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Comparison of the nutritional quality and the fat globule size after six months of lactation of donkey and human milk

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Abstract

Donkey milk is acknowledged as a valuable nutritional source in the human diet, well known for its bioactive and functional properties. Therefore, the main goal of this research was to investigate the similarities between donkey and human milk after six months of lactation with respect to the lipid composition, milk fat globule particle size distribution, antioxidant activity, and mineral content. These components are related to the nutritional properties of milk and they are important for the dairy industry as well to human health. The obtained results showed that the most dominant fatty acids in both types of milk were oleic, palmitic, and linoleic followed by lauric, capric, and alpha-linolenic acids. Donkey milk had a desirable fatty acid composition due to its high alpha-linolenic acid content and especially low omega-6/omega-3 ratio. After the fat globule distribution was analysed, it was found that fat globules smaller than 2 μm had the highest percentage in both human and donkey milk. The antioxidant activity of human milk was significantly higher at 42.95 % compared to donkey milk at 35.83 %. Predominant mineral in both types of milk was Ca, followed by P, Zn, Fe and Cu. Highlighting the similarity between donkey milk and human milk encourages the use of donkey milk as a potential substitute for human milk in the future.

Key words: donkey milk; human milk; nutritional quality; fat globule size distribution

Introduction

Donkey milk (DM) is well known for its beneficial properties on human health (unique nutritional composition, functional and bioactive compounds). Therefore, currently, it takes high attention in the field of food science (Salimei and Fantuz, 2012; Altomonte et al., 2019; Martini et al., 2018). In terms of lactose, lipids, fatty acid, and protein profile, DM has a composition that is more comparable to human milk (HM) than cow milk (Gubić et al., 2015; Altomonte et al., 2019).

Some studies have shown that the absorption and metabolism of DM and HM in human digestion system are very much alike due to their similarities in bioactive compounds (Aspri, 2017; Prasad, 2020). Some studies have shown that the absorption and metabolism of DM and HM in the human digestive system are very similar due to their similarities in bioactive compounds (Aspri, 2017; Prasad, 2020). First, due to the low content of casein, which creates soft flocculation during digestion in infants, it additionally contributes to the faster digestibility of milk (Ragona et al., 2016). Clinical studies have shown that infants tolerate DM well (82.6-88 %) (Ragona et al., 2016; Aspri, 2017). Fats contribute the major portion (45-55 %) of the energy source in HM, with a total fat intake of approximately 5.5 kg in a fully breastfed infant during the first six months of life (Koletzko et al., 2011; Agostoni et al., 2005). Then, in the first 12 months of a child's life, daily lipid intake represents 50 %, and in the period between 12 and 24 months about 40 % (Sarti et al., 2019; WHO, 2010). One of the major differences between DM and HM is related to fat yield. DM has low-fat content and hence it may represent a limitation in the children's diet and needs to be enriched with lipids in order to fulfill the infant's requirements. Generally, during lactation HM contains approximately 3.5 to 4.5 % of fat content, while DM ranges between 0.45 to 1.15 % with an increasing, nonlinear, trend from partum to the end of lactation (Szabo et al., 2010; Gubić et al., 2015). The uniqueness of DM is mainly attributed to the favourable composition of FA, especially to the high PUFA content with linoleic acid (18:2 n-6; LA) and alpha-linolenic acid (C18:3 n-3; ALA) ratio of 2:1 and a balanced ratio of omega-6/omega-3 (n-6/n-3) of 1.17:1 compared to that of HM of 12.45:1.37 (Martemucci and D'Alessandro, 2012; Gubić et al., 2015; Martini et al., 2018; Szabo et al., 2010). In particular, the high value of PUFA (52.2 %), the low n-6/n-3 ratio, and the advantageous values of atherogenic (AI) and thrombogenic indices (TI) could represent a nutritional advantage of DM (Chiofalo et al., 2011; Gastaldi, 2010; Martemucci and D'Alessandro, 2012; Martini et al., 2015).

