Biotechnology in Animal Husbandry 40 (2), 155-168, 2024
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.05'636.18
https://doi.org/10.2298/BAH2402155Z

NUTRITIONAL QUALITY OF DONKEY MILK DURING THE LACTATION

Milica Živkov Baloš, Nenad Popov, Suzana Vidaković Knežević, Sara Savić, Vladimir Gajdov, Sandra Jakšić, Dragana Ljubojević Pelić

Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Republic of Serbia
Corresponding author: Milica Živkov Baloš, milica@niv.ns.ac.rs
Original scientific paper

Abstract: The Domestic Balkan and Banat donkey (*Equus asinus asinus*) are native donkey breeds primarily raised in the Special Nature Reserve 'Zasavica', Serbia. The study's objective was to analyze the composition of donkey's milk during the lactation period (12th to 30th week of lactation), regardless of the donkey's breed. The investigated donkey milk composition is characterized by a low content of dry matter, protein and fat and a high amount of lactose, compared to the milk of other dairy animals. The study revealed that content of dry matter ranged from 7.20 to 9.52%, fat ranged from 0.10 to 1.00%, protein ranged from 1.17 to 2.07%, lactose ranged from 4.52 to 6.71%, ash ranged from 0.28 to 0.50% and pH ranged from 6.82 to 7.46. Dry matter, fat, and ash content progressively lowered during lactation, with some oscillations. Milk protein and lactose content were not affected during the lactation stage. The pH value increased progressively during lactation with slight oscillations during the 18th and 21st week of lactation. Statistically significant differences between lactation weeks were established only for lactose content and pH values.

Key words: donkey milk, milk composition, lactation

Introduction

Donkey milk is considered to have the potential for use as a dietetic food and substitute for human milk. Sensory properties, a high amount of lactose and a low amount of fat and proteins make donkey milk closely similar to mare and human milk (Garhwal et al., 2023; Živkov Baloš et al., 2023; Albertos et al., 2022; Prasad, 2020; Marchis et al., 2018; El-Hatmi et al., 2015). Compared to ruminant milk, donkey milk has a lower content of dry matter, ash, fats and proteins,

especially caseins, and a significantly higher content of lactose, and whey proteins (Martini et al., 2018, 2021; Salari et al., 2019). On the other hand, compared to human milk, donkey milk has a lower content of dry matter and fat, a similar content of lactose, and proteins and a higher content of ash (Martini et al., 2021; Swar, 2011). The data from the literature suggested significant differences in the chemical composition of donkey milk concerning the contents of individual components.

The composition of milk can be attributed to the breed or type, diet, age, lactation number, season of the year, farming system, climate and health status of the animal (Bhardwaj et al., 2020; Nayak et al., 2017; Kučević et al., 2016). The donkey breed and seasonal variations are factors that have the greatest influence on milk composition (Kaskous and Pfaffl, 2022; Bhardwaj et al., 2020). In addition, diet is a very important factor that can affect the content of individual components in milk (Miraglia et al., 2020; Burden and Bell, 2019; Raspa et al., 2019; Lazarević et al., 2017). The donkeys are considered a seasonal polyestrous species, therefore, when adequate planning in the breeding seasons milk could be available throughout the year (Giosuè et al., 2008). The lactation period of donkeys ranges from 180 to 350 days (Giosuè et al., 2008; Guo et al., 2007; Salimei et al., 2004). Lactation peak usually occurs between 4 and 5 weeks from parturition (Centoducati et al., 2012).

The contents of individual components of donkey milk have been determined worldwide, and data from the literature suggested certain differences between donkey breeds. These include Halari donkey from India (Garhwal et al., 2023), Cyprus (Aspri et al., 2019) and Arcadian from Greece and Cyprus (Massouras et al., 2020), Amiata from Italy (Sarti et al., 2019; Salari et al., 2019; Ragona et al., 2016), Jiangye and other Chinese breeds (Li et al., 2018; Guo et al., 2007), Ragusana from Italy (Malacarne et al., 2019; Giosuè et al., 2008; Salimei et al., 2004), Martina-Franca from Italy (Salimei et al., 2004), Zamorano-Leonese from Spain (Albetros et al., 2022), Tunisian breeds (Aroua et al., 2018; El-Hatmi et al., 2015), Domestic Balkan from Serbia (Lazarević et al., 2017) and Litoral Dinaric from Croatia (Ivanković et al., 2009).

