains elements whose biological role, if any, is still not known, and other elements considered potentially toxic such as As, Cd, and Pb (Gaucheron, 2013). The mineral composition of milk depends on endogenous factors, such as species, stage of lactation, and health status of the mammary gland, and on exogenous factors such as diet. Literature data

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about minor trace elements in milk from dairy species are scarce and this topic is not well documented. The biological importance of minor elements such as Ti, V, Rb, and Mo (but the same applies to As, Cd, and Pb) is related to the fact that they can be grouped as occasionally beneficial elements at ultratrace level (estimated dietary requirements usually less than 1 mg/kg, and often less than 50 μg/kg of diet for laboratory animals; Nielsen, 1998; Suttle, 2010). The essentiality of Mo is now supported by substantial evidence and specific biochemical functions have been defined for this element (NRC, 2005). On the contrary, the occasional beneficial effect of Ti, V, As, Rb, Cd, and Pb is based on the fact that, in experimental conditions, a suboptimal biological function due to dietary deprivation of a specific element may be prevented or reversed by an intake of physiological amounts of the element (Nielsen, 1998). Haenlein and Anke (2011) reviewed research focusing on deficiency effects of some elements in experimental ration fed to dairy goats. Based on tissue indicators and on reproductive efficiency, growth, milk production, health, and mortality of goats and their kids, the authors established deficiency and sufficiency dietary levels for Ti, V, As, Mo, and Cd, among others elements. The majority of studies on Sr and Cs in milk dealt with radioactive isotopes, namely Sr^{90} and Cs^{137}, as by-products of nuclear fission, and only little information is available on stable Sr and Cs. Strontium is not classified as an essential trace element but was shown to increase bone formation, and in humans, Sr-ranelate is considered a potential pharmaceutical for the treatment of postmenopausal osteoporosis (NRC, 2005). The aim of this trial was to study the concentration of Ti, V, As, Rb, Sr, Mo, Cd, Cs, and Pb in donkey milk and blood serum, also considering the effect of dietary essential trace element supplementation and stage of lactation.

MATERIALS AND METHODS

Animals, Diet, and Sampling

This on-field experiment was carried out at a private dairy farm producing donkey milk, located in a rural area of Reggio Emilia province, Italy (44°38′9.24″N, 10°28′31.08″E). The research protocol was in accordance with the European Commission guidelines (1986/609/EC) concerning the protection of animals used for experimental and other scientific purposes. Sixteen clinically healthy lactating donkeys (Martina-Franca-derived population; 6 to 12 yr old; 3 to 7 parities; average live weight 205.4 kg; 32 to 58 d after foaling at the beginning of the trial) were used to provide individual milk and blood samples during a 3-mo period. As a part of a larger study focusing on essential trace elements in donkey milk, experimental animals were randomly divided into 2 homogeneous groups: control (**CTL**) and trace element (**TE**). Donkeys in each group had free access to meadow hay and fresh water. Donkeys were fed 2.5 kg of pelleted mixed feed (CP 14.3 g/100 g of DM; NDF 29.4 g/100 g of DM), divided in 2 meals per day. The mixed feed for the TE group had the same ingredients as CTL, but was supplemented with a commercial trace element premix providing 185 mg of Fe (ferrous carbonate), 36 mg of Cu (copper sulfate), 163 mg of Zn (zinc oxide), 216 mg of Mn (manganese oxide), 3.20 mg of I (calcium iodate), 2.78 mg of Co (cobalt sulfate), and 0.67 mg of Se (sodium selenite)/kg of mixed feed. Samples of feedstuff and drinking water were collected at d 0 and 42 from the beginning of the trial. Details on chemical composition of hay and mixed feeds together with details on housing of donkeys were described elsewhere (Fantuz et al., 2013). Individual and bulk milk samples were collected every 2 wk at 1100 h by mechanical milking as described by Salimei et al. (2004). Aliquots of milk samples were frozen and stored at −20°C until analysis. All glassware and polyethylene tubes used for collection, storage, and analysis of samples were previously washed with 3% nitric acid solution (Suprapur quality, Merck, Darmstadt, Germany). Blood samples were collected just after milking by jugular venipuncture in evacuated tubes (Venoject, Terumo Europe NV, Leuven, Belgium) without anticoagulant. Tubes were centrifuged and serum aliquots stored at −20°C until analysis. The health of the mammary gland and the milk hygiene were checked during the trial by monitoring the SCC (Fossomatic 360, Foss, Hillerød, Denmark) and total bacteria (Bactoscan 8000, Foss) in fresh bulk milk samples.

