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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