Milk fat occurs in a form of native milk fat globules (MFG) surrounded by the milk fat globule membrane (MFGM), with a size distribution ranging from 0.2 to 20 μm, which may affect FA composition and additionally provide 40 to 55 % of total energy intake (Martini et al., 2012, 2013a, 2014; Altomonte et al., 2019). The lipid fraction of milk is a "natural solvent" for macronutrients and micronutrients such as minerals (Manoni et al., 2021). The main minerals in the milk lipid fraction MFG are iron, zinc, copper, calcium, and phosphate (soluble and colloidal), which balances are fundamental to the structure and function of the micelles (Lucey and Horne, 2009). Information about the MFG of DM is significant from a nutritional and technological point of view and only a few studies have focused on the morphometric characteristics of MFG in the milk obtained from Italian donkeys (Martini et al., 2012, 2015, 2018). In previous studies, the authors discovered that the size and composition of lipid globules have a significant influence on lipid digestion and metabolization by infants (Mizuno et al., 2009; Martini et al., 2014; Duan et al., 2021). In addition to very small MFG, DM is characterized as a rich source of short-chain FA, which improves fat digestibility and intestinal absorption (Martemucci and D'Alessandro., 2012; Altomonte et al., 2019).

The best milk for an infant is HM, which insures healthy and harmonious child development. About 75 % of mothers choose to breastfeed their infants in the first six months of life, while only 13 % continue to breastfeed after this period (WHO, 2008). Globally, only 36-38 % of infants are exclusively breastfed (WHO-Breastfeeding, 2008). Besides being nutritionally valuable and essential for nutrition, HM improves the performance of the immune system because it has immunomodulatory potential and contains numerous antimicrobial agents (Szabo et al., 2010; Lubetzki et al., 2012). When breastfeeding is not possible, it is very important that infant nutrition fulfills the antioxidant requirements to resemble natural feeding as much as possible (Beghelli et al., 2016). The antioxidant activity (AA) of milk is the result of a complex interaction between antioxidant components such as proteins, carotenoids, flavonoids, and vitamins E and C (Faustini et al., 2014; Simos et al., 2011). AA is related to the prevention of lipid peroxidation, providing that way oxidative stability in milk (Salimei and Fantuz, 2012; Beghelli et al., 2016). Some studies were focused on investigating the antioxidant potential of HM (Zarban et al., 2009). On contrary, there is still a lack of scientific results based on the AA and the influence of the lactation period of DM.

Previous research has highlighted the similarities between DM and HM for infant nutrition (Altomonte et al., 2019; Szabo et al., 2010; Prasad, 2020). In a comprehensive literature review, only a few scientific studies investigated DM and HM obtained during prolonged lactation (Czosnykowska-Łukacka et al., 2018; Szabo et al., 2010). DM has been increasingly investigated as a promising alternative food for infants and children, so this study aimed to improve research data on the nutritional similarities between DM and HM for infant nutrition after six months of lactation.

Material and methods

Animals and nutritional management

DM was supplied from a farm located in the Special Nature Reserve Zasavica (Serbia) within the native area of the Balkan donkey, an autochthonous breed. The donkey was reared outdoors and the diet consisted of *ad libitum* pasture, traditional pasturing practices. In the pasture, the species

consumed by the donkeys were *Lolium perenne, Agropyrum, Alopecurus, Festuca pratensis, Trifolium, Trifolium repens, Medicago sativa, Achillea, Matricaria and Plantago media*. In the winter, when there was insufficient pasture to meet the herd's nutritional requirements, donkeys were fed with meadow hay and maize. During milking, the animals were fed corn twice a day, pasture hay and fresh water available *ad libitum.*

Milk samples

The study was performed on 20 donkeys, which were routinely machine milked twice a day. During the 2 hours prior to milking, foals were physically separated from their mother in an adjacent box to allow visual contact. The mature milk samples were taken from each individual animal after the 6th month of lactation. The samples were stored in sterile plastic bottles at 4 °C until further analysis and samples used for FA determination were freeze-dried and stored at -20 °C until analysis.

Human milk samples

The samples of HM have been collected from 20 healthy women between the ages of 28 to 36 years, after the 6th month postpartum. All samples were delivered from mothers who agreed to participate in the study. Milk samples were removed from the mammary gland with a mechanical breast pump. HM samples were treated similarly to DM samples. The HM samples have been stored at 4 °C and analyzed after 24 h after their collection.

