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Mini Review

Bioactive components of donkey milk

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Abstract

Recently, donkey milk has received a lot of interest due to its similarity to human milk in terms of protein, lactose and milk fat content. The purpose of this review is to summarize the knowledge of research done on the composition of milk, including its functionality and potential therapeutic use. Donkey milk is suitable for use in children who are allergic to cow's milk. The bioactive components contained in donkey milk have antibacterial, antiviral and antifungal effects, as well as anti-inflammatory and antioxidant properties.

Keywords: Donkey milk, functionality, therapeutic use, bioactive components

Abbreviations: MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; SCC – somatic cell count; TBC – total bacteria count

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Introduction

Since ancient times, donkey milk has been known for its therapeutic properties and has been used to heal wounds and treat various diseases such as bronchitis, asthma, joint pain, gastritis (Martini et al. 2018; Karami and Akbari-adergani 2019).

Today it is marketed as a commercial product and is used in newborns (Salimei 2011; Mansueto et al. 2013), people with allergies to cow's milk proteins and elderly people (Karami and Akbari-Adergani 2019). Donkey milk is becoming increasingly attractive as it possesses antimicrobial, antiviral, anti-inflammatory, anti-proliferative (Martini et al., 2018; Aspri et al. 2017) and antioxidant activity (Vincenzetti et al. 2017).

Donkey milk consumption is widespread in the Mediterranean region, and its production in the European Union (EU) is estimated at approximately 300 tons. In the past 10 years, the rearing of donkeys for milk production has increased, along with the number of published scientific articles on its use. Donkey milk farms in the EU are mainly located in Italy, France, Spain and Belgium (Valle et al. 2018).

The purpose of this review is to summarize the knowledge of research done on the composition of milk, including its functionality and potential therapeutic use.

Dairy. Donkey milk production differs significantly from that of traditional dairy farm animals, both in terms of consumer demand and supply (Salimei 2011).

Average daily milk yield for donkeys of the Jiangyue breed for a 180-day lactation period (four times manual milking) is 1.28 kg with fluctuations during lactation within the range of 1.16-1.54 kg. For the Littoral-Dinaric breed in Croatia, an average milking yield (by hand, once in the morning) of 172 ml was found (Ivanković et al. 2009), which was significantly lower than that of Ragusana breeds 0.77 kg (Chiofalo and Salimei 2001) and Martina Franca 0.61-0.76 kg (Salimei et al. 2005) in Italy. In another study for the Ragusana breed, lower values were found for the average milk yield of 0.55-0.70 kg (Alabiso et al. 2008).

The higher milk yield of the Italian breeds is attributed to the provision of concentrated feed (2.5-3.0 $kg.d^{-1}$) in the animal ration. For the Littoral-Dinaric breed, reliable differences in milk yield were found, influenced by the individual characteristics of the animals, while the composition (dry matter, milk fat, protein and lactose) and the hygienic indicators of the milk (SCC and TBC) were not influenced by this factor (Ivanković et al. 2009).

The method of milking - manual or machine - has no effect on the fat content of donkey milk. Variables associated with milk fat content include: lactation stage, with fat content decreasing during lactation; diurnal rhythms, with peaks occurring at night; and the interval between machine milking as milk fat content increases with longer intervals. (Salimei 2011).

Chemical composition. The data summarized by Salimei and Chiofalo (2006) from the research of 17 authors show that the average content of dry matter, milk fat, protein, lactose and mineral substances in donkey milk is in the range of 8.10-11.07%; 0.28-1.82%; 1.36-1.90%; 5.10-7.20 and 0.32-0.50%.

Data from more recent studies show similar variations in values for the above parameters of donkey milk during lactation (Table 1).

Proteins. The protein content of donkey milk varies considerably during lactation. Donkey milk is characterized by low casein content and high whey protein content - 53.03-57.06% of total protein (Guo et al. 2007; Barłowska et al. 2011). It has a low casein/whey protein ratio, averaging 1.3, with values ranging from 0.66 to 1.33 during lactation (Brumini et al. 2015).

The amount of non-protein nitrogen in donkey milk is close to that of cow and mare, ranging from 14 to 16% of total nitrogen (Salimei 2011). In individual samples, values of 0.18-0.41 g (Guo et al. 2011a) and 0.07-0.26 g of non-protein nitrogen per 100 g of donkey milk were found (Malacarne et al. 2019).