Feedstuff, Milk, and Blood Serum Analysis

Ultrapure water obtained from a Millipore Milli-Q system (resistivity 18.2 MΩ cm) was used to prepare all solutions. Mineralization of thawed milk samples (n = 112) was obtained as described by Fantuz et al. (2013). Briefly, 1 mL of sample was placed in a Teflon digestion vessel, followed by 3 mL of HNO_3 (65%, Suprapur quality, Merck). A microwave closed vessel system (Berghof Speedwave 4, Berghof, Eningen, Germany) was used for digestion. Digested solutions were transferred to a 10-mL volumetric flask and diluted with ultrapure water. Digestion solution for feedstuff was made of 0.2 g of ground samples, 3.5 mL of HNO_3 (65%) (suprapur quality, Merck), and 3.5 ml of H_2O_2 (30%; Suprapur quality, Merck). Blood serum samples were diluted 1:20 with an HNO_3 solution (1%; Fantuz et al., 2013). The concentrations of Ti, V, As, Rb, Sr, Mo, Cd, Cs, and Pb in the acid digested solution or in acidified (HNO_3 1%) blood serum and drinking water were measured by inductively coupled plasma-mass spectrometry (Agilent Technologies, 7500 cx series) as previously described (Fantuz et al., 2013). The limits of detection (**LOD**), expressed as concentration (μg/L) of the elements in the digested solution and diluted blood serum, were calculated as 3 times the standard deviation of 10 repeated determinations of the blank (Table 1). When less than 20% of the samples were below the LOD, results below the LOD were replaced by LOD/2. When more than 80% of the samples were below the LOD, only lower bound (results <LOD replaced by 0) and upper bound (results < LOD replaced by LOD) were calculated (EFSA, 2010). Analysis of certified reference material, nonfat milk powder NIST SRM 1549 (National Institute of Standards and Technology, Gaithersburg, MD) and Seronorm Trace Elements Serum L-2 (Sero As, Billingstad, Norway) were regularly performed, within each batch analysis, to validate the accuracy of the analytical procedure. Certified values were available for milk Cd and Pb and only information or approximate values were available for As, Rb, and Mo in NIST SRM 1549, and for other investigated elements in Seronorm Trace Elements Serum L-2. Neither certified nor information values were available for Ti, V, Sr, and Cs in NIST SRM 1549. For the purpose of the present research, analytical results agreed well with certified or approximate values in reference materials (Table 1).

Statistical Analysis

Descriptive statistics were calculated using SPSS 12.0 (SPSS Inc., Chicago, IL). As checked by Kolmogorov-Smirnoff test, milk Ti and Sr and serum Ti and As concentrations had normal distribution. Data on milk Rb and Mo, and on serum Sr and Mo were logarithmic transformed, whereas data on milk Pb and Cs, and on serum V, Rb, and Cs were square root transformed before statistical analysis. To evaluate the effects of stage of lactation (within-subject factor) and dietary treatment (between-subject factors), data were processed by ANOVA for repeated measures (SPSS Inc.). Data from the first sampling time were used as a covariate when significant. In the case of significant effects ($P < 0.05$), differences between means were analyzed by LSD. Association between variables was examined by calculating simple linear correlations. Significant correlations were declared strong (r > 0.7), moderate (r from 0.3 to 0.7), or weak (r < 0.3).