Chemical composition

The contents of total solids were determined according to IDF 21:2010 methods. The concentrations of total nitrogen (TN) were determined according to IDF 20-1:2001. A nitrogen conversion factor of 6.38 was used to calculate protein concentrations of milk samples. The concentration of fat was determined according to IDF 105:2008. Ash content was determined after mineralization of milk at 550 °C for 4 h according to IDF 258, 2001.

Lipid composition

Fatty acid composition from lipid extracts was determined by gas chromatography-flame ionization detection (GC Agilent 7890A system). Supercritical fluid extraction with CO_2 was used for the preparation of fat extracts, described by Ivanov et al., (2012). Extractions were performed on a fat analyzer (LECO TFA 2000) according to procedures (Leco Corporation, 2003). FA methyl esters were prepared by transmethylation method using a 14 wt% boron trifluoride/ methanol solution (Sigma Aldrich, MO, USA) described by Folch et al., (1957) with the slight modification described by Gubić et al. (2015). Results were expressed as the ratio of individual FA or FA group in total identified FA (%, w/w).

Indexes of lipid quality

The atherogenicity (AI) and thrombogenicity (TI) indices were calculated according to the equations [1] and [2] (Senso, 2007), by using data on the FA composition.

Index of atherogenicity (AI) indicates the relationship between the sum of the main saturated FA (SFA) and that of the main classes of unsaturated, the former being considered pro-atherogenic and the latter anti-atherogenic:

AI = [(4 × *C*14:0) + *C*16:0 + *C*18:0]/ ΣMUFA +ΣPUFA – *n*6 +ΣPUFA – *n3* [1]

Index of thrombogenicity (TI) shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic FAs (*MUFAs,* PUFAs – *n6* and *PUFAs – n3*) (Senso, 2007).

The following equation was applied:

TI=(C14:0+C16:0+C18:0)/(0.5MUFA+0.5PUFA-n6+3PUFAn3+ PUFA-n3/PUFA-n6 [2]

The particle size distribution (PSD) of MFG

The particle size distribution of MFG in milk samples was determined by Mastersizer 2000 (Malvern Instruments, England) laser diffraction particle size analyzer using a Hydro 2000G dispersion unit. The samples were added at ambient temperature in water until an adequate obscuration was obtained (10-20 %). The results were quantified as the volume-based particle size distribution by means of the Mastersizer 2000 software. The obtained particle size distribution parameters included following parameters: D[4.3]-volume mean diameter; d(0.5)-mass median diameter of the volume of distribution, indicating that 50 % of the sample volume has a particles with sizes smaller than that value, whereas 50 % has a larger size; d(0.1)- indicating that that 10 % of the sample volume are particles with sizes smaller than that value and 90 % are larger than that value; and parameter d(0.9)-indicates that 90 % of sample volume has a particles with sizes smaller than that value and 10 % are larger than that value and dsr-mean diameter (μm) (Stojanović et al., 2010).

Antioxidant activity

The antioxidant activity (AA) was determined by a 2.2- diphenyl-1-picrylhydrazyl (DPPH) scavenging system described by Alyaqoubi et al. (2014). To prepare the stock solution, 40 mg DPPH was dissolved in 100 mL methanol. By mixing 350 mL of the stock solution with 350 mL methanol, an absorbance of 1.0±0.01 units was obtained using a spectrophotometer (Jenway 6405 UV/Vis) at 517 nm wavelengths. 100 μL of fresh milk extract was mixed with

1 mL methanol DPPH solution and kept in the dark for 2 h to allow the reaction to occur. The percentage of DPPH activity was calculated as follows: DPPH (%) = [(A blank - A sample) / A blank] × 100. A is the absorbance.

Minerals content

Milk samples were mineralized by dry ashing (AOAC method 999.11 B) and content of minerals calcium (Ca), copper (Cu), zinc (Zn), and iron (Fe) was determined by flame atomic absorption spectrometry VARIAN SpectrAA-10, with background correction system (D2-(deuterium lamp) and appropriate cathode lamps. Standard solutions of minerals were prepared immediately before use by dilution (with 0.1 M HNO_3, Merck, Germany) of standards at the concentration of 1 mg/cm (Baker, The Netherlands). Phosphorus content was determined by molecular absorption spectrometry (IDF 42:2006).