A significant number of donkeys worldwide are not systematized but are classified as domestic donkeys. The situation is similar in Serbia, where the domestic Balkan donkey breed is officially recognized. However, genetic research indicates that this breed has different genetic profiles in Serbia. Thus, besides the domestic Balkan donkey, it is believed that there is another breed in Serbia, namely the Banat donkey (Urošević, 2022). In the area of the "Zasavica" special nature reserve, both genotypes are breeding, and from this reserve, the idea and project of separating and forming the Banat donkey breed originated (Mandić et al., 2022). Donkeys have been used as working animals in Serbia for centuries, primarily in agriculture and transport. There is not enough data on the composition of the milk

of domestic breeds, and current Serbian regulations on milk quality (Rule Book, 2017) do not cover donkey milk.

This research focused on the composition and nutritional analyses of Balkan domestic and Banat donkeys, during the middle stage of lactation to obtain data important for donkey milk standardization.

Material and Methods

Milk samples

Milk samples (18 composite samples) were collected from healthy Balkan and Banat jennies from The Special Nature Reserve "Zasavica", Serbia. The donkeys were reared outdoors in extensive livestock farming. The animals grazed on natural pastures and drank water from natural springs. After parturition, and during the first two months, all the milk was left for the foal. Starting from 45-60 days after parturition, the jennies move from pasture to pen, together, that is, there are no individual meals. Their diet includes alfalfa hay and corn. Alfalfa hay is the basic coarse feed, in addition, they feed on raw or spent meadow grass or alfalfa. They get corn in the morning and the afternoon. Raw milk samples were obtained by hand milking of 15 jennies, aged 3 to 15 years. Samples were taken every 3 weeks, from May to October 2023 (12th to 30th week of lactation). The foal stayed with mothers and they were separated only a few hours before milking. The composite milk samples (3 composite samples), of an average volume of 100 ml were collected in glass bottles. The samples were transported under refrigerated conditions (4º C), on the same day to the laboratory of Scientific Veterinary Institute „Novi Sad“, Novi Sad.

Physicochemical analysis

Nutritional analysis of milk was performed in terms of dry matter, fat, total proteins, ash, and lactose content. Dry matter was determined by the oven method (ISO 6731) at 102° C, and ash content by the muffle furnace method at 550^0 C (AOAC 930.30). Fat content was determined by the Gerber method (ISO 11870, 2009). Proteins were determined by the Dumas method (ISO 14891), and total acidity (pH) was measured by a pH meter. Lactose was determined by an HPLC (Jakšić et al., 2022).

Statistical analysis

All measurements were made in triplicate, and the results were expressed as mean value ± standard deviation. To analyze variations of the results, ANOVA was used: Single factor including F-test. The analysis was performed using the software package Microsoft Office Excel 2007. Differences between the means with probability $p<0.05$ were accepted as statistically significant.

Results and Discussion

The composition of Balkan and Banat donkey milk samples are summarized in Table 1. The gross composition is characterized by a low content of dry matter (DM), protein and fat and a high amount of lactose compared to the milk of other dairy animals. The obtained data were compared with the results reported by other authors from our and other countries. Our study revealed that in all examined donkey milk samples, the percentage of dry matter ranged from 7.20 to 9.52% (mean value 8.49%). These results are consistent with other literature data, according to which DM content is in the range from 7.50 to 10.40% (Salimei et al., 2004; Guo et al., 2007; Ivanković et al., 2009; El-Hatmi et al., 2015; Ragona et al., 2016; Lazarević et al., 2017; Li et al., 2018; Aroua et al., 2018; Aspri et al., 2019; Sarti et al., 2019; Salari et al., 2019; Malacarne et al., 2019; Massouras et al., 2020; Albetros et al., 2022).

Table 1. Descriptive statistics of physicochemical properties of Balkan and Banat donkey milk

Composition	Range	Mean values	SD	RSD (%)
DM (%)	7.20 – 9.52	8.49	0.54	14.56
Fat (%)	0.10 – 1.00	0.41	0.28	67.99
NFDM (%)	6.20 – 8.70	8.08	0.63	7.85
Protein (%)	1.17 – 2.07	1.69	0.25	14.56
Lactose (%)	4.52 – 6.71	5.38	0.66	12.36
Ash (%)	0.28 – 0.50	0.37	0.06	16.32
pH	6.82 – 7.46	7.19	0.17	2.43

SD – standard deviation; RSD – relative standard deviation; DM – dry matter; NFDM – non-fat dry matter

Fat content (mean value 0.41%) of all analyzed donkey milk samples ranged from 0.10 to 1.00% and was and consistent with other literature data according to which fat content is in the range from 0.16 to 1.30% (Salimei et al., 2004; Guo et al., 2007; Giosuè et al., 2008; Ivanković et al., 2009; El-Hatmi et al., 2015; Ragona et al., 2016; Lazarević et al., 2017; Li et al., 2018; Aroua et al., 2018; Aspri et al., 2019; Sarti et al., 2019; Salari et al., 2019; Malacarne et al., 2019; Massouras et al., 2020; Albetros et al., 2022). Milk fat in cow milk is considered a risk factor for human health because of its high content of saturated fatty acids. Donkey milk is characterized by low fat content and low energy value, as well as the content of fatty acids that are desired in a balanced human diet (Živkov Baloš et al., 2023). The content of fat in donkey milk is influenced by breed, nutrition, milking technique, milking intervals, lactation stage and number of lactations (Guo et al., 2007).

