

## Comparison of the protein and fatty acid fraction of Balkan donkey and human milk

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### Abstract

The aim of this study was to compare the protein and fatty acid fractions of Balkan donkey and human milk in the early lactation stage (40 and 90 day). This study revealed that donkey milk contains  $\alpha_{s1}$ -casein (1.38-1.89 g/L) and higher concentration of  $\beta$ -casein (0.1-0.55 g/L) in comparison to human milk. The concentration of  $\alpha$ -lactalbumin increased during the lactation phases from 40 to 90 days in both types of milk. Donkey milk contained  $\beta$ -lactoglobulin in low concentrations which decreased to 90<sup>th</sup> day of lactation. Donkey milk was particularly rich in two whey proteins, lactoferrin and lysozyme, which were found to have molecular weight of approximately 76 kDa and 14.9-15.4 kDa, respectively. The content of lysozyme in donkey milk ranged from 2.39 to 2.97 g/L, while human milk contained 30-50 times lower concentrations of lysozyme in comparison to donkey milk. Thus, donkey milk contained also a higher concentration of lactoferrin (0.012-0.25 g/L) than it was found in the human milk. Lysozyme and lactoferrin content in donkey milk increased during the period from 40<sup>th</sup> to 90<sup>th</sup> day of lactation. The percentage of total SFA, MUFA and PUFA was similar in donkey and human milk. The content of essential fatty acids increased during 40-90 days of lactation and was approximately 2.5 times higher in comparison to human milk.

*Key words:* Balkan donkey milk, human milk, proteins, fatty acids

### Introduction

It is well known that the best nutritional option for newborns is human milk which contains higher amount of protective agents compared to bovine milk. However, some infants cannot be exclusively breast fed during the first months of life, and many clinical studies indicate concerns about lactation and nutrition issues as the mothers' most frequent reasons for stopping breastfeeding during the first two months (Ruowei et al., 2008). Therefore, there is a need for another substitute of similar composition and properties as human milk. Other

types of milk have been proposed as substitute for human milk, such as mare (Park et al., 2006), sheep (Haenlein and Wendorff, 2006) and buffalo (Shamsia, 2005) milk, but little information is available on the use donkey milk for this purpose.

Donkey milk has lower protein content (13 to 28 g/L) than other mammalian milks, and a proteomic profile similar to human milk (D'Auria et al., 2005). It has an acceptable taste, it does not cause an overload of solutes in the kidney (Milonis and Polidori, 2011) and two case reports in pediatrics quoted it to be less expensive than infant formula.

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Such features make it an ideal substitute for human milk, whenever the mother cannot or will not breastfeed, or the child is intolerant to cow's milk, providing the nutritional and health needs of the infant (Mansueto et al., 2013). Lien (2003) reported that infant formula is the most appropriate, nutritionally adequate substitute during the first year of life and closely mimics the ratio of total whey to casein in human milk (approximately 60:40). The concentration of  $\alpha$ -lactalbumin (the dominant protein in human milk) is relatively low in formula, whereas  $\beta$ -lactoglobulin, a protein not found in human milk, is the most dominant whey protein in formulas.

Milk contains a number of antimicrobial factors, especially milk proteins, which play a major role in the immune-modulating system of milk. The highest content of lactoferrin (1-2 g/L) is found in human milk (Floris et al., 2003), followed by equine milk (0.99 g/L) and bovine milk (0.02-0.3 g/L). Donkeys' milk is regarded as a very rich source of lysozyme (1 g/L) (Vincenzetti et al., 2008) and has a significantly higher content than human (0.4 g/L) and bovine milk (0.13 mg/L), while the level of lysozyme in donkey milk is quite similar to that in equine milk (0.4-1 g/L) (Floris et al., 2003). Šarić et al. (2012) investigated the antibacterial properties and the protein profile with an emphasis on the lysozymes and lactoferrin of raw donkey milk from an autochthonous Serbian breed. The high content of protective antimicrobial factors in donkey milk suggests its beneficial impact on gut health in persons who have a reduced immune system.