Statistical analysis

All analyses were performed in duplicate and results were expressed as mean ± standard deviation. The data were processed statistically using the software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) and Tukey's HSD test for comparison of sample means were used to analyze variations for significance ($p<0.05$). In the milk samples frequency distribution of the total counted and measured MFG (Fig. 1) was performed according to Martini et al. (2012), where their size were divided into three size categories of fat globules: small globules (SG) with a diameter <2 μm, medium-sized globules (MG) with a diameter from 2 to 5 μm and large globules (LG) with a diameter >5μm.

Results and discussion

The basic compositions of DM and HM are presented in Table 1. Donkey milk showed lower values of total solids and fat, while it had the highest values for protein and ash. Although the variation in protein and ash percentages between milk types was greater, $p<0.05$ did not indicate that there was a statistically significant difference. In the present study, the main difference between the types of milk was the fat content. Similar results were obtained for composition of mature HM according to Czosnikovska-Łukacka et al. (2018). The results of showed that HM produced above 1 year of lactation is extremely rich in fat and has a higher energy content than HM produced during the first 6 months of lactation Lubetzki et al. (2012). Fat content of DM show decreasing tendency especially after 6th month of lactation (Massouras et al., 2017; Martemucci and D'Alessandro, 2012).

The FA composition of the observed DM and HM milk samples is presented in Table 2. Accordingly, the total SFA content was higher in HM, while the concentrations of MUFA and PUFA were found to be higher in DM (Mesias et al., 2021).

Table 1. *Chemical composition during the follow-up period of DM and HM*

Composition	DM	HM
Total solids, %	9.28±0.57^a	11.22±0.43^b
Protein, %	1.46±0.04^a	1.30±0.10^a
Fat, %	0.66±0.13^a	3.35±0.23^b
Ash,%	0.54±0.11^a	0.40±0.20^a

Data are expressed as mean ± SEM. Mean values of the observed parameters are written as the result of three measurements (n=3). Values with different superscripts (a,b) are significantly different ($p<0.05$)

Previous studies (Chiofalo et al., 2011; Salimei and Fantuz, 2012) already indicated that DM and HM have different FA compositions, but the SFA are those that are present in the highest concentration in both kinds of milk. The SFA were the most prevalent in the milk of Nordestina donkeys (48.9 %) after 120 days of lactation compared to MUFA (31.1 %) or PUFA (19.7 %), which had lower concentrations than the milk under study here. However, a similar trend was obtained in another study of SFA where donkey milk up to 210 days of lactation was examined (Massouras et al., 2017; Lazarević et al., 2017; Martemucci and D'Alessandro, 2012). The results showed that the most dominant FA in both types of milk was oleic acid (C18:1), palmitic acid (C16:0), LA, followed by lauric acid (C12:0), capric acid (C10:0) and ALA. The FA composition obtained in this study is in agreement with the results of human mature milk (Lubetzky, 2012).

Among the PUFA group, ALA and LA fatty acid dominated in both types of milk, with the fact that compared with HM, DM contained larger amounts of ALA and LA which is in keeping with the previous research (Massouras et al., 2017; Nayak et al., 2017). Also, a higher content of PUFA n-3, PUFA n-6, and n-6/n-3 ratio was found in DM. The results reported by Massouras et al. (2017) revealed that during late lactation period n-6/n-3 ratio shows an increasing tendency in DM. According to O'Connell et al. (2017) the high PUFA n-3 content and low n-6/n-3 ratio in HM are more beneficial to human health, which PUFA n-3 and n-6 and their proportions are correlated with human diseases.

Furthermore, a low amount of ALA resulted in higher AI and TI in the HM rather than in DM. Regarding the FA composition, with a sufficient amount of LA, ALA, PUFA n-3, and PUFA n-6 FA, such as DHA, AA as well as MUFA, DM showed to be suitable to meet childs' growth and metabolic needs. This observation was in agreement with results obtained by Bobinski and Bobinska (2020), who found that the first six months of a child's life is one of the most dynamic developments of a nervous system and the above-mentioned FA play a mandatory role in its development (Bobinski and Bobinska, 2020). Some studies have also demonstrated that the diet of breastfeeding mothers and donkeys greatly influences the FA composition of milk (Agostoni, 2005). However, it is found that only small parts of FA originate from the direct resorption of food while the greater part originates from the body's stock which is partially compensated by oscillations in food intake (Agostoni, 2005). Furthermore, poor levels of PUFA n-3 in HM