The range of protein content (mean value 1.69%) in the investigated donkey milk samples were between 1.17 and 2.07%, similar to the results of other authors (from 1.22 to 2.14%) (Salimei et al., 2004; Guo et al., 2007; Giosuè et al., 2008; Ivanković et al., 2009; El-Hatmi et al., 2015; Ragona et al., 2016; Lazarević et al., 2017; Li et al., 2018; Aroua et al., 2018; Aspri et al., 2019; Sarti et al., 2019; Salari et al., 2019; Malacarne et al., 2019; Massouras et al., 2020; Albetros et al., 2022). Casein fraction in the total protein content of donkey milk is considerably lower as compared to bovine milk, which contributes to the low allergenicity of donkey's milk (Kaskous and Pfaffl, 2022; Bhardwaj et al., 2020; Martini et al., 2021). The content of whey proteins, lactose and casein in donkey milk is similar to that in human milk, though significantly different from cow, goat and camel milk. The casein/whey protein ratio is higher in donkey milk than in human milk. On the other hand, casein/whey protein ratio in ruminant milk is four times higher than that of donkey milk, and seven times higher as compared with human milk (Vincenzetti et al., 2017; Derdak et al., 2020). Gubić et al. (2016) reported that Balkan donkey milk represents a source of antibacterial proteins such as lysozyme and highly digestible proteins such as whey protein, α-lactalbumin and lactoferrin.

According to research data from Serbia and other countries, the lactose content in donkey milk ranges from 5.90 to 7.23±0.243% (Salimei et al., 2004; Guo et al., 2007; Giosuè et al., 2008; Ivanković et al., 2009; Lazarević et al., 2017; Ragona et al., 2016; Aroua et al., 2018; Sarti et al., 2019; Salari et al., 2019; Malacarne et al., 2019; Massouras et al., 2020; Albetros et al., 2022). Our study revealed that in all examined donkey milk samples, the percentage of lactose ranged from 4.52 to 6.71% (mean value 5.38%). The lactose concentration in donkey milk is similar to that in human milk. However, compared to bovine milk, donkey milk contains significantly more lactose (Martini et al., 2018; Ragona et al., 2016). Lactose stimulates intestinal absorption of calcium and phosphorus contributing to the homeostasis of these elements, which is especially important for bone mineralization and prevention of osteoporosis (Martini et al., 2021; Vincenzetti et al., 2017; Nayak et al., 2017; Massouras et al., 2017). Lactose is a nutritional factor and a potential probiotic because it stimulates the development of intestinal enteroflora (Yvon et al., 2016).

The mean ash content in investigated donkey milk samples was 0.38%, with a range of 0.28 to 0.50% (Table 1), similar to the results of other authors (from 0.32 to 0.51%) (Salimei et al., 2004; Guo et al., 2007; El-Hatmi et al., 2015; Ragona et al., 2016; Lazarević et al., 2017; Li et al., 2018; Aroua et al., 2018; Sarti et al., 2019; Salari et al., 2019; Malacarne et al., 2019; Massouras et al., 2020; Albetros et al., 2022). Cow's milk has two times higher ash content than donkey's milk. The lower mineral concentration in donkey milk has a favorable effect on the function of the kidneys since they are not burdened by minerals (Martini et al., 2018). Calcium, phosphorus, sodium, and magnesium contents are higher compared to human milk, but lower than those in cow, buffalo, goat and sheep

milk (Bhardwaj et al., 2020). Calcium, manganese, iron, selenium and zinc contents are similar in horse and donkey milk, whereas the concentrations of sodium, potassium and copper are significantly different (Bilandžić et al., 2014). Compared to bovine milk, calcium/phosphorus ratio in donkey milk is more suitable for human nutrition (Malacarne et al., 2019; Li et al., 2018; Massouras et al., 2017). The most prevalent microelement in donkey milk is zinc, with concentrations above the levels detected in human milk. Donkey milk contains twofold amount of copper as compared with human and cow milk. The content of selenium in donkey milk is ten times higher than that in cow and sheep milk, and seven times higher than in human milk (Albertos et al., 2022).