The lipid content of donkey milk is poor, and it has a low caloric value with respect to human milk and other mammalians milk (Salimei et al., 2004). However, this milk was characterized by a higher content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), similarly to human milk. Moreover, in terms of percentage, fatty acid composition and especially PUFA content of donkey milk is higher than that of cow milk, which is the main point of interest in this milk (Gastaldi et al., 2010). The well-balanced ratio of n-6/n-3 of 1.17:1 in donkey milk when compared to human milk makes it an interesting product for human nutrition. As a species, humans are generally deficient in n-3 fatty acids and have excessive levels of n-6 series fatty acids, which are associated with the pathogenesis of cardiovascular, inflammatory and autoimmune diseases (Simopoulos, 2002). The importance of high percentage of lauric (C12:0) and myristic acid (C14:0) in the diet of human milk-fed infants has been shown in its multiple roles as an

antibacterial and antiviral agent (Carpo et al., 2007). To our knowledge, there have been no comparative studies so far regarding the protein and fatty acid composition of donkey and human milk during the first three months of lactation. The aim of this work was to study and compare concentrations of protein fractions and fatty acid profile of donkey and human milk in the early lactation stage (40 and 90 day), in order to evaluate its nutrition values with respect to infants feeding.

## Materials and methods

### Sample collection

The research was conducted on Balkan donkey, an autochthonous breed, from the "Zasavica" Special Nature Reserve (Kugler et al., 2008). Raw milk samples were obtained from 10 female donkeys, after parturition on 40<sup>th</sup> and 90<sup>th</sup> day of lactation. Raw human milk samples were collected from 10 lactating women individually, during the same period of lactation as the donkey's milk. Milk samples were collected from both breasts before the start and after breastfeeding. The samples were cooled down to 4 °C. Concentrations of total protein were determined immediately after sample transportation to laboratories.

### Chemical analyses

The protein content was determined on the basis of total nitrogen, using Kjeldahl method (IDF ISO 8968-1:2001). Milk fat content was determined by a butyrometric method according to Gerber (IDF ISO 488:2008).

### Determination of protein profile

The electrophoretic characterization of milk samples was performed on the Agilent 2100 bioanalyser (Agilent Technologies, Santa Clara, CA, USA) by using of the Protein 80 Plus Lab Chip kit. The obtained electrophoregrams were analysed using the 2100 expert software. The samples were prepared according to Tidona et al. (2011) by modifying the method through applying the dilution ratio 1:1.5 (v/v) instead of 1:2 (v/v), sample: buffer (0.125M Tris-HCL, 4 % SDS, 2 % glycerol, 2 %  $\beta$ -mercaptoethanol, pH 6.8).

### Determination of fatty acids

Fatty acid methyl esters were prepared from the extracted lipids with method that uses 14 % boron

(III)-fluoride in methanol solution, as recommended method for this type of substrates (Folch et al., 1957). The obtained samples were analysed by a GC Agilent 7890A system with flame-ionization detector (FID), auto injection module for liquid samples, equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50  $\mu$ m). Helium was used as a carrier gas (purity >99.9997 vol%, flow rate = 1.26 mL/min). The fatty acid peaks were identified by comparing retention times of samples to retention times of standards from Supelco 37 component fatty acid methyl ester mix (Sigma-Aldrich, EU) and to data from internal data library, based on previous experiments. Results were expressed as a mass of fatty acid or fatty acid group percentage (% w/w) of fatty acids.

### Statistical analysis

The experiments were a completely randomized design with four replicates. The obtained data were subjected to analysis of variance (ANOVA) and mean values were separated by a Duncan's multiple range test at  $p < 0.05$  significance level.