RESULTS

Milk hygiene and health of the mammary gland were good as demonstrated by the low somatic cell count (7,300 cells/mL) and total bacteria (3,400 cfu/mL) in bulk milk. The concentrations of Ti, V, As, Rb, Sr, Mo, Cd, Cs, and Pb in drinking water and feedstuff are reported in Table 2. Descriptive statistics on investigated elements in donkey milk and blood serum are summarized in Tables 3 and 4, respectively. The percentage of milk samples below the LOD was 89.2% for V, 80.3% for As, and 92.8% for Cd. The lower bound calculated for milk V, As, and Cd was the same, 0.03 μg/L, for the 3 elements; the upper bound was calculated at 0.23, 0.10, and 0.31 μg/L; and the maximum value was observed at 0.54, 0.15, and 0.51 μg/L, respectively. The percentage of serum samples below the LOD was 88.4% for Cd and 81.2% for Pb. The lower bound calculated for serum Cd and Pb was 0.04 and 0.16, and the upper bound was 0.13 and 0.56 μg/L, respectively. The maximum serum Cd concentration was observed at

Table 1. Trace element concentration (±SD) in certified reference materials (nonfat milk powder NIST 1549, National Institute of Standards and Technology, Gaithersburg, MD; and blood serum Seronorm Trace Elements Serum L-2, Sero As, Billingstad, Norway) and limits of detection (LOD)

	NIST 1549,[1] mg/kg			Seronorm, μg/L		
Element	Found	Certified value	Milk LOD, μg/L	Found	Approximate value	Serum LOD, μg/L
Ti	1.95 ± 0.34		0.126	27.3 ± 2.3	12.9	0.112
V	0.002 ± 0.0002		0.023	2.01 ± 0.22	1.01	0.011
As	0.0023 ± 0.0003	(0.0019)	0.010	0.94 ± 0.13	0.67	0.009
Rb	10.7 ± 0.85	(11)	0.025	7.50 ± 0.12	9.73	0.065
Sr	3.40 ± 0.24		0.351	31.5 ± 0.46	36.3	0.071
Mo	0.32 ± 0.02	(0.34)	0.026	1.09 ± 0.05	0.9	0.017
Cd	0.0006 ± 0.0001	0.0005 ± 0.0002	0.030	0.16 ± 0.02	0.13	0.005
Cs	0.014 ± 0.001		0.007	<LOD	0.024	0.003
Pb	0.021 ± 0.004	0.019 ± 0.003	0.042	2.45 ± 0.56	1.11	0.025

[1]NIST 1549 information values in parentheses.

Table 2. Concentration of minor and potentially toxic elements in drinking water and in feedstuff

Element	Water, μg/L	Meadow hay, mg/kg of DM	Mixed feed,[1] mg/kg of DM	
			CTL	TE
Ti	0.03	3.1	11.3	10.5
V	0.11	0.27	1.4	1.5
As	0.11	0.04	0.14	0.16
Rb	0.58	10.4	8.8	8.8
Sr	371.3	60.3	32.5	29.4
Mo	0.58	1.4	1.0	1.7
Cd	0.03	0.07	0.09	0.10
Cs	0.005	0.03	0.16	0.14
Pb	0.07	0.10	0.42	0.44

[1]CTL mixed feed was composed of barley, dehydrated alfalfa (15% CP), wheat, wheat bran, pea, calcium carbonate, bicalcium phosphate, sodium chloride. In TE mixed feed, 0.25% trace element premix was included, providing 163 mg of Zn (zinc oxide), 185 mg of Fe (ferrous carbonate), 36 mg of Cu (copper sulfate), 216 mg of Mn (manganese oxide), 0.67 mg of Se (sodium selenite), 2.78 mg of Co (cobalt sulfate), and 3.20 mg of I (calcium iodate)/kg.

