

INFLUENCE OF THE AREA AND LACTATION ON PHYSICO-CHEMICAL PARAMETERS AND THE CONTENT OF HEAVY METALS IN THE DONKEY MILK

Adina Lia LONGODOR, Vioara MIREȘAN, Camelia RĂDUCU, Aurelia COROIAN

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Animal
Science and Biotechnology, 3-5 Manastur Street, Cluj-Napoca, Romania

Corresponding authors emails: vmiresan@yahoo.com; coroian.aurelia@gmail.com

Abstract

Donkey milk is considered a substitute for breast milk. Because of its nutritional and compositional properties it is very close to breast milk. Equine asinus can be considered suitable for feeding newborns, against cow's milk, which can cause intolerance. We determined the physico-chemical parameters of donkey milk with CombiFoss FT. The total germ count (TNG) was analyzed by BactoScan and the total number of somatic cells (TNS) by Fossomatic FC. Determination of heavy metals in milk was performed by mass spectrometry to identify Pb, Cd, Cu and Zn. Heavy metals are present in the environment but also in food and can cause serious health problems to the consumer. For the consumer, the main source of cadmium exposure is the food they consume, water and smoking. Cd contamination of dairy milk may also be due to the use of Cd-based chemicals, fertilizers, feed and waste water.

Key words: donkey, heavy metals, lactation, milk, protein.

INTRODUCTION

The donkey milk is a complex aliment, due to its chemical composition (protein, lizozim, fat acids, vitamins) and it represents an important nutritive contribution to the human organism (Shibamoto et al., 2009). Milk is a beneficial aliment for the human organism, present in daily nourishment, but it requires increased attention, due to toxic chemical compounds that may be present (microbiologic contaminants, total number of germs, total number of somatic cells). Among others, steel production, as well as burning coal and waste can increase the concentration of zinc in the environment. It can be inhaled along with dust and smoke, taken in through alimentation, or through exposure to various work places. The quantity that protrudes through the skin is relatively small. Zinc can be stored in the entire organism (ATSDR, 2007; Dash et al., 2013; Mukhopadhyay et al., 2005). All these factors (pollution, industrial activities, consumption of contaminated aliments, energy production) influence the human body over a prolonged period of time and may cause a vast array of health issues (Reimann et al., 2005; Concha et al., 1998; Aposhian et al., 2004). It can be said

that heavy metal particles are causing a large number of drastic illnesses. Heavy metals find their way into the human organism through alimentation and cause concerning effects due to bioacumulation (Vinodhini et al., 2008; Coroian et al., 2017; Dash et al., 2013; Piver et al., 1989).

The presence of heavy metals like Cd in aliments is due to usage Cd based fertilizers and residual waters (EFSA, 2009), as well as packing various foods in metal cans. The symptoms of prolonged intoxication with relatively large quantities of Cd are mainly coughing, irritability and pain associated with chest burns.

Clinic analyses have highlighted the apparition of pneumonia, respiratory obstruction, affected pulmonary function as well as a decrease of the organisms vital capacity (EFSA, 2009). For acute intoxications we can observe: nausea, looseness of bowels and dizziness (Muzy et al., 2001; Vantroyen et al., 2004). Oral Cd intoxications lead to severe irritation of the gastro-intestinal epithelium accompanied by dizziness, nausea, excessive salivation, abdominal pains, cramps, looseness of bowels (Andersen et al., 1988). Pb intoxication cause symptoms such as abdominal pains,

constipation, cramps, nausea and anorexia (Rosenman et al., 2003). The purpose of the research was to determine the physico-chemical composition, the TGN and SCN present in donkey milk under the influence of lactation and geographical location, as well as assessing the concentration of heavy metals present in donkey milk.

MATERIALS AND METHODS

Physico-chemical analysis and TGN (Total Germ Number) and SCN (Somatic Cell Number) analysis of donkey milk

Donkey milk samples have been gathered from traditional farms located in two areas: Sălaj and Cluj. A number of five samples have been taken for each lactation and each location. The milk has been harvested using traditional methods (manual) in an appropriate hygienic environment. The samples have been stored in sterile containers, the ones allocated for the physico-chemical and microbiological analysis being refrigerated, while the ones assigned for heavy metals analysis have been frozen. The equipment used to conduct the physico-chemical analysis was CombiFoss FT, BactoScan for the TGN and Fossomatic FC for the SCN.