## Results and discussion

### Protein identification and characterization

The protein gel image separated by electrophoresis for fractions of Balkan donkey and human milk during 40 and 90 day lactation period are presented

in Figure 1 (a, b). The protein profile of donkey and human milk is presented in Table 1.

The content of casein in the donkey milk ranges from 6.4 to 10.3 g/kg i.e. 37.56 to 39.98 % of the total protein content (Salimei et al., 2004). Guo et al. (2007) reported that the content of whey proteins in human milk is in the range of 6.8 to 8.3 g/kg and 4.9 to 8.0 g/kg (53.03 to 57.06 % of total protein) in donkey milk. The obtained  $\alpha_{s1}$ -casein concentration in tested donkey milk samples were 1.38-1.89 g/L, whereas  $\beta$ -caseins contents were 0.10-0.55 g/L. These results are in accordance with literature data (Milonis and Polidori, 2011; Polidori and Vicenzetti, 2012). The  $\alpha_{s1}$ -casein was detectable only in some of the analysed human milk samples which is in agreement with Goldfarb et al. (1989), who analysed 125 individual human milk samples using a two-dimensional electrophoresis. Donkey milk showed the absence of intense  $\alpha_{s2}$ -casein band on 40<sup>th</sup> day of lactation and very low concentration (0.11 g/L) of lowest molecular weight proteins (about 28 kDa) in the sample on 90<sup>th</sup> day. The chip-based separations profile of soluble proteins in human milk showed other types of caseins, such as  $\alpha_{s2}$ -casein (0.021-0.056 g/L) and  $\beta$ -casein (0.23-0.35 g/L), which were identified during the lactation. In equine milk, the caseins were highly degraded in gastric lumen and only 30 % remained undigested after 30 min. In human milk, about 39 % undigested casein was observed in gastric lumen, and further digestion by the duodenal juice

Table 1. The protein profile of Balkan donkey and human milk

Protein fraction	Content in Balkan donkey milk (g/L)		Molecular weight (kDa)	Content in human milk (g/L)		Molecular weight (kDa)	p-value		
	40 day	90 day		40 day	90 day		40 days	90 days	
Caseins	$\alpha_{s1}$ -casein	1.38 $\pm$ 0.02	1.89 $\pm$ 0.21	~30.2	ND	ND	ND	S	S
	$\alpha_{s2}$ -casein	ND	0.11 $\pm$ 0.02	~27.6/28.3	0.02 $\pm$ 0.01	0.05 $\pm$ 0.04	~27.6/28.3	S	S
	$\beta$ -casein	0.10 $\pm$ 0.02	0.55 $\pm$ 0.03	~35.2	0.23 $\pm$ 0.04	0.35 $\pm$ 0.06	~35.5	S	S
Whey proteins	$\alpha$ -lactalbumin	1.57 $\pm$ 0.09	2.73 $\pm$ 0.14	~12.3	0.54 $\pm$ 0.08	0.62 $\pm$ 0.05	~12.3	S	S
	$\beta$ -lactoglobulin	0.26 $\pm$ 0.04	0.20 $\pm$ 0.03	~19.6	ND	ND	ND	S	S
	serum albumin	0.11 $\pm$ 0.03	0.11 $\pm$ 0.03	~66.2	0.10 $\pm$ 0.02	0.10 $\pm$ 0.01	~66.2	NS	NS
	lysozyme	2.97 $\pm$ 0.75	2.49 $\pm$ 0.06	~14.9	0.04 $\pm$ 0.06	0.08 $\pm$ 0.04	~15.4	S	S
	lactoferrin	0.01 $\pm$ 0.01	0.25 $\pm$ 0.08	~76.0	0.01 $\pm$ 0.30	0.01 $\pm$ 0.01	~76.4	NS	S

Notes: Data are represented as mean  $\pm$  SEM. Differences between the values are marked as: NS-not significant at a level of  $p < 0.05$  in both the groups and lactating days; S-significant at a level of  $p < 0.05$  in both the groups and lactating days

showed that most of the caseins were degraded after 30 min (Inglstad et al., 2010). According to Cunsolo et al. (2009), considerable differences might be found between the primary structure of donkey and bovine  $\alpha$ <sub>s1</sub>-casein, which could be related to the already demonstrated low allergenic properties of donkey milk and could contribute to better tolerance of donkey milk.