0.78 μg/L and that of Pb at 1.47 μg/L. The transfer of the analyzed elements from blood into milk appeared to occur at a different rate for the different elements: the average milk concentrations of Ti, Rb, Sr, Mo, and Cs were 600, 458, 346, 16, and 294%, respectively, than those of blood serum. Yet, Cs concentrations were in the same order of magnitude in milk and serum. Although precise relationships between milk and serum concentrations were not established for V, As, Cd, and Pb, it can be argued that Pb concentration in donkey milk was higher than that in serum. Moderate to strong correlation coefficients were observed between milk and blood serum concentrations for Ti ($r = 0.34$; $P = 0.01$), Rb ($r = 0.84$; $P = 0.001$), Sr ($r = 0.75$; $P = 0.001$), and Cs ($r = 0.52$; $P = 0.001$). The dietary supplementation with Zn, Fe, Cu, Mn, Se, Co, and I did not significantly affect the concentration of the investigated elements in milk and blood serum (results not shown). The effect of stage of lactation was significant for all the investigated elements in milk (Table 5) and blood serum (Table 6). The concentrations of milk Ti, Mo, and Cs, and of serum V, As, and Cs showed small changes and the trends of milk Pb and serum Ti, Mo, and Cs did not show consistency. The concentrations of Rb and Sr in milk and blood serum were lower during the second part of the trial. The effect of treatment × stage of lactation interaction was not significant for all variables.

DISCUSSION

The concentrations of the analyzed elements in water were within the range reported for tap water in Italy and did not give concerns about contamination with potentially toxic elements (Dinelli et al., 2012). The difference between analyzed elements in CTL and TE mixed feed was small except for Mo whose concentration was about 70% higher in TE mixed feed compared with CTL, likely because of Mo residues associated with the trace element premix included in the mixed feed. Current results are substantially consistent with the available data on Ti, V, Rb, Sr, and Mo in feedstuff (NRC, 2005; Suttle, 2010; Van Paemel et al., 2010). The concentrations of As, Cd, and Pb in feedstuff did not exceed the limits indicated by the European rules for hay and complementary feeds with 88% DM and are consistent with data available for noncontaminated feedstuff (Patra et al., 2008; Van Paemel et al., 2010). Assuming the DMI at 3.2% BW (Salimei, 2011) and considering the known amount of mixed feeds fed to the donkeys, the daily intake of hay was estimated at

Table 3. Descriptive statistics for selected trace elements (μg/L) in donkey milk

Item	Ti	Rb	Sr	Mo	Cs	Pb
Mean	77.3	339.1	881.7	4.5	0.49	3.2
Median	76.1	320.9	829.9	3.9	0.47	2.6
SD	7.7	82.1	270.4	1.6	0.09	2.7
95% CI[1]	75.7–78.8	322.7–356.2	826.6–936.8	4.05–4.72	0.47–0.51	2.8–4.1
Minimum	65.3	156.1	307.7	1.8	0.34	<0.42
Maximum	104.5	502.1	1,728.7	12.4	0.78	12.5
1st quartile	71.3	268.1	680.4	3.3	0.42	1.3
3rd quartile	81.5	408.2	1,082.7	5.0	0.56	4.2

[1]Confidence interval for the mean.

Table 4. Descriptive statistics for selected trace elements (μg/L) in blood serum of lactating donkeys

Item	Ti	V	Rb	Sr	As	Mo	Cs
Mean	12.4	1.0	74.1	254.8	0.49	28.5	0.17
Median	11.8	0.88	74.9	247.5	0.47	25.5	0.16
SD	3.7	0.38	15.2	56.0	0.12	13.8	0.05
95% CI[1]	11.6–13.2	0.90–1.10	70.9–77.2	243.3–251.9	0.47–0.52	25.5–31.4	0.16–0.18
Minimum	5.9	0.40	48.1	160.2	0.26	10.9	0.09
Maximum	23.9	2.0	67.2	416.7	0.82	79.6	0.49
1st quartile	9.9	0.69	60.6	207.3	0.40	17.6	0.13
3rd quartile	13.8	1.4	88.0	292.5	0.56	35.5	0.20

[1]Confidence interval for the mean.

