Timisoara, 25-26 May

COMPARISON OF THE PROTEIN PROFILE AND MILK SUGARS OF DONKEY'S MILK WITH THAT OF HUMAN MILK

BOLDURA O.M.1, TUDOR B.A.M.1, MARC S. 1, TULCAN C. 2, POPESCU S. 2, HUȚU I. 1, MIRCU C. 1
1 University of Life Sciences, Faculty of Veterinary Medicine, 300645, Calea Aradului, 119, Timișoara, România
2 University of Life Sciences, Faculty of Engineering and Applied Technologies
e-mail: oanaboldura@usab-tm.ro

Abstract: *Donkey milk is considered a potential substitute for human milk for infants affected by cow's milk protein allergy. To expand our knowledge about this valuable food, we explored the protein profile of donkey milk using the ELFO technique by comparing it with that of human milk, and the milk sugars were detected by the Brix method. Donkey milk showed a protein and sugar content similar to human milk and essentially different from cow's milk. The results of the performed analysis indicated that the protein profile of donkey's milk is more similar to human milk than that of cow's milk-based formulas, with higher content in sugars and lower in proteins.*

• Introduction

Donkey milk is white in colour with a slightly sweet taste with a very low number of somatic cells and germs (NTG) . The protein composition of donkey milk is significantly different from cow's milk: the total content is lower (1.5-1.8 g / 100g), and quite similar to that of human milk and mare milk. The higher proportion of whey protein in donkey milk by 35–50%, compared to only 20% of bovine milk, makes it more easily absorbed by the human body. The major components of whey proteins present in donkey milk include β-lactoglobulin, α-lactalbumin, lactoferrin, serum albumin, lysozyme and immunoglobulins, and other proteins of low abundances, such as enzymes and proteins that bind metals. As one of the most important milk allergens, β-lactoglobulin accounts for less than 30% of all whey protein, which has been suggested to be lower than that of bovine milk (>50%), but higher than that of human milk (negligible). The second major whey protein, α-lactalbumin, is a small-molecule protein that binds to calcium ions, with a very close amount of donkey milk to human milk and works with anti-inflammatory, antiviral and anti-tumour properties. Furthermore, a high content of lysozyme is present in donkey milk (0,67-4,00 g/kg), which is much higher than that of human milk (0,04 to 0,5 g/kg) and bovine milk (negligible). Along with immunoglobulins, lactoferrin and lactoperoxidase, lysozyme works as one of the most important antimicrobials to reduce harmful bacteria in donkey's milk and the incidence of gastrointestinal infections in infants. Donkey milk has a high content of lactose (5.8-7.4), which is higher than in cow's milk and much more similar to human milk. The high lactose content also stimulates intestinal absorption of calcium and probiotic activity because it is an ideal substrate for developing intestinal lactobacilli. Lactose gives a good taste to donkey milk and is also a precious source of galactose, essential for developing the nervous system. Donkey milk is lower in fat compared to human milk. The effects of donkey milk consumption on health are related to low allergenicity, antimicrobial activity, iron homeostasis regulation, anti-inflammatory activity and modulation of the immune system, antihypertensive, antidiabetic, antitumor, stimulates development, anti-stress and anti-oxidant activity, and anti-osteoporosis. Several studies show that donkey milk is a suitable alternative for children suffering from allergies to cow's milk proteins due to its low composition in casein, which is the main allergenic component of milk. Sarti et al. (2019) showed that donkey milk has no negative influence on infants and children.

• Material and method

The milk samples from E. asinus and Bos taurus species were collected from authorised farms in Arad county, and the human milk sample was collected under a donation agreement by the subject in a volume of approximately 2 ml each.
On-chip protein electrophoresis was performed on Bioanalyzer 2100 (Agilent Technologies, Santa Clara, US) Agilent patented and standardised technology following the laboratory standard proposed by Rainer Nitsche, 2018, under the guidance of the manufacturer of the analysis kit, using the Protein 80 kit, which uses electrophoresis with microfluidic capillary gel with laser-induced fluorescence (LIF) detection.
Quantification of the sugar content of milk was carried out using the refractometric Brix method, which is designed to measure the percentages in a liquid solution and express them in "degrees Brix". One degree of Brix is defined as 1 gram of sugars in 100 grams of solution. The analysis was performed with the BRIX refractometer - model DR201-95, KRUSS Optronic, Germany.

Results of the analysis by comparison of protein concentrations in cow's milk, donkey milk and human milk

Protein fractions	Cow's milk (g/dl)	Donkey milk (g/dl)	Human milk (g/dl)
Total protein	3.4	1,74	1.16
α-lactalbumina	0.15	0.16	0.12
beta-lactoglobulina	0,22	0.21	0.25
beta-cazeina	0.7	-	-
α- cazeina	0.73	0.3	0.23
K-cazeina	0.9	-	-
lizozim	-	0.12	0.1
lactoferrin	-	0.21	0.24
serum albumin	0,1	0.14	0.16

Refractometric analysis data

Biological sample	Dry matter (g/dl)	Sugar content (g/dl)
Cow milk	6.7	4.7
Donkey milk	8.8	6.8
Human milk	9.1	7.1

• Results and discussions

Regarding the use of the microfluidic electrophoresis method on the chip (patented Agilent technology) for the detection and quantification of the protein fractions in the solution, from what is known until now, it has not been used on the donkey milk, as a biological matrix.