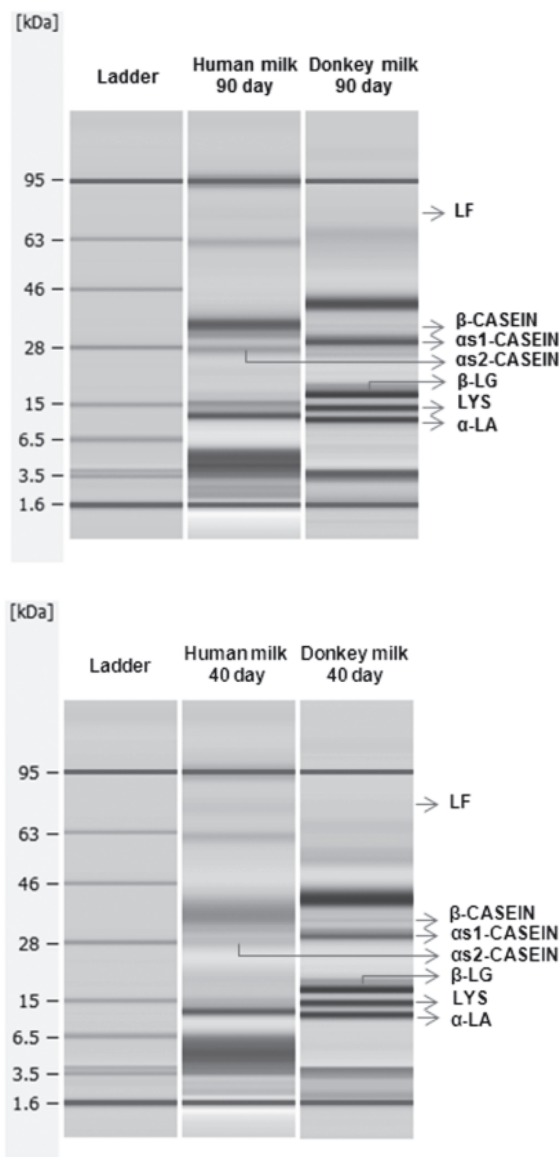


Figure 1. Lab-on-a-Chip gel images of Balkan donkey milk and human milk during lactation period: a) 40 day and b) 90 day. Arrows indicate the position of  $\alpha$ <sub>s1</sub>-casein,  $\alpha$ <sub>s2</sub>-casein,  $\beta$ -casein,  $\alpha$ -lactalbumin ( $\alpha$ -LA),  $\beta$ -lactoglobulin ( $\beta$ -LG), lysozyme (LYS) and lactoferrin (LF)