The analysis of heavy metals in donkey milk

Heavy metals in donut milk were analyzed by inductively coupled plasma mass spectrometry or ICP-MS used to identify and quantify Pb, Cd, Cu, and Zn elements. The samples have been taken through the mineralization process as following: microwave digestion of 1 ml of milk, using 8 ml HNO₃ 65% and 2 ml H₂O₂ 30%. The digestion process consisted of four stages, as following: stage 1 - temperature - 145°C; pressure - 30 (bar); ramp time - 5 min; maintenance time - 15 min; stage 2 - temperature -180°C; pressure - 30 (bar); ramp time - 1 min; maintenance time -10 min; stage 3 - temperature -120°C; pressure - 30 (bar); ramp time - 1 min; maintenance time - 15 min; stage 4 - temperature -100°C; pressure - 0 (bar); ramp time - 1 min; maintenance time - 10 min. The equipment used was a Berghoff MWS-3 (Eningen, Germany) microwave digester. The sample was cooled to room temperature, dissolved in 25 ml of ultrapure

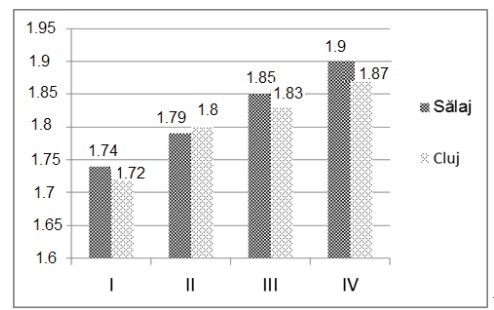
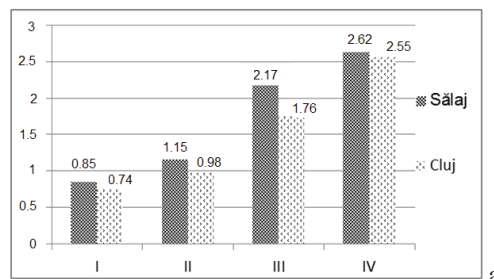
water then filtered through 0.45 µm cellulose membrane. The concentration of heavy metals were determined using an ICP-MS ELAN DRC II Perkin-Elmer.

RESULTS AND DISCUSSIONS

The fat content of the donkey milk varies according to lactation and the harvesting area as follows: in lactation I, the fat content has the lowest average values (0.74-0.85%) (Figure 1. a). The highest values can be seen in lactation IV (2.55-2.62).

Similar results have been reported for fat (Coroian et al., 2016). The protein varies similarly to fat, showing the lowest mean values in lactation I (1.72-1.74%) and the highest values in lactation IV (1.87-1.90%) (Figure 1. b).

Lactose varies according to lactation as follows: 6.71% in lactation I in the Sălaj area and the highest values are in lactation IV 6.89% of Sălaj area (Figure 1. c). The water content ranges from 86.5% in lactation I, Sălaj and 89.81% in lactation IV (Figure 1. d). The results obtained for physicochemical parameters are in agreement with other studies such as those reported by Gastaldi et al., 2010, Husby et al., 2014, Cotte et al., 2003, El-Hatmi et al., 2015.



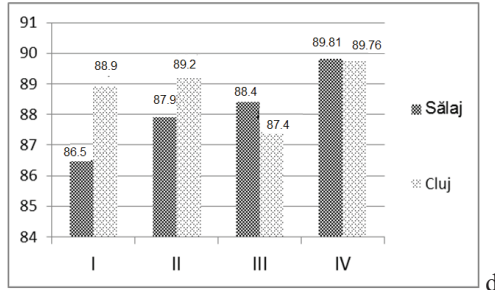
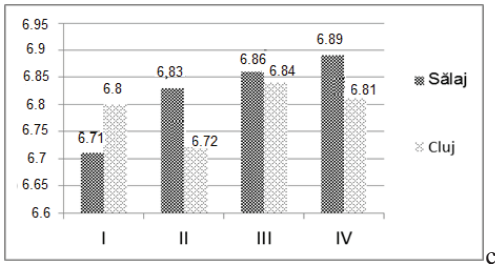


Figure 1. Fat (a), protein (b), lactose (c), water (d) content of lactating milk according to lactation (I-IV) and sampling area (Sălaj and Cluj)

TNG varied in the 48.5 range, the lowest values in lactation I, Sălaj area and 85.5, the highest values in lactation IV, Sălaj area. TNS varied in the range 230.1, lactation I, Sălaj and 320.9, lactation IV Cluj area (Figure 2. a, b).