4.62 kg of DM. Consequently, the calculated concentrations of the investigated elements in the CTL diet (mg/kg of DM) were as follows: 5.8 Ti, 0.65 V, 0.07 As, 9.9 Rb, 50.7 Sr, 1.3 Mo, 0.08 Cd, 0.07 Cs, and 0.2 Pb. These concentrations are lower than the maximum tolerable levels of V, As, Sr, Mo, Cd, and Pb in diets for horses (NRC, 2005). The dietary Mo concentration in the CTL and TE group did not raise concerns for interactions with S and Cu. Interactions between Mo, S, and Cu are of great importance in ruminant nutrition because dietary Mo and S concentrations as low as 5 mg/kg of DM and 0.3%, respectively, can decrease Cu availability and increase the risk of Cu deficiency (NRC, 2005). On the contrary, although the maximum tolerable level of dietary Mo for horses (derived from interspecies extrapolation) was established at the same level as that of cow and sheep (5 mg/kg of DM), interactions between Cu and Mo are reported to be minimal in nonruminants (NRC, 2005), and in horses, exposure to Mo does not impair Cu metabolism or raise Cu requirements (Suttle, 2010). In the study by Rieker et al. (2000), a dietary Mo concentration of 20 mg/kg of DM did not appear to interfere with Cu utilization in geldings. It should also be noted that dietary concentration of Cu in TE group was 2.6 times higher than that in CTL group (Fantuz et al., 2013).

Among the investigated elements, on the basis of available knowledge, only Mo can be considered essential in nutrition. Bacteria require Mo for nitrogen fixation and for reduction of nitrate to nitrite and Mo has been identified as a component of 3 mammalian enzymes: xanthine oxidase, aldehyde oxidase, and sulfite oxidase (NRC, 2005). No data are available on Mo in donkey milk, but the observed average concentration was similar (3.5 μg/L; Björklund et al., 2012) or lower (17 μg/L) than published values for human milk (Anderson, 1992) and lower than horse (16 μg/L) and cow milk (22 to 45 μg/L; Anderson, 1992; Rey-Crespo et al., 2013). Observed Mo concentration is consistent with values reported for goat milk (1.9 to 7.1 μg/L) (Trancoso et al., 2009). The concentration of Mo in goat milk can be increased when goats are fed control (0.5 mg/kg of DM) as opposed to Mo-deficient diet (0.02 mg/kg DM; Haenlein and Anke, 2011). It is reported that Mo requirements in livestock are easily met feeding practical diets, although Mo requirements are not well established. A dietary requirement of 0.1 mg Mo/kg of DM was indicated for beef cattle and dietary level of 0.5 mg/kg of DM for sheep and from 0.1 to 1 mg/kg of DM for goats were indicated as general recommendations (Van Paemel et al., 2010). Therefore, it can be assumed that in the current study Mo concentration in the CTL diet met or exceeded dietary Mo requirements for donkeys. The fact that the higher dietary Mo in TE group did not increase the milk and the blood serum Mo concentration is in agreement with findings by Fantuz et al. (2013), who observed that the concentrations of some essential trace elements in donkey milk are not related to dietary intake, at least when dietary requirements are fulfilled. Moreover, the

Table 5. Selected trace elements (μg/L) in donkey milk during lactation

	Days of lactation						
Item	46–72	60–86	74–100	88–114	102–128	116–142	SEM
Ti	72.6[b]	73.3[b]	74.1[b]	87.0[a]	83.1[a]	74.1[b]	1.86
Rb	402.0[b]	425.1[a]	388.4[b]	242.3[d]	295.9[c]	281.3[c]	10.1
Sr	1,178.0[a]	1,185.1[a]	772.9[b]	700.1[b]	689.6[b]	767.9[b]	30.9
Mo	4.5[a]	4.7[a]	4.8[a]	4.7[a]	4.3[a]	3.4[b]	0.35
Cs	0.62[a]	0.61[a]	0.46[b]	0.39[c]	0.46[b]	0.55[b]	0.02
Pb	0.95[c]	3.3[b]	6.1[a]	4.9[ab]	3.3[b]	1.8[bc]	0.78

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

Table 6. Selected trace elements (μg/L) in blood serum of lactating donkeys during lactation