have been correlated with the development of symptoms of allergic disease in children followed up to 18 months of age (Chiofalo et al., 2011; Gastaldi et al., 2010). DM with higher PUFA n-3 levels and a more favorable FA profile are adequate for children's nutrition, given that, following the nutritional guidelines of various international organizations, a child's fat intake should be gradually decreased from 60 % to 35 % after age 6 months (Sarti et al., 2019; Martini et al., 2021). In particular, despite major concerns regarding the use of DM as the sole source of nutrition (if not adequately supplemented), the results of the research by Sarti et al. (2019) indicate that it could be considered a valid alternative in weaning infants (older than 5-6 months), considering that solid food should be introduced at the latest at the age of six months (Kostecka and Kostecka-Jarecka, 2021). Results obtained by Martemucci and D'Alessandro (2012) showed that n-6/n-3 ratio was approximately 2:1 in DM, with values <1 during the last period of lactation. Additionally, they suggested application of DM, as a functional food, in human nutrition, especially for the elderly and with potential utilization in infant nutrition (Martemucci and D'Alessandro, 2012; Prasad, 2020). Results obtained in this study showed that the ratio of AI and TI values in DM and HM are very similar. However, a statistical differences and higher values were detected for AI and TI in HM (Table 2). Increasing the value of AI and TI may be due to the reduction of MUFA, PUFA n-3 and PUFA n-6 as antithrombogenic acids (Ulbricht and Southgate, 1991). Previous studies in mares' milk have shown that the high and equilibrate essential FA content together with low AI and TI indicate the immune modulatory properties of milk (Chifalo et al., 2011; Pikul and Wójtowski, 2008).

The PSD of MFG and minerals content in DM and HM

Laser diffraction was used to analyse the PSD of MFG in DM and HM. Milk fat is present in a form of globules with a diameter in the range of 0.1-15 μm (average diameter ~ 4 μm) (Wiking et al., 2004). Regarding the results of PSD parameters of MFG (Figure 1), it was found that the fat globules had a different amount of MFG size between two types of milk for the same period of lactation. DM has smaller sizes of MFG compared to HM, as shown in Figure 1. Mastersizer 2000 software showed that 38.42 % of DM volume has globules in the range of 0.1 to 1 μm, while only 12.15 % of the HM volume contains globules in that interval. On the other hand, 56.81 % of DM volume has particles in the range of 1 to 10 μm and 80.49 % of HM volume has globules in that range. Compared to Amiata donkey milk analyzed by Martini et al. (2014), the average distribution percentage of globules in the range of 0.1 to 1 μm was 25.98 % of the total measured MFG, which is a smaller proportion than that obtained in our study. Several other studies have shown that the average size of MFG can vary during lactation, due to changes in the total amount of milk fat produced during lactation (Chang et al., 2015). However, other authors indicate that an increase in the fat content of milk leads primarily to an increase in the number of MFG, and not in size (Mizuno et al., 2009).