The gels showed similar bands, with molecular weight of approximately 12.3 kDa in donkey and human milk, suggesting the presence of  $\alpha$ -lactalbumin. Donkey milk contained a higher quantity of this protein (1.57-2.73 g/L) than human milk (0.54-0.62 g/L) during the analysed period. The concentration of  $\alpha$ -lactalbumin increased during the lactation phases (40 to 90 day) in both types of milk. The lowest content was recorded at the beginning of lactation, but increased during the three month period. The  $\alpha$ -lactalbumin is important in lactose synthesis (Chatterton et al., 2006) and needed for proper iron transport (Calil and Falcão, 2003). The  $\beta$ -lactoglobulin fraction was also found in donkey milk, although in low concentration which further decreased until towards the 90<sup>th</sup> day of the lactation (0.26 to 0.20 g/L).  $\beta$ -lactoglobulin found in donkeys' milk is a monomer, while it is mainly a dimer in ruminants milk (Polidori and Vincenzetti, 2012). Owing to that, donkey milk is regarded as highly digestible (70 %) and is digested as twice as much in comparison to cow milk (Inglstad et al., 2010; Tidona et al., 2011). Also, equine  $\beta$ -lactoglobulin was digested significantly faster in comparison to bovine and caprine  $\beta$ -lactoglobulin (Inglstad et al., 2010). Human milk is devoid of  $\beta$ -lactoglobulin. On contrary, donkey milk contains  $\beta$ -lactoglobulin (Malacarne et al., 2002) but it is characterised by different structure which makes it highly digestible and bioavailable, thus being very nutritious for a newborn (Barłowska et al., 2011; Lönnnerdal, 2003). A number of health characteristics have been attributed to  $\beta$ -lactoglobulin, including antiviral and anticarcinogenic effects (Chatterton et al., 2006). Other authors demonstrated that  $\beta$ -lactoglobulin forms complexes with folic acid, suggesting that these complexes might be used as an effective carrier of folic acid in functional foods and neonatal acquisition of passive immunity (Liang et al., 2008).

Donkeys' milk is particularly rich in two whey proteins, lactoferrin and lysozyme, whose bands were found to have molecular weight of approximately 76 kDa and 14.9-15.4 kDa respectively. Also, the obtained results showed that during the first three months of lactation donkey milk contained a high concentration (0.01-0.25 g/L) of lactoferrin, which was similar to human milk (0.01 g/L). The present study showed that the content of lysozyme (2.97-2.49 g/L) in donkey milk was

30-50 times higher than in the human milk (0.043-0.081 g/L). Higher content of lysozyme in donkey milk compared to human milk was also found in some previously conducted studies. Šarić et al. (2012) found that lysozyme and lactoferrin content in the donkey milk samples were 1.31 g/L and 4.80 mg/L, respectively. Also, Balkan donkey milk showed antibacterial activity against *E. coli* and *S. enteritidis* at different tested temperatures (Šarić et al., 2012). In the present study, lysozyme and lactoferrin levels increased from 40<sup>th</sup> to 90<sup>th</sup> day of lactation, which might suggest an important immune-protective role of this enzyme in breast-fed infants during the late lactation (Polidori and Vincenzetti, 2012). With regard to the content of the main protein fractions identified in donkey milk, it can be concluded that protein fractions of human milk share more similarity to those of donkey milk than to those of ruminant animals' milk (Polidori and Vincenzetti, 2012; Lönnderal, 2013). Unlike other human milk substitutes, donkey milk, as a natural food, may allow the infants to build a normal and complete immune system.

#### *Fatty acids characterization*

The results of fatty acids composition, as well as total SFA, MUFA and PUFA content of donkey and human milk samples during lactation time are shown in Table 2. Saturated fatty acids constitute 41.65 % of the total fatty acids in the lipids of Balkan donkey milk and 41.30 % of the total fatty acids in the lipids of human milk after 40 day of lactation period. Similar to human milk (Susmita et al., 2012), donkey milk was characterized by low amounts of short chain and by high quantities of long chain fatty acids. Saturated fatty acids were the dominant class in donkey and human milk, followed by MUFA and PUFA which were present in lower concentrations (Malacarne et al., 2002). Total unsaturated fatty acids (UFA) content in the lipids of Balkan donkey milk (48.44 %) and human milk (50.99 %) after 40 days of lactation period was higher in comparison to SFA constituents in both types of milk. The obtained results of the human milk are in accordance with literature data (Susmita et al., 2012). Susmita et al. (2012) also analysed 135 samples of Bengali mothers' milk. They found that SFA constituted 35 % of the total fatty acids while UFA accounted for 51 % of the total fatty acids in the lipids of the