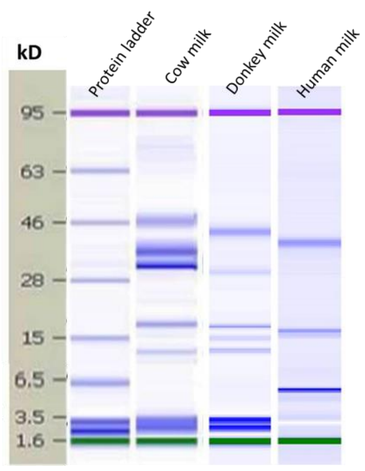

Fig. 1. Protein fingerprinting in the three types of milk. L – Molecular weight marker (Protein Ladder, Protein Kit 80; 1 - Cow's milk; 2 - Donkey milk; 3 - Human milk;

The total protein content of donkey milk varies between 1 – 2.3 g/dl, and the casein fraction is about 35–45%, much lower than ruminant milk (>70%), but more similar to human milk (<30%).

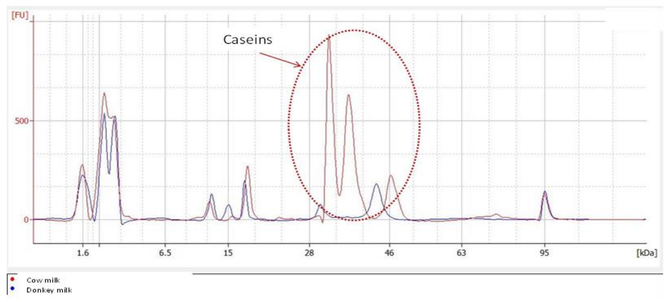

Fig. 2. Overlapped electropherograms of cow's milk (in red) and donkey milk (in blue).

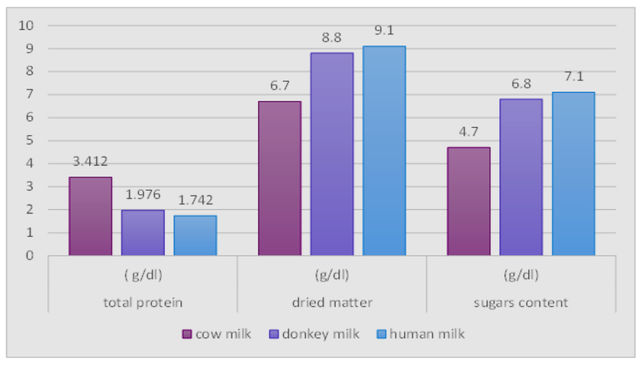

Fig. 3. Graphical representation of biochemical composition data of each sample collected in this experiment.

The available knowledge of donkey milk caseins is limited compared to conventional dairy species, and a complete characterisation has also been complicated by their heterogeneity, partly due to post-translational processes, genetic polymorphism, and non-allelic deletic forms. However, a combination of electrophoretic, chromatographic and proteomic methods allowed the identification of the four casein fractions (αs1-, αs2-, β- and k-casein). It is known that α-casein is the main protein in cow's milk, while donkey milk contains lactoferrin - approximate relative molecular weight of 75 kDa, serum albumin, about 60 kDa, casein (M - 21 to 35 kDa), β-lactoglobulin (M about 18 kDa), lysozyme (M about 15 kDa), α- lactoalbumin (M about 14 kDa). Of these, the three major proteins in whey from donkey milk are α-lactalbumin, β-lactoglobulin and lysozyme. An overlap of the electropherograms obtained in the case of cow's and donkey milk made it possible to highlight the differences between the two types of milk in terms of casein content. Thus, depending on the molecular weights characteristic for the studied protein fractions, it can be seen that the β – casein and κ – casein are totally absent from donkey milk, and α - caseins are present in considerably reduced quantities. Donkey milk contains a large amount of lactose (5.8-7.4 g%), which is higher than cow's milk (4.6 – 4.8 g/%) and much more similar to human milk (approx. seven g%). The high content is responsible for the good palatability of milk and facilitates the intestinal absorption of calcium, which is essential for the bone mineralisation of the infant.

• Conclusions

- In this study, two methods were used that allowed the identification and quantification of three biochemical parameters that ensure the nutritional quality of milk, but the amount of which can also be the source of some nutritional imbalances for the people who consume them.
- The method of microfluidic electrophoresis on the chip allows the identification and quantification of milk proteins and has been successfully used to compare the protein fingerprints of the three types of biological samples. Thus, it was found that κ-casein (the major protein of cow's milk) is missing in the case of donkey milk and human milk. At the same time, cow's milk lacks lysozyme and lactoferrin, which are present in the milk of the other two species analysed.
- The Brix refractometry method was used to quantify the sugar content and dry matter in milk and made it possible to identify noticeable differences in these parameters as well. Thus, the content in the two biochemical parameters is higher in donkey and human milk compared to cow's milk.
- The data shows that donkey milk can be a quality substitute for human milk compared to cow's milk.