Average pH value of milk samples was 7.19 and ranged from 6.82 to 7.46, similar to the results of other authors (7.01- 7.35) (Guo et al., 2007; El-Hatmi et al., 2015; Aroua et al., 2018; Aspri et al., 2019; Salari et al., 2019; Malacarne et al., 2019; Albetros et al., 2022). Aroua et al. (2018) reported that donkey milk has an almost neutral pH (7.09), whereas the pH values of cow and goat milk are 6.65 and 6.50, respectively. The almost neutral pH of donkey milk is attributed to the lower casein and phosphate levels (Salimei et al., 2004).

Table 2. Physicochemical properties of Balkan and Banat donkey milk during the 12th to 30th week of lactation (mean values ± standard deviation)

Week of lactation	DM (%)	Fat (%)	NFDM (%)	Protein (%)	Lactose (%)	Ash (%)	pH
12	9.03±0.70	0.70±0.28	8.33±0.42	1.56±0.11	5.20±0.49	0.39±0.04	7.12±0.17
15	8.55±0.01	0.65±0.00	7.90±0.01	1.80±0.04	5.28±0.30	0.39±0.01	7.15±0.08
18	8.18±0.91	0.50±0.44	7.68±1.31	1.62±0.23	4.60±1.52	0.43±0.07	6.94±0.11
21	8.63±0.32	0.37±0.21	8.26±0.49	2.01±0.07	5.49±0.80	0.37±0.05	7.24±0.03
24	8.70±0.08	0.17±0.06	8.53±0.14	1.51±0.37	6.26±0.40	0.34±0.07	7.24±0.03
27	8.06±0.46	0.23±0.06	7.83±0.41	1.63±0.06	4.69±0.14	0.30±0.03	7.43±0.03

DM – dry matter; NFDM – non-fat dry matter

Physicochemical properties of Balkan and Banat donkey milk during the 12th to 30th week of lactation are displayed in Table 2. and Figure 1. An analysis of variance indicates that the values between the groups, defined based on the lactation period of the donkeys, did not show statistically significant differences for the examined parameters: protein, dry matter, non-fat dry matter and ash, since obtained values for the F-test were lower than the F-critical ones. Statistically significant differences in the measured lactose and pH values were noted between the formed groups. The F-test values were significantly higher than the F-critical ones. This is in correlation with the results in Table 2., where the measured values

of lactose and pH values at different weeks of lactation have a slightly wider range of measured values.

Dry matter, and consequently and non-fat dry matter, showed a progressive lowering, with some oscillations, from the 12. week of lactation (9.03%) to the 27. week of lactation (8.06%) (Table 2). Ivanković et al. (2009) reported that the lactation stage of Littoral-Dinaric donkeys significantly influences DM content in the milk. The highest dry matter concentrations were established at the early lactation stage. A significant positive correlation was observed between the milk yield, dry matter and fat contents. Malacarne et al. (2019) reported a progressive decrease in DM content throughout the lactation period, with minor oscillations from the early to the late lactation period. However, Martini et al. (2014) concluded that DM in the milk of Amiata donkeys did not change significantly during lactation.

A progressive lowering trend was found for fat (from 0.70 to 0.23%) (Table 2., Figure 1.). The fat content varies throughout the donkey's lactation period, whereby it shows a decreasing tendency, especially after the 150th lactation day (Massouras et al., 2017; Martemucci and D'Alessandro, 2012). However, Lazarević et al. (2017) state that fat content in Balcan donkey milk did not vary significantly within the period of lactation and was slightly higher in the early lactation. Salimei et al. (2004), Guo et al. (2007) and Aspri et al. (2019) reported a similar trend in fat content decrease in donkeys' milk after 100 days of lactation.

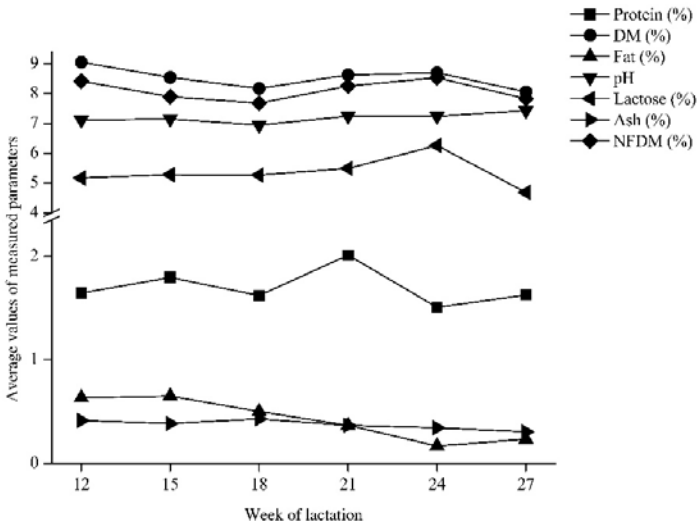

Figure 1. Lactation stage effect on physicochemical properties of Balkan and Banat donkey milk