In terms of protein composition, donkey milk is closest to mother's milk (Tesse et al. 2009; Marletta et al. 2016). αs1- and β-casein fractions in donkey milk have been isolated and identified (Vincenzetti et al. 2005a; 2005b). In some studies, αs2-, γ- and κ-casein fractions were not detected, probably due to their very low content in this type of milk (Vincenzetti et al. 2007; Criscione et al. 2009). αs1-casein consists of 202 amino acid residues, and αs2 - of 221. k-casein is characterized by great heterogeneity and is difficult to detect, due to its low content in donkey milk (Marletta et al. 2016).

Table 1. Composition of donkey milk

Indexes	Content	Authors
Dry matter, %	8.45-9.13	Salimei et al. 2004
	8.80-11.70	Guo et al. 2007
	7.92-9.82	Ivanković et al. 2008
	7.60-9.00	Massouras et al. 2017
Milk fat, %	0.10-1.40	Salimei et al. 2004
	0.30-1.80	Guo et al. 2007
	0.12-0.96	Ivanković et al. 2008
	0.70-1.30	Massouras et al. 2017
Protein, %	1.25-2.18	Salimei et al., 2004
	1.50-1.80	Guo et al. 2007
	1.03-2.16	Ivanković et al. 2008
	1.20-1.70	Massouras et al. 2017
Lactose, %	6.03-7.28	Salimei et al. 2004
	5.80-7.40	Guo et al. 2007
	5.61-6.97	Ivanković et al. 2008
	5.80-6.10	Massouras et al. 2017
Ash, %	0.36-0.44	Salimei et al. 2004
	0.30-0.50	Guo et al. 2007
	0.35-0.48	Nartini et al. 2007
	0.25-0.48	Malacarne et al. 2019

The size of casein micelles in donkey milk varies in a wide range 100-1000 nm (Salimei 2011) - an average of 298.5 nm, which shows that they exceed the sizes of micelles in other milks. There is a negative correlation between the size of casein micelles in donkey milk and the stage of lactation, which is not characteristic of cow's milk (Tidona et al. 2010). In individual samples of donkey milk, variation in the content of total protein, casein and whey proteins was found in the range of 1.31-1.83%, 0.42-0.98% and 0.43-0.85%, respectively, and of the size of casein micelles in the range 275.5-318.2 nm (Tidona et al. 2014).

α-Lactalbumin (α-La), β-lactoglobulin (β-Lg) and lysozyme (Lys) are the main whey proteins in donkey milk (D'Alessandro et al. 2011; Polidori and Vincenzetti 2012). The content of α-lactoalbumin in donkey milk marked a significant increase until the 3rd month of lactation to reach a stable value of 1.8-2.1 $mg.mL^{-1}$ in the middle of lactation - day 100-150. The content during lactation can vary from 0.8-1.3 to 2.7 $mg.mL^{-1}$ (Gubić et al. 2014; Brumini et al. 2015).

α -La in donkey milk contains 123 amino acid residues and only one genetic variant with two isoforms (Vincenzetti et al. 2012; Brumini et al. 2015). The α -La of donkey milk is resistant to gastric and duodenal enzymes and 95% of it reaches the intestine almost intact (Tidona et al. 2014).

The average content of β-Lg in donkey milk is in the range of 1.3-5.5 mg.mL^{-1} (Brumini et al. 2015), but there are data that during lactation this component can reach even lower values - 1.39 - 2.63 mg.mL^{-1} (Gubić et al. 2014). β-lactoglobulin consists of two components – β-Lg I (main) consisting of 162 amino acid residues and β-Lg II (secondary) - 163 amino acid residues. β-Lg I accounts for about 80% of the total share of β-Lg and has only two genetic variants (A and B), while β-Lg II (20% of the β-Lg fraction) has five - A, B, C D E (Brumini et al. 2015).

70% of the β-lactoglobulin in donkey milk is absorbed, which is twice as much as in cow's milk. Thus, in gastric bioactivity, a higher percentage of digestible β-lactoglobulin may also increase the production of peptides - mainly from αs1- and β-casein, as well as β-lactoglobulin (Tidona et al., 2010; Gubić et al., 2014).