mature human milk of Bengali mothers, with most of the unsaturated fatty acids being MUFA (35 %). Palmitic acid (C16:0) accounted for 51 % and 35 % of the total SFA of the donkey milk after 40 and 90 days of lactation period, respectively. Also, amount of palmitic acid in total SFA of the human milk was 47 % after 40 and 44 % after 90 days of lactation period, respectively. Among the fatty acids of nutritional interest, the highest percentage was observed for the palmitic acid (C16:0), in the donkey milk and the human milk. Susmita et al. (2012) found that palmitic acid (C16:0) accounted for 60 % of the total SFA in mature human milk of Bengali mothers, while results of Mayer and Fiechter (2013) showed that palmitic acid accounted of 40 % and 39 % of the total SFA of the sheep milk and goat milk, respectively. The content of capric acid (C10:0) in donkey milk was higher than in human milk. The fatty acids from C4:0 to C11:0 and C13:0 to C16:0 showed a decreasing trend toward the 90<sup>th</sup> day, while the contents of C12:0, C14:0 and C18:0 increased. The percentages of lauric (C12:0) and stearic acid (C18:0) were higher and myristic (C14:0) moderately higher in human milk. Regarding the total concentration of SFA, there was an increasing trend during lactation in donkey and human milk, but the increase in concentration was more pronounced in donkey milk with 41.65 % to 49.67 %.

Unsaturated fatty acids accounted for 48.44 % of donkey milk and 50.99 % of human milk after 40 days of lactation period. Most of UFA was MUFA, which contributed to 34.04 % and 37.23 % of the total fatty acids of donkey and human milk after 40 days of lactation period, respectively. Also, Chiofalo et al. (2004) did not find significant variability throughout lactation in the donkey milk. The donkey and human milk showed significantly higher percentages of oleic acid (C18:1 n-9c) in comparison to the other identified MUFA. Among the MUFA, amount of oleic acid (C18:1 n-9c) was significantly higher and increased during lactation period in donkey milk, while it decreased in human milk. The second major fatty acid fraction in milk was represented by palmitoleic acid (C16:1 n-7).

Polyunsaturated fatty acids of linoleic acid series (n-6) were 52.47 % and 84.59 % in donkey milk and human milk after production, respectively.  $\alpha$ -linoleic acid (ALA) and the n-6 essential linoleic fatty acid (LA), increased significantly during the

Table 2. The comparison typical fatty acid composition in the Balkan donkey and human milk during the lactation period