Table 2. *Fatty acid composition during the follow-up period of DM and HM*

Fatty acid	Content (% of total fatty acids)	
	DM	HM
SFA	48.72±0.01^a	51.17±0.02^b
C4:0	0.22±0.01^a	0.20±0.01^a
C6:0	1.35±0.02^a	1.81±0.01^b
C8:0	4.06±0.01^a	5.92±0.08^b
C10:0	8.95±0.01^a	9.90±0.18^a
C11:0	2.69±0.02^a	3.47±0.04^b
C12:0	9.26±0.01^a	9.28±0.08^b
C14:0	0.30±0.02^a	0.35±0.03^a
C16:0	19.77±0.03^a	18.61±0.19^a
C18:0	1.85±0.03^a	1.33±0.04^a
C20:0	0.22±0.01^a	0.20±0.05^a
C22:0	0.05±0.01^b	0.10±0.03^a
MUFA	27.38±0.06^b	23.83±0.18^a
C10:1	0.08±0.01^a	0.05±0.01^a
C12:1	0.18±0.01^a	0.16±0.02^a
C14:1	0.34±0.02^a	0.44±0.02^b
C16:1	4.06±0.03^a	3.35±0.06^a
C17:1	0.12±0.02^a	0.12±0.1^b
C18:1	21.59±0.1^b	18.43±1.1^a
C20:1	0.28±0.02^b	0.38±0.01^a
C22:1 n-9	0.54±0.02^a	0.60±0.02^b
C24:1 n-9	0.19±0.01^b	0.30±0.01^b
PUFA	21.63±0.05^a	18.75±0.04^b
PUFA n-3	7.33±0.03^b	6.41±0.03^b
C18:3 n-3	6.44±0.02^b	5.57±0.07^a
C18:4 n-3	0.20±0.01^a	0.26±0.01^a
C20:3 n-3	0.10±0.01^a	0.08±0.01^a
C20:4 n-3	0.07±0.01^a	0.12±0.01^b
C20:5 n-3	0.25±0.02^b	0.19±0.02^a
C22:5 n-3	0.07±0.01^a	0.06±0.01^a
C22:6 n-3	0.20±0.01^a	0.13±0.01^a
PUFA n-6	14.30±0.02^a	12.34±0.02^b
C18:2 n-6	11.57±0.06^b	9.53±0.05^a
C18:3 n-6	0.58±0.02^a	0.52±0.02^b
C20:2 n-6	1.10±0.01^b	1.20±0.01^a
C20:4 n-6	1.05±0.01^a	1.09±0.02^a
n6/n3 ratio	1.95±0.02^a	1.92±0.02^b
AI	0.62±0.01^a	0.69±0.02^b
TI	0.51±0.02^b	0.54±0.01^a

Data are expressed as mean ± SEM. Mean values of the observed parameters are written as the result of three measurements (n=3). Values with different superscripts (a,b) are significantly different ($p<0.05$)

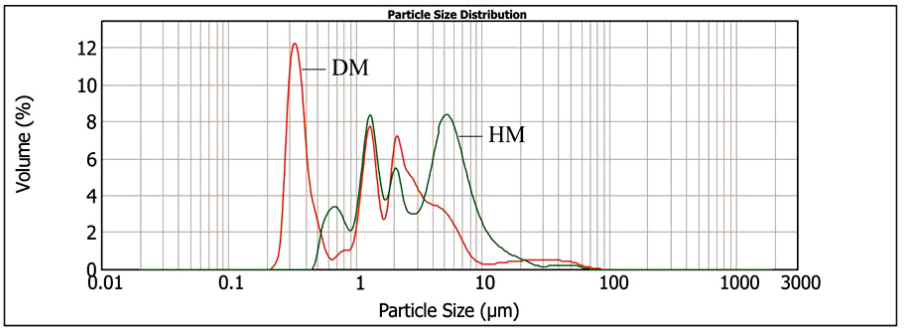

Figure 1. Particle size distribution of DM and HM

DM has significantly ($p<0.05$) lower values of all particle size parameters compared to HM, as shown in the Table 3. The volume mean diameter of MFG in DM is 3.20 µm while HM has volume mean diameter of 4.56 µm. Moreover, the same lactation period in both types of milk led to a greater ($p>0.05$) synthesis of SG globules, which are most accounted and subsequently followed by MGs and LGs. The AA of HM was significantly higher 42.95 % compared to DM 35.83 % after the 6th month's lactation. In our study, DM showed an AA lower than DM found in previous research by Bučević Popović et al. (2014). Compared to the results of the present study, higher AA in DM compared to HM could be observed (Beghelli et al., 2016). When HM is unavailable, DM may be one of the finest alternatives, not only for its nutritional qualities but also for its antioxidant capabilities, which may help prevent oxidative stress-mediated illness in disease in early human life (Beghelli et al., 2016).

Table 3. Particle size distribution (PSD) of milk fat globules (MFG) and antioxidant activity (AA) in dokey milk (DM) and human milk (HM) in the 6th month of lactation

Parameters	DM	HM
d0.1 µm	0.31[a]	0.86[b]
d0.5 µm	1.31[a]	3.36[b]
d0.9 µm	5.48[a]	8.80[b]
D [4,3]µm	3.20[a]	4.56[b]
SG%	69.46[a]	54.13[b]
MG%	22.72[a]	29.77[b]
LG%	7.83[a]	16.10[b]
AA%	35.83[a]	42.95[b]

Data are expressed as mean ± SEM. Values with different superscripts (a,b) are significantly different ($p<0.05$).