Turganbaeva and Isakova (2021) found that the amino acid composition of donkey milk changes slightly and remains relatively constant throughout the lactation period. Donkey milk is characterized by a higher content of essential amino acids - 44.3% of the total their amount, which exceeds their content in the cow and mother (Table 2.).

Unlike other types of milk, the tryptophan content in donkey milk is negligible (Li et al., 2018). Leucine (0.30-0.34 g.100g^{-1}) and phenylalanine (0.19-0.25 g.100g^{-1}) have the highest content among the other essential amino acids in donkey milk: tyrosine (0.13 -0.18 g.100g^{-1}), lysine (0.18-0.23 g.100g^{-1}), isoleucine (0.09-0.13 g/100g^{-1}), methionine (0.01-0 .02 g.100g^{-1}), threonine (0.08-0.13 g.100g^{-1}) and valine (0.14-0.17 g.100g^{-1}), the amount of which is close to that of mare and lower than the cow (Nayak al., 2020).

Of the essential amino acids in donkey milk with the highest content, arginine (0.39-0.42 g.100g^{-1}) and glutamic acid (0.35-0.41 g.100g^{-1}) are distinguished, followed by proline (0.27-0.32 g.100g^{-1}), histidine (0.19-0.24 g.100g^{-1}), serine (0.19-0.22 g.100g^{-1}), alanine (0.16-0.21 g.100g^{-1}), glycine (0.16-0.20 g.100g^{-1}), aspartic acid (0.11-0.15 g.100g^{-1}), cystine (0.02-0.06 g.100g^{-1}) and tryptophan (0.01-0.07 g.100g^{-1}) (Nayak al. 2020).

Table 2. Amino acid composition of donkey and breast milk

Amino acids	**Donkey milk** (Nayak et al. 2020), g.100g^{-1}	**Breast milk** (Feng et al. 2016), mg.dL^{-1}
Essential amino acids		
Histidine	0.23	26
Isoleucine	0.11	59
Lysine	0.21	72
Methionine	0.10	17
Phenylalanine	0.22	41
Threonine	0.11	49
Triptophan	0.04	21
Tyrosine	0.15	48
Valine	0.16	60
Leucine	0.32	107
Non essential amino acids		
Alanine	0.17	40
Aspartic acid	0.13	90
Proline	0.29	95
Arginine	0.40	42
Glutamic acid	0.38	187
Serine	0.20	50
Glycine	0.18	25
Cystine	0.04	23

Donkey lactoferrin (Lf) is an iron-binding glycoprotein of the transferrin family. This multifunctional protein exerts several biological activities that may be different in different species (Vincenzetti et al. 2012; Brumini et al. 2015).

The Lf content of donkey milk is higher than that of ruminants, but much lower than that of mare and mother's milk. Its average content in donkey milk has a wide range of variation from 0.080-0.0048 $g.L^{-1}$ in breeds in Italy (Vincenzetti et al. 2012) and Serbia (Šarić et al. 2012; Šarić et al. 2014b). For donkey milk of the Balkan breed, Šarić et al. (2014a) reported that Lf can have both very low and very high values (0.054 $g.L^{-1}$).

Lysozyme. Donkey milk is particularly rich in the enzyme lysozyme (LZ) (Martini et al. 2019), which destroys the peptidoglycan layer of gram-positive bacteria. The average concentration of lysozyme in donkey milk is about 1.07 $g.L^{-1}$ (Licitra et al. 2019), which is similar to the content in human milk (0.3-1.1 $g.L^{-1}$; (Lönnerdal et al. 2017) and higher than cow's milk, in which negligible amounts of lysozyme have been reported. Lysozyme activity for donkey milk ranges from 1670 to 11531 $U.mL^{-1}$ (Addo 2015; Martini et al. 2019), while in cow it is 0.0292 $U.mL^{-1}$, and the highest is in breast milk (about 39,000 $U.mL^{-1}$) (Martini et al. 2018a).