	Days of lactation						
Item	46–72	60–86	74–100	88–114	102–128	116–142	SEM
Ti	15.0^{a}	15.3^{a}	12.8^{b}	9.16^{d}	11.4^{bc}	11.1^{c}	0.65
V	1.4^{a}	1.5^{a}	1.2^{b}	0.61^{c}	0.69^{c}	0.68^{c}	0.06
As	0.54^{ab}	0.61^{a}	0.42^{d}	0.45^{cd}	0.40^{d}	0.50^{bc}	0.03
Rb	90.8^{a}	92.8^{a}	80.5^{b}	57.5^{d}	62.9^{c}	61.6^{c}	1.32
Sr	301.7^{b}	334.3^{a}	212.2^{d}	246.2^{c}	201.8^{d}	257.0^{c}	6.51
Mo	39.9^{b}	15.7^{d}	19.8^{c}	57.2^{a}	23.6^{c}	34.9^{b}	2.62
Cs	0.22^{a}	0.21^{a}	0.14^{bc}	0.16^{b}	0.12^{c}	0.16^{b}	0.01

$^{a–d}$Means within a row with different superscripts differ ($P < 0.05$).

difference in Mo concentration between CTL and TE diet could have been too low (1.3 vs. 1.5 mg/kg of DM) to affect Mo concentrations in milk and blood serum.

Compared with data from the current experiment, cow milk contains a similar concentration of Ti (54 to 111 μg/L; Anderson, 1992; Dobrzanski et al., 2005), and higher V (6.3 μg/L) and Cs levels (9.3 μg/L; Khan et al., 2014). Low V concentration (0.07 μg/L) was observed in human milk, whereas the Cs concentration was higher (1.1 to 11 μg/L) compared with current results (Rossipal and Krachler, 1998; Björklund et al., 2012). Average Ti concentration in human and horse milk was reported at 25 and 145 μg/L, respectively (Anderson, 1992). Based on findings by Fantuz et al. (2013) and Bilandzic et al. (2014), Sr and Rb in donkey milk represent, respectively, the second and third highest concentrations among trace elements after that of Zn. Donkey milk Sr concentration was higher than that observed in cow (417 to 698 μg/L) and horse milk (442 μg/L), whereas Rb concentration was higher in cow milk (2,088 to 2,330 μg/L; Anderson, 1992; Khan et al., 2014). Dobrzanski et al. (2005) observed Rb in cow milk ranging from 290 to 840 μg/L depending on the area of milk collection. Human milk contains lower Sr (33 to 60 μg/L) and higher Rb concentration (714 μg/L; Anderson, 1992; Björklund et al., 2012) than donkey milk. In the current experiment Sr was systematically dominant over Rb concentration, whereas in the mentioned studies Rb was higher than Sr. Data on Rb and Sr in donkey milk need to be confirmed with other diets and in different geological areas. Indeed, donkey milk samples from another dairy farm located in central Italy contained 897 and 497 μg/L, respectively, for Rb and Sr (F. Fantuz, 2015, unpublished data).

The only study available in literature for potentially toxic elements in donkey milk (Potorti et al., 2013) reported higher concentrations for As (ranging from 13.9 to 142.5 μg/L), Cd (1.1 to 20.4 μg/L), and Pb (2.4 to 59.8 μg/L) than those observed in the current experiment. However, comparison with As, Cd, and Pb levels in feedstuff described by Potorti et al. (2013) is difficult because of the composition, including grass silage, of the different diets and lack of information on DM content of diets. More data are available for potentially toxic elements in milk from human and other species. Similar to the current study, milk As and Cd concentrations below the LOD or averaging less than 1 μg/L were observed in human (Leotsinidis et al., 2005; Björklund et al., 2012; Chao et al., 2014), cow (Rahimi, 2013; Rey-Crespo et al., 2013), and goat milk (Trancoso et al., 2009). Low average milk Pb concentration (<10 μg/L) was also observed in human (Leotsinidis et al., 2005; Björklund et al., 2012), cow (Rey-Crespo et al., 2013; Khan et al., 2014), and goat milk (Trancoso et al., 2009; Rahimi, 2013). However, variable results on potentially toxic elements were reported in literature for cow milk (Dobrzanski et al., 2005; Bilandzic et al., 2011). For instance, high concentrations of Cd and Pb were measured in milk samples collected from cows reared around industrial units (Swarup et al., 2005; Patra et al., 2008). It should be noted that only few studies on potentially toxic trace elements in milk from dairy species report data on element concentration in feedstuff and water. Although a direct relationship between milk concentration and dietary intake of potentially toxic elements was not yet established unambiguously, the current low levels of potentially toxic elements in donkey milk agree with the low levels in feedstuff and drinking water. Furthermore, Ca supplementation to the diet of experimental donkeys may have limited Cd and Pb absorption (NRC, 2005; Suttle, 2010).