The protein content did not vary significantly during the middle and late lactation (Table 2., Figure 1.). The research of Martini et al. (2014) conducted on Amiata donkeys revealed a progressive decrease in milk protein content during first 6 months of lactation, and consequent stabilization of the concentration at 1.50%. Similar results are reported by Lazaravić et al. (2017). They state that the highest concentrations of protein were determined during the early lactation period, and gradually decreased from the beginning to the end of the lactation. Gubić et al. (2016) in their study analyzed proteins of Domestic Balkan donkey milk during the lactation period (from the 45th to the 280th day). They reported that protein reached the highest value of 1.92% on the 60th day of the lactation stage. The protein concentration decreased until the end of the lactation period and reached 1.40%. Barbosa Dos Santos et al. (2023) concluded that donkey age and lactation stage affect milk composition. Milk total solids and protein fraction content decrease with the lactation stage. Aspri et al. (2019) reported a decreasing trend in the protein level of donkey milk during lactation. They concluded that this phenomenon may occur due to the different expression of genes responsible for milk protein synthesis during the lactation period.

The lactose content was influenced by the stage of lactation (Table 2., Figure 1.) and was uniform in the 12th and 15th weeks, but there was a decrease in the 18th week. After that, in the 21st and 24th weeks of lactation, it increased slightly, but in the 27th week, the content significantly decreased. Martini et al. (2014) reported that lactose levels in the milk of Amiata donkeys decreased during the first 60 days of lactation and then remained constant until the end of lactation. Malacarne et al. (2019) in their research conducted on the Ragusano donkey breed established a progressive decrease of lactose content in donkey milk throughout the lactation period.

The ash content showed a progressive lowering (from 0.39 to 0.30%) (Table 2., Figure 1.). Malacarne et al. (2019), concluded that ash content in the milk of Ragusano donkeys (Italy) decreases during lactation. These results are comparable with the results of Albertos et al. (2022) reported for Zamorano-Leonese donkeys (Spain). Martini et al. (2014) reported a decrease in ash content in the milk of Amiata donkeys during the lactation period. Salimei et al. (2004) based on the results of their study about milk quality from Martina Franca and Ragusana donkey breed concluded that ash content was constant throughout the experimental period (28 - 150 days after parturition). According to the results of Lazaravić et al. (2017), statistically higher ash content was observed in the middle period of lactation, compared to other stages of lactation.

A progressive increasing trend was found for pH value (from 7.12 to 7.43) (Table 2., Figure 1.). Salari et al. (2019) in their study about milk from the Amiata donkey breed, noticed that the pH increased in the late lactation stage. They stated that an increase of pH value at later lactation stages is associated with a decrease in casein level, and an increase of urea content in the milk (Salari et al., 2019).

However, Aspri et al. (2019) stated that the pH value of donkey milk does not significantly change during the lactation period.

Conclusions

Banat and Balkan donkey milk did not show a large variability of physicochemical properties. The homogeneity of the milk produced during lactation is important when donkey milk is intended for sensitive categories of consumers. Donkey milk is not standardized, so it is necessary to develop regulations that would regulate its quality. Further research is needed on donkey's nutrition and milking. In addition, thanks to favorable climatic conditions and soil characteristics, Serbia has great potential for developing extensive livestock production, including donkey farming. The breeding of donkeys and the production of milk, due to the modest needs of donkeys in nutrition and care, can be additional and profitable activities for farmers.

Nutritivna vrednost mleka magaraca tokom laktacije

Milica Živkov Baloš, Nenad Popov, Suzana Vidaković Knežević, Sara Savić, Vladimir Gajdov, Sandra Jakšić, Dragana Ljubojević Pelić

Rezime

Domaći balkanski i banatski magarac (*Equus asinus asinus*) su autohtone rase magaraca, koje se prvenstveno gaje u Specijalnom rezervatu prirode „Zasavica“ u Srbiji. Cilj studije je bio da se analizira sastav mleka magarica obe rase, u periodu laktacije (od 12. do 30. nedelje laktacije). Sastav ispitivanog magarećeg mleka karakteriše nizak sadržaj suve materije, proteina i masti i visok sadržaj laktoze, u poređenju sa mlekom ostalih životinja namenjenih za proizvodnju mleka. Ispitivanjem je utvrđeno da je sadržaj suve materije varirao od 7,20 do 9,52%, masti od 0,10 do 1,00%, proteina od 1,17 do 2,07%, laktoze od 4,52 do 6,71%, pepela od 0,28 do 0,50% i pH vrednosti od 6,82 do 7,46. Sadržaj suve materije, masti i pepela se progresivno smanjivao tokom laktacije, uz izvesne oscilacije. Sadržaj proteina i laktoze nije bio pod uticajem faze laktacije. pH vrednost se progresivno povećavala tokom laktacije sa blagim oscilacijama tokom 18. i 21. nedelje laktacije. Statistički značajne razlike posmatrano po nedeljama laktacije, utvrđene su samo za sadržaj laktoze i pH vrednosti.