The content of lysozyme in donkey milk during lactation is 0.69 $mg.ml^{-1}$, reaching a maximum value of 0.76 $mg.ml^{-1}$ at the 4th month, after which it gradually decreases to 0.47 $mg.ml^{-1}$ – 8th month. The content of the major whey proteins in donkey milk α -La, β -Lg and Lys are influenced by lactation stage and season. In general, the best quality is the milk during the first 4 months of lactation, after which these 3 components of whey proteins decrease successively, reaching at 8 months 40% of their highest value. This shows that as lactation progresses, the ability of the mammary gland to synthesize these whey proteins decreases and in order to maintain a good nutritional quality of the milk, it is useless to prolong the lactation of donkeys too much. The highest concentration of the most important whey proteins in milk in spring, leads to the hypothesis that the quality of donkey milk is influenced by the availability and/or quality of food (D'Alessandro et al. 2011).

The high concentration of Lys can play as the main antimicrobial agent in the whey proteins of donkey milk and is responsible for the low total bacteria in it (Chiavari et al. 2005; Šarić et al. 2012; Brumini et al. 2015).

Milk fat and fatty acid profile. The diameter of fat globules in milk varies from 1 to 10 μm, their size distribution is similar to cow (Salimei 2011). 70% of the fat globules in donkey milk have an average diameter of 2 μm, similar to that of a mare, but smaller than that of a mother`s milk - 4 μm (Carminati and Tidona 2017).

The fatty acid composition of donkey milk depends on the feed used. In the donkey and other animals with a simple stomach, the hydrogenation of fatty acids characteristic of ruminants does not occur. In donkey milk, the average content of n3 polyunsaturated fatty acids is higher than in ruminant milk (7.4-9.6 $g.100\ g^{-1}$ fatty acids). The high levels of these fatty acids are well balanced with n6 polyunsaturated fatty acids – 10.0-13.0 $g.100\ g^{-1}$ fatty acids (Salimei 2011; Wang et al. 2022).

According to summarized literature data of Salimei and Chiofalo (2006) from several studies by different authors, the content of saturated fatty acids in the milk fat of donkey milk can vary from 46.8 to 56.2%, monosaturated - 15.3-35.0%, polyunsaturated 15.2-30.5% of the total amount of fatty acids.

The n3/n6 ratio is 0.86, and the unsaturated/saturated ratio is 0.48 (Chiofalo et al. 2001), which in various studies is found in the range of 0.48-1.07 (Salimei and Chiofalo 2006).

The mean SFA content of Martina Franca milk was higher (51.98%) than MUFA (28.00%) and PUFA (20.02%). Palmitic acid (C16:0) is characterized by the largest amount of SFA - on average 19.94%,

followed by capric acid (C10:0 - 10.78%), lauric acid (C12:0 - 8.78%), myristic acid (C14:0 - 6.98% on average) and caprylic (C8:0 - 6.20%) acids, with the lowest content being stearic (C18:0 - 1.80%). Of the MUFA with the highest content, oleic acid (C18:1 n-9) (average 21.50%) – 27.57%. (Martemucci and D'Alessandro 2012).

The fatty acid composition of the milk of the Ragusana breed is characterized by a greater amount of saturated fatty acids caprylic (C8:0 - 12.80%), capric (C10:0 - 18.65%) and palmitic (C16:0 -11.47%) acids. Among the fatty acids of nutritional interest and found in small quantities, the highest concentrations were observed for myristic (C14:0 - 5.77%) and stearic (C18:0 - 1.12%). Saturated fatty acids occupy 67.57% of the total share of fatty acids. Monounsaturated fatty acids occupy 15.82% of the total share. Of these, the largest amount is found in oleic (C18:1 - 9.65%). Caprinooleic (C10:1 - 2.20%) and palmitoleic (C11:1 - 2.37%) fatty acids are found in smaller amounts. A similar content of n3 (7.45%) and n6 (8.65%) polyunsaturated fatty acids was found. Among them, linolenic (C18:3 - 6.32%) and linoleic (C18:2 - 8.15%) acids are the most abundant components of polyunsaturated fatty acids (PUFA), the amount of which reaches 16.60% (Chiofalo et al. 2001). Among the "minor" PUFAs (<0.5% of the total share) in donkey milk, are eicosadiene (C20:2 n6), arachidonic (C20:4 n6), eicosapentaenoic (C20:5 n3) and docosaenoic (C22:6 n3) acids (Chiofalo and Salimei 2001), which in mare's milk are in the form of traces (Gastaldi et al. 2010).