To the authors' knowledge, blood serum references are not available for the investigated trace elements in dairy animals nor are data available about the relationship between minor trace elements in milk and blood serum, and only little information is available for humans. Current data on donkeys are partially in agreement with findings by Krachler et al. (1999) who reported higher concentrations of Rb, Sr, Cs, and Pb in human colostrum compared with maternal blood serum, but opposite to this study, Krachler et al. (1999) reported higher Mo concentration in colostrum than in blood serum. Previously, higher Zn, Mn, and I, and lower Fe, Cu, Se, and Co compared with respective serum levels were observed in donkey milk (Fantuz et al., 2013). The presence of mechanisms able to uptake elements into the mammary gland with subsequent secretion into milk was described for essential elements such as Ca, P, K, and Zn, and the homeostatic control of mammary gland on milk Fe and Cu was also reported (Shennan and Peaker, 2000; Lonnerdal, 2007). Current observations support the hypothesis that the mammary gland regulates the milk content of some minor trace elements, promoting the transfer of Ti, Rb, Sr, and Cs from blood into milk and inhibiting the transfer of Mo. On the other hand, considering the significant and positive correlations observed between milk and serum Ti, Rb, Sr, and Cs concentrations, passive transfer can also be hypothesized for such elements. On the contrary, the absence of relationship between Mo in milk and blood serum suggest that Mo concentration in donkey milk is independent from maternal Mo status and no passive transfer occurred across the mammary epithelium. Positive correlation coefficient between Sr concentration in human colostrum and blood serum was previously reported (Krachler et al., 1999). Nevertheless, the relationship between trace element in blood and milk likely depends also on the actual blood level of a specific elements: Swarup et al. (2005) observed significant correlation between cow milk and blood Pb only with blood Pb concentration above 0.20 mg/L.

Although the effect of stage of lactation was significant for all elements, only Rb and Sr concentrations showed decreasing trends in milk and blood serum. It should be noted that Rb and Sr show chemical properties similar to K and Ca, respectively, sharing regulating and transport mechanisms (NRC, 2005). In this regard, the concentration of Ca in donkey milk, as well as those of P, Mg, Cu, and Se, declines as lactation progresses, but that of K does not follow a consistent trend (Fantuz et al., 2012, 2013). The concentrations of others investigated elements showed only small, albeit significant, changes or inconsistent trends. A decline of Cd, As, and Pb was observed in human milk until 2 mo from parturition (Chao et al., 2014). Rossipal and Krachler (1998) reported higher concentrations of Pb in human colostrum or transitory milk compared with mature milk, whereas the concentrations of Cs and Sr remain unchanged during the course of lactation.

CONCLUSIONS

The current study provided novel information on minor trace elements and added new data on potentially toxic elements in donkey milk. When compared with published data on human milk, donkey milk generally shows similar or lower V and Mo concentrations, higher Ti and Sr, and lower Cs and Rb. Compared with cow milk, donkey milk contains similar or lower Ti, lower V, Mo, Rb, and Cs, and higher Sr. The current study also provided first data on minor and potentially toxic elements in blood serum of lactating donkeys. Donkey milk shows a lower concentration of Mo and higher concentrations of Ti, Rb, Sr, and Cs compared with their respective blood serum concentrations, suggesting that the mammary gland plays a role in determining the milk concentrations of such elements. In the current experimental conditions, in agreement with the low levels in drinking water and feedstuff, donkey milk concentration of potentially toxic elements was very low and did not raise health concerns for human consumption.

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