The relationship between different groups of the FA and the MFG size, subdivided into SG, MG, LG, and dsr in both types of milk, are presented in Table 4. The Pearson correlation analysis was performed to find out how MFG in both types of milk are related to their FA content. Only the SG group positively correlated with the MUFA and PUFA n-6, but the relationship coefficient varied. The dimensional parameters as MG, LG, and dsr are negatively correlated to the percentages of the MUFA, PUFA n-3, and PUFA n-6 FA and positively related to SFA and n-6/n-3 ratio. The SFA concentrations were positively correlated and higher in larger MFG in an earlier study that looked at the composition of small fatty acids and MFG in HM (Agrov et al., 2008). In line with results of some previous studies (Agrov et al., 2008; Faustini et al., 2014; Mesilati-Stah et al., 2011), many correlations between fat globules dimensional parameters and FA percentages could be due to a different composition along the range of PSD. It is considered that the morphometric characteristics of MG may be related to the FA composition of the fat which means that a higher number of small MFG implies a greater abundance in these of MUFA and PUFA acids (Agrov et al., 2008; Faustini et al., 2014; Mesilati-Stah et al. 2011).

The concentration of minerals in DM and HM is presented in Table 5. In both types of milk, Ca and P were the most abundant micronutrients, followed by Zn, Fe and Cu. The obtained results of the DM are lower than the literature data (Fantuz et al., 2016). According to previous studies, in HM 10 to 16 % of Ca, and 2 % of P were reported to be associated with the fat fraction (Fox et al., 2015). The Ca content in milk is correlated to MFG size and smaller MFG are characteriyed than larger Ca content (Manoni et al., 2021). Also, the major minerals found in the milk lipid fraction lead to an increase in their bioavailability and bioaccessibility (Baldi et al., 2008). The studies by Li et al. (2018) and Malacarne et al. (2019) showed the most abundant element is Ca followed by P, K, Na, Mg, Zn, Fe, and Cu in DM, which is similar to the distribution of elements in HM (Ballard et al. 2013). Ca is the most abundant mineral in DM and HM and half of the phosphorus present is associated with enhanced calcium absorption from the organism (EFSA, 2013).

Conclusion

The increasing interest in the use of donkey milk as a natural product proved to be a good opportunity for its research as a substitute for human milk. The obtained results show similarity in nutrition quality, particle size distribution of fat globules and AA between DM and HM obtained after six months of lactation. DM had a high level of PUFA, especially n-3 FA, a favorable ratio of n-6/n-3 fatty acids, low AI and TI index values compared to HM. Since DM is considered a good substitute for HM, these data support the growing interest in further research of DM as an alternative in infant nutrition, which has adequate levels of FA, bioavailable minerals and

Table 4. Relationship coefficients between dimensional parameters PSD of MFG and percentage of FA in DM and HM