Lactose. The main component in donkey milk and the main energy source is lactose. The average lactose content of donkey milk (7.0-7.2%) is close to that of mother's milk. The high lactose content improves the taste of donkey milk. In addition, it stimulates and optimizes the intestinal absorption of calcium, thereby improving bone mineralization in newborns (Vincenzetti et al. 2007; Carminati and Tidona 2017).

The lactose content of donkey milk varies from 5.61 to 7.40% (Guo et al. 2007; Ivanković et al. 2009). The results of research by Malissiova et al. (2016) demonstrated a fairly wide range of lactose variation in donkey milk samples from Northern Greece – 3.54-8.46%, against 6.64-8.46 and 6.82-7.33%, for milk from Central Greece and Cyprus. In contrast, Guo et al. (2007) found almost constant lactose content (6.01-6.60%) in donkey milk for 180 days of lactation. Despite the high lactose content, donkey milk has a lower energy value (1750 kJ. kg^{-1}) than cow's and mother's milk (Salimei 2011).

Among the minor carbohydrates with an indirect positive effect on digestibility, donkey milk contains the same oligosaccharides (3-sialyllactose - 12.4-16.9 $\mu g.ml^{-1}$, 6-sialyllactose - 12.9-20.0 $\mu g.ml^{-1}$ and disialyl-lacto-N-tetraose - 5.0-1.8 $\mu g.ml^{-1}$; 3-SL: 3-sialyllactose; 6-SL: 6-sialyllactose; DSLNT-disialyl-lacto-N-tetraose) found in breast milk (196.3-839.6 $\mu g.ml^{-1}$, 46.4-98.0 $\mu g.ml^{-1}$ and 455.5-805.7 $\mu g.ml^{-1}$), but in lower amounts (Monti et. al., 2015; Carminati and Tidona, 2017). The presence of these oligosaccharides confirms the suitability of donkey milk as a food for infants, as they have the potential to modulate the growth of intestinal flora, influence various gastrointestinal and inflammatory processes and provide protection against bacterial and viral infections (Kunz and Rudolff 2006; Carminati and Tidona 2017).

Mineral composition. The average ash content (0.36%) occupies 4.40% of the total dry matter share of donkey milk (Salimei 2011), and even its components are significantly influenced by the lactation period (Salimei 2011).

The average content of Ca, P, K, Na and Mg in donkey milk are in the range of 54.36-68.90; 41.00-43.44; 65.70-110.27; 37.00-43.77 and 6.13-8.89 $mg.100g^{-1}$ (Li et al. 2018; Malacarne et al. 2019) (Table 3).

The most abundant element is K (65.70-110.27 $mg.100g^{-1}$), followed by Ca, P, Na. The content of

Mg is 6.13-8.89 (Malacarne et al. 2019), which is similar to the distribution of elements in breast milk (Kim et al. 2020). The trace elements Zn, Fe and Cu were found in small amounts or as on traces.

The initial high content of mineral substances is associated with the requirements of the newborn during the initial stage of its development (Fantuz et al. 2012), and their changes are probably also related to the decrease in total protein and casein during lactation (Giosuè et al. 2008; Fantuz et al. 2012).

Table 3. Mineral composition of donkey and breast milk

Minerals	Donkey milk (Malacane et al. 2019), mg.100 g^{-1}	Breast milk (Kim et al. 2020), mg.L^{-1}
Ca	54.36-68.90	200-250
P	41.00-43.44	120-140
K	65.70-110.27	400-450
Na	37.00-43.77	150-250
Mg	6.13-8.89	30-35

Vitamins. Vincenzetti et al. (2020) first identified vitamin B6 (pyridoxine) in donkey milk. The average content of the remaining vitamins of this group is: thiamine (B1) 0.661 μM, riboflavin (B2) 0.168 μM, pyridoxine (B6) – 5.38 μM and folic acid (B9) – 0.83 μM ± 3.0. Donkey milk contains a large amount of nicotinic acid (niacin) - 18.75 μM of vitamin group B3. It is known for its lipid-lowering effect, suppresses the release of free fatty acids from adipocytes, and reduces their amount in plasma and very low density lipoprotein synthesis. The sufficient amount of vitamins B6 and B9 in donkey milk is very important for the growth of children. Folic acid is involved in nucleic acid synthesis, accelerates cell division and is important for fetal growth and development.