Ključne reči: magareće mleko, sastav mleka, laktacija

Acknowledgment
This research was funded by the Science Fund of the Republic of Serbia, #Grant No 3535, Project Health Protection and Biodiversity Conservation of domestic donkeys in the Special Nature Reserve "Zasavica"- PROTECTDonkey

Conflict of interest
The authors declare that they have no conflict of interest.

References

Albertos I., Lòpez M., Jiménez J-M., Cao M.J., Corell A., Castro-Alija M.J. 2022. Characterisation of Zamorano-Leonese Donkey Milk as an Alternative Sustainably Produced Protein Food. *Frontiers in Nutrition*, 9, 872409. doi: 10.3389/fnut.2022.872409.

AOAC Official Method 930.30. 2012. Ash of Milk Powder, Official Method of Analysis of AOAC International, 19th Edition, Editor dr. George W. Latimer, Jr.

Aroua M., Jemmali B., Ben Said S., Touati I., Mahouachi M. 2018. Milk composition comparison between donkey, goat and cow breeds. *Journal of New Sciences, Sustainable Livestock Management*, 9(5), 202-206.

Aspri M., Souroullas K., Ioannou C., Papademas P. 2019. Physico-chemical Composition and Antimicrobial Protein Content of Early Lactation Donkey Milk. *International Journal of Food Studies*, 8, 68-75. 10.7455/ijfs/8.1.2019.a6

Barbosa dos Santos I.C., Rangel A.H.N., Ribeiro C.V.M., de Araujo Oliveira C.A. 2023. Donkey Milk composition is altered by lactation stage and jennies age. *Journal of Food Composition Analysis*, 115:104971. doi: 10.1016/j.jfca.2022.104971

Bhardwaj A., Pal Y., Lengha R.A., Sharma P., Nayan V., Kumar S., Tripathi H., Tripathi B.N. 2020. Donkey milk composition and its therapeutic applications. *Indian Journal of Animal Sciences*, 90(6), 837-841.

Bilandžić N., Sedak M., Đokić M., Solomun Kolanović B., Varenina I., Božić Đ., Končurat A. 2014. Differences of the essential mineral element levels in the milk of Croatian Coldblood horse and Littoral-Dinaric donkey. *Mljekarstvo*, 64(1), 12-18.

Burden F.A., Bell N. 2019. Donkey nutrition and malnutrition. *Veterinary Clinics of North America: Equine Practica*, 35, 469-479.

Centoducati P., Maggiolino A., De Palo P., Tateo A. 2012. Application of Wood's model to lactation curve of Italian Heavy horse mares. *Journal of Dairy Science*, 95, 5770-5775. https://doi.org/10.3168/jds.2012-5513

Derdak R., Pop O.L., Sakoui S., Muresan C., Vodnar D.C., Addoum B., Suharoschi R., Vulturar R., Soukri A., El Khalfi B. 2020. Donkey Milk Bioactive Proteins and Peptides, Health and Food Applications – A review. Preprints

(www.preprints.org), not per-reviewed, posted 7 july 2020. doi:10.20944/preprints202007.0119.v1

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*, 65(3), 159-167.

Garhwal R., Sangwan K., Mehra R., Bhardwaj A., Pal Y., Nayan V., Legha R.A., Tiwari M., Chauhan M.S., Iquebal M.A., Jaiswal S., Kumar H. 2023. Comparative metabolomics analysis of Halari donkey colostrum and mature milk throughout lactation stages using 1H Nuclear Magnetic Resonance. *LWT-Food Science and Technology*, 182, 114805. https://doi.org/10.1016/j.lwt.2023.114805.