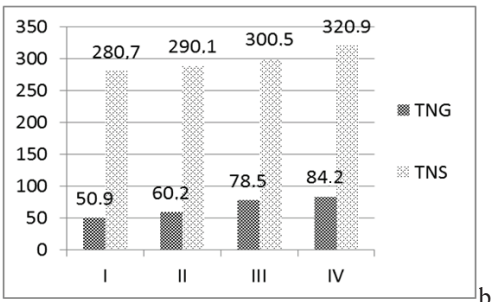
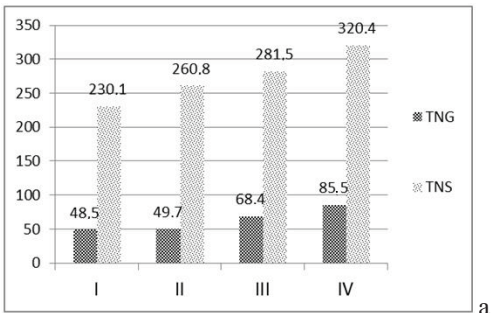


Figure 2. TNG and TNS from lactating milk according to lactation and area Sălaj (a) and Cluj (b)

The Pb ranged from 29.7 ($\mu\text{g/L}$) for lactation I, in the Sălaj area, showing the lowest values, and 48.2 ($\mu\text{g/L}$) for lactation IV. The level of Pb in donkey milk in the Cluj area varied in the range of 19.59 ($\mu\text{g/L}$), lactation II and 32.6 ($\mu\text{g/L}$) in lactation IV (Figure 3, a and b). The Cd ranged from 4.19 ($\mu\text{g/L}$), lactation I and 5.25 ($\mu\text{g/L}$), lactation 3, in the Sălaj area. The values for Cd are lower for donkey milk in Sălaj compared to the Cluj area. Cd in donkey milk in the Cluj area varies between 5.66 ($\mu\text{g/L}$), lactation I, and 6.89 ($\mu\text{g/L}$) in lactation IV. Zn in lactation III in the Sălaj area shows the lowest level, 2761 ($\mu\text{g/L}$), and highest in lactation IV, 3431 ($\mu\text{g/L}$). In the Cluj area, the lowest level for Zn is 2019 ($\mu\text{g/L}$), in lactation 3 and highest in lactation I, 2430 ($\mu\text{g/L}$). The level of Cu varied within the range, 196 ($\mu\text{g/L}$), lactation III, and 320 ($\mu\text{g/L}$), lactation I, Salaj area, and with lower values in the Cluj area, with variations of 127 ($\mu\text{g/L}$), lactation IV and 266 ($\mu\text{g/L}$), lactation I (Figure 3 a, b). These results are similar to those reported by Coroian et al., 2017, for cow's milk.

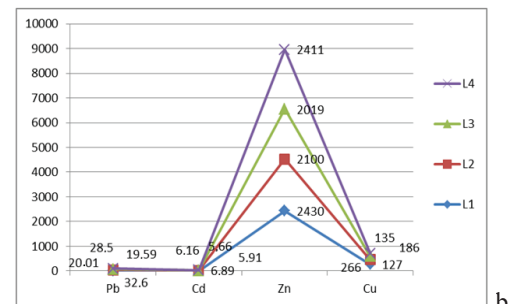
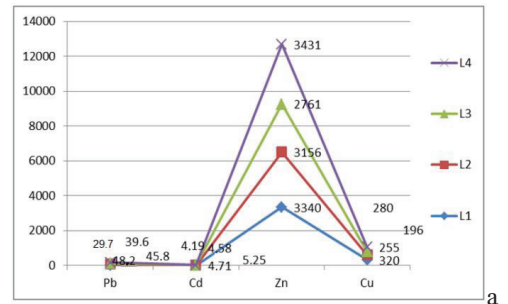


Figure 3. The content of heavy metals (Pb, Cd, Zn and Cu) from lactation milk in the Sălaj area (a) and Cluj area (b)

Heavy metals studies have also been reported by Malag et al., 2005, Caggiano et al., 2005,