Fatty acid carbon numbers	Common names (acid)	% of total acids (w/w)						Sig-nificant level
		Donkey (n=10)		Human (n=10)		p-Value	p-Value	
		40 day	90 day	40 day	90 day	40 days	90 days	
SFA		41.65±0.04	49.67±0.07	41.30±0.05	42.08±0.06	0.002	0.004	S
C4:0	Butyric	0.09±0.00	0.13±0.01	0.07±0.01	0.09±0.00	0.001	0.000	S
C6:0	Caproic	0.17±0.01	1.15±0.02	0.11±0.02	0.20±0.01	0.000	0.000	S
C8:0	Caprylic	3.14±0.02	4.07±0.01	3.06±0.02	3.10±0.02	0.000	0.000	S
C10:0	Capric	6.18±0.01	7.83±0.01	1.98±0.02	2.20±0.01	0.001	0.000	S
C11:0	Undecanoic	1.46±0.01	2.49±0.01	1.12±0.00	1.23±0.01	0.001	0.001	S
C12:0	Lauric	4.42±0.01	8.26±0.00	6.98±0.02	7.40±0.00	0.001	0.001	S
C13:0	Tridecanoic	0.16±0.01	0.35±0.01	0.09±0.01	0.11±0.01	0.000	0.001	S
C14:0	Myristic	3.80±0.01	6.30±0.01	5.50±0.01	5.96±0.01	0.000	0.001	S
C16:0	Palmitic	21.26±0.01	17.77±0.02	19.50±0.04	18.62±0.03	0.000	0.003	S
C18:0	Stearic	0.96±0.01	1.35±0.02	2.90±0.06	3.18±0.02	0.000	0.003	S
MUFA		34.09±0.07	34.44±1.06	37.23±0.54	32.91±0.55	0.150	0.714	NS
C14:1 n-5	Myristoleic	0.36±0.03	0.34±0.02	0.30±0.01	0.36±0.02	0.012	0.344	S
C16:1 n-7	Palmitoleic	6.63±0.01	3.44±0.13	3.90±0.07	3.62±0.06	0.010	0.047	S
C18:1 n-9c	Oleic	26.34±0.11	29.98±1.14	32.40±0.59	28.36±0.54	0.046	0.042	S
C22:1 n-9	Erucic	0.76±0.04	0.68±0.02	0.62±0.04	0.58±0.06	0.010	0.014	S
PUFA		14.35±0.49	17.53±0.52	13.76±0.26	16.10±0.13	0.154	0.145	NS
C18:2 n-6	Linoleic	7.08±0.06	9.52±0.10	11.13±0.10	13.25±0.40	0.000	0.001	S
C18:3 n-3	Linolenic	6.60±0.41	7.56±0.62	1.93±0.34	2.10±0.20	0.014	0.001	S
C20:2 n-6	Eicosadienoic	0.21±0.08	0.11±0.02	0.01±0.00	0.01±0.00	0.404	0.751	NS
C20:3 n-3	Eicosatrienoic	0.23±0.03	0.17±0.05	0.20±0.05	0.18±0.06	0.100	0.102	NS
C20:4 n-6	Arachidonic	0.24±0.07	0.17±0.01	0.50±0.11	0.58±0.12	0.001	0.001	S
n-6/n-3		1.07±0.18	1.27±0.23	5.88±0.42	6.25±0.56	0.012	0.020	S
UFA		48.44±0.46	51.97±1.35	50.99±0.54	49.01±0.49	0.130	0.126	NS
UFA/SFA		1.16±0.03	1.05±0.20	1.23±0.05	1.16±0.09	0.020	0.035	S

Notes: Data are represented as mean ± SEM. Total amounts of MUFA, PUFA, UFA and certain fatty acids such as C20:2 n-6 and C20:3 n-3 are bolded. Differences between the values are marked as: NS-not significant at a level of  $p < 0.05$  in both the groups and lactating days; S-significant at a level of  $p < 0.05$  in both the groups and lactating days

lactation period from 40<sup>th</sup> to 90<sup>th</sup> day. Among PUFA, the linolenic acid (C18:3) amounted approximately 6.60 % and 1.93 % in donkey milk and human milk after production, respectively. Polyunsaturated fatty acids of linoleic acid series (n-3) were 47.52 % and 15.41 % in donkey and human milk after 40 days of lactation period and decreased in both milk samples during 90 day of lactation. The value of total PUFA fraction in human milk was slightly higher than that detected in the European population (10-16.6 %),

with the predominance of PUFA from n-6 series (Krešić et al., 2013). Values of the n-3 essential fatty acids showed significantly higher values in donkey milk (about 2.5 times) when compared to the human milk. During the studied period of lactation, contents of LA and ALA increased in both types of analysed milk samples. The low concentration of eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3) and arachidonic acid (C20:4) was found in both types of milk samples.