Giosuè C., Alabiso M., Russo G., Alicata M.L., Torrisi C. 2008. Jennet milk production during the lactation in a Sicilian farming system. *Animal*, 2, 10, 1491-1495. https://doi.org/10.1017/S1751731108002231

Gubić J., Tasić T., Tomić J., Torbica A., Iličić M., Tasić T., Šarić Lj., Popović S. 2016. Characterization of several milk proteins in domestic Balkan donkey breed during lactation, using lab-on-a-chip capillary electrophoresis. *Chemical Industry and Chemical Engineering*, 22(1), 9-15. https://doi.org/10.2298/CICEQ150105013G

Guo H. Y., Pang K., Zhang X. Y., Zhao L., Chen S. W., Dong M. L., Ren F. Z. 2007. Composition, Physiochemical Properties, Nitrogen Fraction Distribution, and Amino Acid Profile of Donkey Milk. *Journal of Dairy Science*, 90, 1635–1643. doi:10.3168/jds.2006-600

ISO 11870. 2009. Milk and milk products, Determination of fat content.

ISO 21424. 2018. Milk, milk products, infant formula and adult nutritionals - Determination of minerals and trace elements - Inductively coupled plasma mass spectrometry (ICP-MS) method

ISO 6731. 2010. Milk, cream and evaporated milk — Determination of total solids content

ISO 14891. 2002. Milk and milk products - Determination of nitrogen content - Routine method using combustion according to the Dumas principle.

Ivanković A., Ramljak J., Štulina I., Antunac N., Bašić I., Kelava N., Konjačić M. 2009. Characteristics of the lactation, chemical composition milk hygiene quality of the Littoral-Dinaric ass. *Mljekarstvo*, 59 (2), 107-113. Available on: https://hrcak.srce.hr/37624

Jakšić S., Popov N., Mihaljev Ž., Živkov Baloš M. 2022. Determination of lactose in milk and dairy products by HPLC-RID method. *Journal of Agroalimentary Processes and Technologies*, 28 (1), 5-10.

Kaskous S., Pfaffl M.W. 2022. Milk Properties and Morphological Characteristics of the Donkey Mammary Gland for Development of an Adopted Milking Machine – A Review. *Dairy*, 3, 233-247. https://doi.org/10.3390/dairy3020019

Kučević D., Trivunović S., Bogdanović V., Čobanović K., Janković D., Stanojević D. 2016. Composition of raw milk from conventional and organic dairy farming. *Biotechnology in Animal Husbandry*, 32 (2), 133-143. doi: 10.2298/BAH1602133K

Lazarević J., Tasić T., Popović S., Banjac V., Đuragić O., Kokić B., Čabarkapa I. 2017. Changes in milk composition of domestic Balkan donkey's breed during lactation periods. *Acta Periodica Technologica*, 48, 187-195. https://doi.org/10.2298/APT1748187L

Li L., Liu X., Guo H. 2018. The nutritional ingredients and antioxidant activity of donkey milk and donkey milk powder. *Food Science and Biotechnology*, 12, 27(2), 393-400. doi: 10.1007/s10068-017-0264-2.

Mandić R., Urošević M., Drobnjak D. 2022. Specijalni rezervat „Zasavica". In *Prikaz uzgoja i pokazatelja stanja i dobrobiti Banatskog magarca i magaraca koji se uzgajaju u specijalnom rezervatu prirode „Zasavica"*, Ed. Sava Lazić, Naučni institut za veterinarstvo „Novi Sad", 5-23.

Malacarne M., Criscione A., Franceshci P., Bordonaro S., Formaggioni P., Marletta D., Summer A. 2019. New Insights into Chemical and Mineral Composition of Donkey Milk throughout Nine Months of Lactation. *Animals*, 9, 1161. https://doi.org/10.3390/ani9121161

Martini M., Altomonte I., Licitra R., Salari F. 2018. Nutritional and nutraceutical quality of donkey milk. *Journal of Equine Veterinary Science*, 65,33-37. https://doi.org/10.1016/j.jevs.2017.10.020

Martini M., Altomonte I., Salari F., Caroli A.M. 2014. Short communication: Monitoring nutritional quality of Amiata donkey milk: Effects of lactation and productive season. *Journal of Dairy Scie*nce, 97, 6819-6822. https://dx.doi.org/10.3168/jds.2014-8544

Martini M., Altomonte I., Tricò D., Lapenta R., Salari F. 2021. Current Knowledge on Functionality and Potential Therapeutic Uses of Donkey Milk. *Animals*, 11, 1382. https://doi.org/10.3390/ani11051382

Marchis Z., Odagiu A., Coroian A., Oroian I., Mirza M., Burduhos P. 2018. Analysis of Environmental Factors Impact on Donkey's Colostrum Quality. *Sustainability*, 10, 2958. doi:10.3390/su10092958

Martemucci G., D'alessandro A.G. 2012. Fat content, energy value and fatty acid profile of donkey milk during lactation and implications for human nutrition. *Lipids in Health and Disease*, 11, 113. http://www.lipidworld.com/content/11/1/113