Coni et al., 1996, and observe high amounts depending on the area, feed, and the product analyzed. The elimination of lead in the body can be achieved through urine and faeces, and in very small amounts is in the hair, nails, saliva, human milk, sweat (Dale et al., 1994; Hsu et al., 1981; ATSDR, 2007). Regarding Cu, daily intake of food is up to 3 mg. With it, it has an essential role in the body. The sources of contamination are mostly pesticides and copper treatments and copper-made machinery (Suharoschi, 2013). The highest exposures to Cd are from food and tobacco, tobacco leaves accumulate large amounts of Cd. Exposures can also occur through contaminated water consumption. Cd is able to bind to various sulfhydryl groups in proteins, being active in plasma albumin (Voulvoulis et al., 2010). Once food or water contaminated with cadmium is consumed, it is eliminated in the faeces, it is not absorbed in the body in the intestinal tract, but it can be absorbed in the skin, muscles, bones in the kidneys and the liver (Shaikh et al. 1980). Concerning the contamination with Cu, the legislation imposes the following values expressed in mg/kg: 0.5 mg/kg in milk; 1.5 mg/kg in smoked and salted stalks; 2 mg/kg in eggs; 3 mg/kg in cheeses, meat, canned and semiconservé of peas in tomato sauce or vinegar (Suharoschi, 2013). Plant products can accumulate much larger quantities of heavy metals. Plants can accumulate Cd in concentrations ten times higher than soil concentrations. Leaves of plants grown near industrial, polluted areas can contain heavy metals such as Cd that reach values higher than 570 mg/100 g (EFSA, 2009).

Cd poisoning causes anemia, hypoproteinemia, hypoalbuminemia (Jablonska et al., 1971; Solecki et al., 1991). Assessing the level of heavy metals in milk is important because of the risks they can cause to the human body. It is important to establish the level of heavy metals in the donut milk as it is used in the diet of susceptible persons and those suffering from food allergies.

CONCLUSIONS

The average values for heavy metals varied depending on the lactation and the milk sample area. Protein is a very important parameter,

especially when talking about milk processing in dairy products. The highest protein values are for samples from lactation 3th and 4th lactation, and the lowest values are for lactation of lactation 1 and 2. The lactation and sample collection area also affects the fat and lactose content of the donkey.

Heavy metals showed the highest average values in donkey milk samples in the Sălaj area and the lowest are the Cluj area. The donkey milk in Sălaj area showed the highest content in Pb, Cu and Zn and the lowest in Cd. High levels of Zn and Cu in the donut milk were highlighted in all analyzed samples.

REFERENCES

- Andersen O., Nielsen J.B., Svendsen P., 1988. Oral cadmium chloride intoxication in mice: effects of dose on tissue damage, intestinal absorption and relative organ distribution. *Toxicology*, 48, 225-236.
- Aposhian H.V., Zakharyan R.A., Avram M.D., Sampayo-reyes A., Wollenberg M.L., 2004. A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxification of the trivalent arsenic species. *Toxicol. Appl. Pharmacol.*, 198, 327-335.
- Caggiano R., Sabia S., D'Emilio M., Macchiato M., Anastasio A., Ragosta M., Paino S., 2005. Metal levels in fodder, milk, dairy products, and tissue sampled in ovine farms of Southern Italy. *Environmental Research*, 99, 48-57.
- Concha G., Vogler G., Lezciano D., Nermell B., Vahter M., 1998. Exposure to inorganic arsenic metabolites during early human development. *Toxicol. Sci.*, 44, 185-190.
- Coni E., Bocca A., Coppolelli P., Caroli S., Cavallucci C., Trabalza Marinucci M., 1996. Minor and trace element content in sheep and goat milk and dairy products. *Food Chem.*, 57, 253-260.
- Coroian A., 2014. *Toxicologia alimentelor*. Editura Bioflux.
- Coroian A., Mireşan V., Cocan D., Răducu C., Longodor A.L., Pop A., Feher G., Andronie L., Marchiş Z., 2017. Physical-chemical parameters and the level of heavy metals in cow milk in the Baia Mare area. *Banat's Journal of Biotechnology*, VIII(16), 69.
- Coroian A., Mireşan V., Odagiu A., Andronie L., Răducu C., Marchiş Z., Coroian C.O., 2016. Influence of Season on Physico-Chemical Composition of Donkey Milk from Primiparous and Multiparous. *ProEnvironment*, 9, 400 – 403.
- Cotte F., Calabro A., Monsu G., 2003. Il latte di asina: alimento per il futuro. *Il Progresso Veterinario*, 2, 63-68.
- Dale L.S., 1994. Percutaneous absorption of inorganic lead compounds. *Sci. Total Environ.*, 145, 55-70.
- Dash H.R., Mangwani N., Chakraborty J., Kumari S., Das S., 2013. Marine bacteria: potential candidates for