Vitamin C is an important antioxidant that traps free radicals. Fresh donkey milk contains 57 mg.L^{-1} of vitamin C, close to that of mother's milk - 60 mg. L^{-1} and significantly higher compared to the vitamin C content of cow's milk (Vincenzetti et al. 2021).

Donkey milk has higher average vitamin D content than cow's and mother's milk. The total vitamin D content of raw donkey milk was 2.23 μg.100 mL^{-1} and decreased to 1.68 μg.100 mL^{-1} after pasteurization at 63°C for 30 min. Vitamins D2 and D3 are also present in raw milk in amounts of 1.68 and 0.60 μg.100 mL^{-1}, respectively, which after pasteurization decrease to 1.38 and 0.30 μg. 100 mL^{-1} (Martini et al. 2018).

Microbiological quality of donkey milk. The studies by Zhang et al. (2008) showed a low total bacterial count of 4.34 log cfu.ml^{-1} and an absence of Salmonella and Shigella in all samples, which was attributed to the antibacterial properties of donkey milk. Furthermore, the relatively good growth of lactic acid bacteria and fungi at 20°C suggests that the natural antimicrobial substances in donkey milk cannot control these types of microorganisms and thus donkey milk can be used as a fermented milk beverage production.

Raw donkey milk shows an overall good hygienic quality when the observed values are compared to the EC Dir. 92/46 and EC Reg. 853/2004. The established values for TBC emphasize the importance of washing and disinfection as a factor for the good quality of donkey milk, regardless of the method of milking, manual or mechanized - 3.66-5.87 log cfu.ml^{-1} (Salimei and Chiofalo 2006).

A low somatic cell count (3.52-4.55 log SCC/ml) in donkey milk suggests good mammary gland health (Salimei and Chiofalo 2006; Malacarne et al 2019). Breed (Ivanković et al 2009) and season (Cavallarin et al. 2015) did not significantly influence the total number of somatic cells in donkey milk.

Functionality and Potential Therapeutic Uses. Since ancient times, donkey milk has been known for its therapeutic properties and has been used to heal wounds and treat various diseases such as bronchitis, asthma, joint pain, gastritis (Martini et al. 2018; Karami and Akbari-adergani 2019).

Low risk of allergy. An advantage of donkey milk is that it is similar to mother's milk. The gold standard for infant feeding is in terms of average protein content (about 18 and 21 $g.L^{-1}$ in colostrum and breast milk, respectively) (Altomonte et al. 2019). Caseins, which are the main allergenic components of cow's milk, are in smaller amounts in donkey's and mother's milk (56% and 30% of the total protein against 80% of cow's milk). In simulated in vitro feeding, casein in donkey milk was found to degrade faster and have almost complete digestibility in both artificial (Li et al. 2020; Aspri et al. 2018) and natural gastrointestinal fluids (Tidona et al. 2011; 2014). This may also explain the reduced allergenicity of donkey milk, since the allergenicity of food proteins is related to their survival in the gastrointestinal tract. On the other hand, the major protein fraction in donkey milk consists mainly of whey proteins, which have various beneficial metabolic and antimicrobial properties (Mignone 2015).

Donkey milk, however, should be used with caution in some allergic people, as some cases of hypersensitivity reactions to milk have been observed in a small number of children and adults (Peeters et al. 2017; Martini et al. 2018).

Antimicrobial action. Donkey milk usually does not contain pathogenic microorganisms that may exist in other types of milk. Some authors confirm the efficacy of donkey milk in inhibiting the growth of specific foodborne pathogens in vitro, in particular Gram positive bacteria such as: a) *Listeria monocytogenes* (Saric et al. 2014; Altomonteet al. 2019, Ebrahimi et al. 2021); b) *Staphylococcus aureus* (Saric et al. 2014, Ebrahimi et al. 2021); (c) *Enterococcus faecalis* (DSM 2352) (Nazzaro 2010).