Variable	SG	MG	LG	dsr
SFA	-0.997	0.996	0.998	0.983
C4:0	0.787**	-0.865*	-0.782**	-0.811**
C6:0	0.849*	-0.908*	-0.849*	-0.901*
C8:0	0.997+	-0.965+	-0.996+	-0.971+
C10:0	0.995+	-0.961+	-0.994+	-0.964+
C11:0	-1.000+	0.969+	1.000+	0.979+
C12:0	-0.995+	0.968+	0.997+	0.985+
C14:0	-0.736**	0.679	0.741**	0.731**
C16:0	0.592	-0.561	-0.584	-0.510
C18:0	0.280	-0.366	-0.275	-0.312
C20:0	0.698	0.763	-0.798	-0.676
C22:0	0.680	-0.568	-0.675	-0.410
MUFA	0.995	-0.959	-0.969	-0.978
C10:1	0.664	-0.510	-0.672	-0.599
C12:1	0.664	-0.510	0.672	-0.599
C14:1	0.990+	-0.977+	0.990+	0.981+
C16:1	0.664	-0.510	-0.672	-0.599
C17:1	0.994+	0.975+	-0.992+	-0.959+
C18:1	0.996+	-0.973+	-0.997+	-0.984+
C20:1	0.991+	-0.984+	0.991+	-0.992+
C22:1 n-9	0.958+	-0.918**	-0.959+	0.937+
C24:1 n-9	0.991+	0.924+	-0.991**	-0.992+
PUFA	0.998	-0.963	-0.998	0.976
PUFA n-3	0.996	-0.953	-0.978	-0.996
C18:3 n-3	0.991+	-0.984+	-0.991+	-0.992+
C18:4 n-3	-0.990+	-0.984+	-0.990+	-0.976+
C20:3 n-3	0.998+	-0.984+	-0.991+	-0.992+
C20:4 n-3	0.965+	-0.901*	-0.970+	-0.959+
C20:5 n-3	-0.990+	-0.995*	-0.991+	-0.992+
C22:5 n-3	0.965+	-0.901*	-0.968+	0.937+
C22:6 n-3	0.990+	-0.984+	-0.995+	0.995+
PUFA n-6	0.998	-0.964	-0.999	-0.979
C18:2 n-6	0.965+	-0.901*	-0.968+	-0.937+
C18:3 n-6	0.999+	-0.969+	-0.999+	-0.979+
C20:2 n-6	0.991+	-0.984+	-0.991+	-0.992+
C20:4 n-6	0.179	-0.118	-0.174	0.978
n6/n3 ratio	-0.998	0.972	0.998	0.978

+Correlation is statistically significant at p<0.01 level,
**Correlation is statistically significant at p<0.05 level,*
***Correlation is statistically significant at p<0.10 level*

Table 5. The concentration of minerals in donkey (DM) and human milk (HM)

Mineral content (mg/L)	DM	HM
Ca	250.80±0.05[a]	140.80±0.10[b]
P	120.20±0.10[a]	75.30±0.20[b]
Cu	0.32±0.05[b]	0.22±0.03[a]
Zn	1.90±0.02[a]	1.65±0.05[a]
Fe	0.98±0.12[a]	0.80±0.10[a]

Data are expressed as mean ± SEM. Mean values of the observed parameters are written as the result of three measurements (n=3). Values with different superscripts (a,b) are significantly different (p<0.05).

antioxidants to meet the needs of infants. The information presented in this study may be useful for further research on DM as a substitute for breast milk after 6 months of age.

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Usporedba nutritivne kvalitete i veličine čestica mliječne masti mlijeka magarice i humanog mlijeka nakon šest mjeseci laktacije

Sažetak

Mlijeko magarice je poznato po svojim bioaktivnim i funkcionalnim svojstvima zbog čega se smatra visokovrijednom namirnicom u ljudskoj prehrani. Stoga je glavni cilj ovog istraživanja bio ispitati sličnosti između mlijeka magarice i humanog mlijeka nakon šest mjeseci laktacije s obzirom na sastav masti, raspodjelu veličine čestica mliječne masti, antioksidativno djelovanje i udjel mineralnih tvari. Ovi parametri povezani su s nutritivnim svojstvima mlijeka i važni su za mliječnu industriju kao i za ljudsko zdravlje. Dobiveni rezultati pokazali su da su u obje vrste mlijeka dominantne masne kiseline oleinska, palmitinska i linoleinska, a zatim laurinska, kaprinska i alfa-linolenska kiselina. Mlijeko magarice imalo je vrlo poželjan sustav masnih kiselina zbog visokog sadržaja alfa-linolenske kiseline i posebno niskog omjera omega-6/omega-3. Nakon analize raspodjele masnih kuglica, utvrđeno je da su masne kuglice manje od 2 μm najzastupljenije u humanom i mlijeku magarice. Antioksidacijska aktivnost humanog mlijeka (42,95 %) bila je značajno viša na u usporedbi s mlijekom magarice (35,83 %). Ca je bio najzastupljenija mineralna tvar mineral u obje vrste mlijeka, a slijede ga P, Zn, Fe i Cu. Isticanje sličnosti između mlijeka magarice i humanog mlijeka potiče upotrebu mlijeka magarice kao potencijalne zamjene za humano mlijeko u budućnosti.

Ključne riječi: mlijeko magarice; humano mlijeko; nutritivna kvaliteta; raspodjela veličine čestica mliječne masti

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