- enhanced bioremediation. *Appl. Microbiol. Biotechnol.*, 97, 561-571.
- EFSA, 2009. Cadmium in food. Scientific opinion of the Panel on Contaminants in the Food Chain.
- El-Hatmi H., Jrad Z., Salhi I., Aguibi A., Nadri A., Khorchani T., 2015. Comparison of composition and whey protein fractions of human, camel, donkey, goat and cow milk, *Mljekarstvo*, Gabes, 159-167.
- Gastaldi D., Bertino E., Monti G., Baro C., Fabris C., Lezo A., Medana C., Baiocchi C., Mussap M., Galvano F., Conti A., 2010. Donkey's milk detailed lipid composition, *Frontiers in Bioscience*, 2, 537-546.
- Hsu J.M., 1981. Lead toxicity as related to glutathione metabolism. *J. Nutr.*, 111, 26-33.
- Husby S., Olsson C., Ivarsson A., 2014. Celiac Disease and Risk Management of Gluten, *Risk Management for Food Allergy*, Odense, 129-152.
- Malhat F., Hagag M., Saber A., Fayz A.E., 2012. Contamination of cow's milk by heavy metal in Egypt.
- Mukhopadhyay J., Gutzmer, J., Beukes, N.J., 2005. Organotemplate structures in sedimentary manganese carbonates of the Neoproterozoic Penganga Group, Adilabad, India. *J. Earth Syst. Sci.*, 114, 247-257.
- Muzi G., Dell'Omo, M., Madeo, G., 2001. Arsenic poisoning caused by Indian ethnic remedies. *J. Pediatr.*, 139(1), 169.
- Perfus-Barbeoch L., Leonhardt N., Vavasseur A., Forestier C., 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, 32, 539-548.
- Piver J.L., 1989. Medical consequences of stratospheric ozone depletion. *Can. Fam. Physician*, 35, 2283-2284.
- Reimann C., de Caritat, P., 2005. Distinguishing between natural and anthropogenic sources for elements in the environment: regional geochemical surveys versus enrichment factors. *Sci. Total Environ.*, 337, 91-107.
- Rosenman K.D., Sims A., Luo Z., 2003. Occurrence of lead-related symptoms below the current occupational safety and health act allowable blood lead levels. *J. Occup. Environ. Med.*, 45(5), 546-555.
- Shaikh Z.A., Smith J.C., 1980. Metabolism of orally ingested cadmium in humans. *Dev. Toxicol. Environ. Sci.*, 8, 569-574.
- Shibamoto T., Bjeldanes L.F., 2009. *Introduction to Food Toxicology*. Academic Press Inc., CA, USA.
- Solecki R., Hothorn L., Holzweissig M., 1991. Computerized analysis of pathological findings in long term trials with phenylmercuric acetate in rats. *Arch. Toxicol. Suppl.*, 14, 100-103.
- Suharoschi R., 2013. *Toxicologia alimentelor*, Editura Academic Press, Cluj-Napoca.
- Timreck C., 2012. Modeling the climatic effects of large explosive volcanic eruptions. *Wiley Interdiscip. Rev. Clim. Change*, 3, 545-564.
- Vantroyen B., Heilier J.F., Meulemans A., 2004. Survival after a lethal dose of arsenic trioxide. *J. Toxicol. Clin. Toxicol.*, 42(6), 889-895.
- Vantroyen B., Heilier J.F., Meulemans A., 2004. Survival after a lethal dose of arsenic trioxide. *J. Toxicol. Clin. Toxicol.*, 42(6), 889-895.
- Vinodhini R., Narayanan M., 2008. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). *Int. J. Environ. Sci. Tech.*, 5, 179-182.
- WHO, 1997. *Environmental Health Criteria 196: Methanol*. World Health Organization, Geneva.
- ATSDR, 2007. *Toxicological Profile for Lead*. US Department of Health and Human Services, 1-582, Atlanta, Georgia.

REPRODUCTION,
PHYSIOLOGY,
ANATOMY