Massouras T., Bitsi N., Paramithiotis S., Manolopoulou E., Drosinos E.H., Triantaphyllopoulous K.A. 2020. Microbial profile, antibacterial properties and chemical composition of raw donkey milk. *Animals*, 10, 2001. doi: 10.3390/ani10112001

Massouras T., Triantaphyllopoulous K.A., Theodossiou I. 2017. Chemical composition, protein fraction and fatty acid profile of donkey milk during

lactation. *International Dairy Journal*, 75, 83-90. http://dx.doi.org/10.1016/j.dairyj.2017.06.007

Miraglia N., Salimei E., Fantuz F. 2020. Equine milk production and valorization of marginal areas – A Review. *Animals*, 10, 353. doi:10.3390/ani10020353

Nayak M.C., Ramachandra C.T., Udaykumar N., Sharnagouda H., Nagraj N., Jagjivan R. 2017. Composition, Characteristics, Nutritional value and Health Benefits of Donkey Milk - A Review. *Dairy Science & Technology*, EDP sciences/Springer, hal-01538532.https://hal.archives-ouvertes.fr/hal-01538532

Prasad B. 2020. Nutritional and Health Benefits of Donkey Milk. *Journal of Food Science and Nutrition Therapy*, 6(1):022-025. https://doi.org/10.17352/jfsnt.000022

Ragona G., Corrias F., Benedetti M., Paladini I., Salari F., Altomonte I., Martini M. 2016. Amiata donkey milk chain: animal health evaluation and and milk quality. *Italian Journal of Food Safety*, 5:5951. doi:10.4081/ijfs.2016.5951

Raspa F., Cavallarin L., Mclean A.K., Bergero D., Valle E. 2019. A Review of the Appropriate Nutrition Welfare Criteria of Dairy Donkeys: Nutrition Requirements, Farm Management Requirements and Animal-Based Indicators. *Animals*, 9, 315. doi:10.3390/ani9060315

Rulebook.2017. Rulebook on raw milk quality. *Official Gazette of the RS*, 106/2017.

Salari F., Ciampolini R., Mariti C., Millanta F., Altomonte I., Licitra R., Auzino B., D' Ascenzi C., Bibbiani C., Giuliotti L., Papini R.A., Martini M. 2019. A multi-approach study of the performance of dairy donkey during lactation: preliminary results. *Italian Journal of Animal Science*, 18(1), 1135-1141. doi: 10.1080/1828051X.2019.1623094

Salimei E., Fantuz F., Coppolac R., Chiofalod B., Polidori P., Variscoe G. 2004. Composition and characteristics of ass's milk. *Animal Research*, 53, 1, 67-78. https://doi.org/10.1051/animres:2003049

Sarti L., Martini M., Brajon G., Barni S., Salari F., Altomonte I., Ragona G., Mori F., Muscas G., Belli F., Corrias F., Novembre E. 2019. Donkey's milk in the management of children with cow's milk protein allergy: nutritional and hygienic aspect. *Italian Journal of Pediatrics*, 45:102. https://doi.org/10.1186/s13052-019-0700-4

Swar M.O. 2011. Donkey milk-based formula: A substitute for patients with cows milk protein allergy. *Sudanese Journal of Paediatrics*, 11(2), 21-24.

Urošević M. 2022. Kratka istorija domestikacije magaraca. In *Prikaz uzgoja i pokazatelja stanja i dobrobiti Banatskog magarca i magaraca koji se uzgajaju u specijalnom rezervatu prirode „Zasavica"*, Ed. Sava Lazić, Naučni institut za veterinarstvo „Novi Sad", 24-26.

Vincenzetti S., Pucciarelli S., Polzonetti V., Polidori P. 2017. Role of proteins and some bioactive peptides on the nutritional quality of donkey milk and their impact on human health. *Beverages*, 3,34. doi:10.3390/beverages3030034

Yvon S., Olier M., Leveque M., Jard G., Tormo H., Haimoud-Lekhal D.A., Peter M., Eutaméne H. 2016. Donkey milk consumption exerts anti-inflammatory properties by normalizing antimicrobial peptides levels in Paneth's cells in a model of ileitis in mice. *European Journal of Nutrition*, doi: 10.1007/s00394-016-1304-z.

Živkov Baloš M., Ljubojevic Pelić D., Jakšić S., Lazić S. 2023. Donkey milk: An overview of its chemical composition and main nutritional properties or human health benefit properties – Rewiev Article. *Journal of Equine Veterinary Science*, 121, 104225. https://doi.org/10.1016/j.jevs.2023.104225

Received 6 November 2024; accepted for publication 9 December 2024