Donkey milk was characterized by the presence of arachidonic (C20:4 n-6) (AA) acid, particularly represented in the membranes of the nervous cells and essential during the neonatal development (Cocchi, 2000), although the content of AA in donkey milk was lower in comparison to that of the human milk. The levels of PUFA in donkey milk were higher when compared to monogastric herbivores. The absence of hydrogenation of fatty acids in the digestive tract before absorption, which is occurring in ruminants, might in fact explain the high content of linoleic and linolenic acid (Jenkins et al., 1996). The amount of unsaturated long chain fatty acids in milk was related to the amount consumed with forages. Donkey consumes grass, which is rich in ALA and poorer in oleic acid compared to human milk. Generally low content of n-3 fatty acids in human milk indicates that supplementation of lactating women with n-3 FA is highly recommendable (Arsić et al., 2009) while donkey milk is a very good source of essential n-3 fatty acids. Levels of LA and ALA in human milk were in accordance with results of Susmita et al. (2012), who found that urban Bengali mother's milk contained 11.05 % LA and 1.68 % ALA. The n-6/n-3 ratio was 1.07 and 1.27 in donkey milk after 40<sup>th</sup> day and 90<sup>th</sup> day of lactation period, respectively, which was significantly lower in comparison to human milk (5.88 % and 6.25 %). The total ratio of n-6 to n-3 fatty acids showed a significant increase during lactation, which could be attributed to a noticeable decrease in the total n-6 and n-3 fatty acid levels. In human milk a much higher (about 60 times) content of n-6 was than n-3 fatty acids was recorded during the analysed period of lactation.

## Conclusions

Regarding the obtained results of protein characterization, donkey milk appeared to be more similar to human milk in comparison to ruminant animals' milk. Balkan donkey milk also showed to be a rich source of  $\alpha$ -lactalbumin which is important in lactose synthesis and needed for proper iron transport. The presence of  $\beta$ -lactoglobulin in monomer form in donkey milk enables its higher digestibility (70 %) in comparison to cow milk. Balkan donkey milk was also characterised by high concentrations of lysozyme and lactoferrin which are known as antimicrobial agents. High amount of n-3 essential fatty acids were found in donkey milk (about 2.5 times higher than in human milk) which makes donkey milk an adequate substitute for human milk, especially during the early lactation period. The obtained

results indicated that Balkan donkey milk might be considered as a potential substitute for human milk. However additional characterization of donkeys' milk nutritional profile is required in order to elucidate its health benefits.

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## *Usporedba frakcija proteina i masnih kiselina mlijeka balkanskog magarca i humanog mlijeka*

## Sažetak

Cilj ovog istraživanja bio je usporediti frakcije proteina i masnih kiselina balkanskog magarca i humanog mlijeka u ranoj fazi laktacije (40 do 90 dana). Ova studija pokazala je da magareće mlijeko sadrži  $\alpha_{s1}$ -kazein (1,38-1,89 g/L) i veće koncentracije  $\beta$ -kazeina (0,1-0,55 g/L) u odnosu na humano mlijeko. Koncentracija  $\alpha$ -laktalbumina povećala se tijekom laktacije u periodu između 40 i 90 dana u obje vrste mlijeka. Magareće mlijeko sadržavalo je niske koncentracije  $\beta$ -laktoglobulina koje su se snižavale do 90-og dana laktacije. Magaričino mlijeko bogato je dvama proteinima sirutke - laktoferinom i lizozimom, za koje je utvrđeno da imaju molekulsku težinu od približno 76 kDa, odnosno 14,9-15,4 kDa. Sadržaj lizozima u magaričinom mlijeku iznosio je od 2,39 do 2,97 g/L, dok je humano mlijeko sadržavalo 30 do 50 puta niže koncentracije. Magaričino mlijeko sadržavalo je i veću koncentraciju laktoferina (0,012-0,25 g/L) nego humano mlijeko. Sadržaj lizozima i laktoferina u magaričinom mlijeku povećao se u razdoblju između 40 i 90 dana laktacije. Udio ukupnih SFA, MUFA i PUFA bio je sličan u magaričinom i humanom mlijeku. Sadržaj esencijalnih masnih kiselina povećao se između 40. i 90. dana laktacije i to 2,5 puta u odnosu na humano mlijeko.

*Ključne riječi:* mlijeko balkanskog magarca, majčino mlijeko, proteini, masne kiseline

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