Donkey milk possesses a rich set of protective factors and has an inhibitory effect against pathogens (Zhang et al. 2008; Brumini et al. 2015) as: *Bacillus cereus* DSM4384 (Nazzaro et al. 2010), *Bacillus cereus* RT INF01 (Tidona et al. 2011), *Enterococcus faecalis* DSM2352 (Nazzaro et al. 2010), *Escherichia coli* ATCC 10536 (Šarić et al. 2012; 2014c), *Escherichia coli* ATCC 8739 (Šarić et al. 2014c), *Escherichia coli* (EPEC) 10208355 (Tidona et al. 2011), *Listeria monocytogenes* ATCC 19111 (Šarić et al. 2014a), *Listeria monocytogenes* 2230 /92 (Tidona et al. 2011), *Salmonella choleraesuis* CGMCC1.1859 (Zhang et al. 2008), *Salmonella enteritidis* ATCC 13076 (Šarić et al. 2012; 2014b), *Salmonella livingstone* (Šarić et al. 2014b), *Salmonella typhimurium* ATCC 14028 (Šarić et al. 2014b), *Shigella dysenteriae* (CGMCC 1.1869) (Zhang et al. 2008), *Staphylococcus aureus* ATC C 25923 (Šarić et al. 2014a), *Staphylococcus aureus* DSM25923 (Nazzaro et al. 2010).

Immunomodulatory effects. Donkey milk has shown immunological activity in in vitro tests and randomized controlled trials in animal models (Jiang et al. 2018) and humans (Amati et al. 2010) in which it induces the release of certain cytokines that regulate the inflammatory and immune response to infections. Donkey milk has been shown to increase cytokines involved in the regulation of innate immunity: interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) both in vitro (Mao et al. 2009) and in vivo (Amati et al. 2010).

Antioxidant activity of donkey milk. Donkey milk is known to have an antioxidant effect, which gives it oxidative stability, providing protection. It has a high ability to scavenge anionic superoxide radicals and eliminate hydroxyl free radicals generated by the body's metabolism (Li et al. 2017). Simos et al. (2011) determined the antioxidant activity of donkey milk using the oxygen radical absorbance method and found that the main contributors to this activity were caseins and hydrophilic antioxidant compounds such as uric acid and vitamin C contained in donkey milk.

Anti-inflammatory and antitumor activity of donkey milk. Donkey milk is a matrix rich in lactoferrin, which has antimicrobial and antitumor activity, interferon γ, which stimulates macrophages, natural killer cells, and cytotoxic T cells (Jirillo and Magrone 2014; Schroder et al. 2004). Donkey milk can induce the release of anti-

inflammatory cytokines, maintaining a state of immune homeostasis (Simos et al. 2011).

Administration of donkey milk has an anti-inflammatory effect on the liver by improving liver mitochondrial functions (Trinchese et al. 2015). Trinchese et al. (2018) showed that TNF-α and IL-1 decreased while IL-10 levels increased in the serum and tissues of rats fed donkey milk compared to control rats fed cow's milk. In addition to its anti-inflammatory activity, donkey milk has other physiological functions such as immunoregulatory and antitumor activity (Ibrahim and Aoki 2003). Mao et al. (2009) found that many fractions in donkey milk could stimulate the production of cytokines IL-2, IFN-γ, IL-6, TNF-α and IL-1β by lymphocytes and macrophages. These cytokines affect anti-proliferation by inducing apoptosis of A549 tumor cells (human lung cells) and the differentiation of these A549 tumor cells into normal cells. Lysozyme has a strong antiproliferative effect and may be a promising molecule in the treatment of lung cancer (Mao et al. 2009).

Consumption of donkey milk is beneficial in enhancing the immune response in immunocompromised elderly patients (Amati et al. 2010; Kapila 2017).

Antidiabetic activity of donkey milk. In addition to its antioxidant, antimicrobial, anti-inflammatory, and antitumor activity, donkey milk also has antidiabetic effects (Simos et al. 2011; Li et al. 2020; Mao et al. 2009).

Type 2 diabetes, also known as non-insulin-dependent diabetes, is a metabolic disease characterized by chronic excess blood sugar (hyperglycemia). The leading causes of type 2 diabetes include obesity, β cell dysfunction, and insulin resistance from peripheral tissues and cells (Weng et al. 2016). Because of its higher whey protein content, donkey milk can help prevent and treat diabetes by improving glucose metabolism and insulin resistance. An exceptional feature regarding the content of donkey milk with effects on the metabolic status of the cell is the high content of vitamin B12 (cobalamin) in it: 110 (μg.100 g^{-1}) compared to other types of milk - 0.07 in breast milk, 0, 4 and 0.7 or 0.16 (μg.100 g^{-1}) in cow, sheep or goat milk, respectively (Alichanidis et al. 2016).

Li et al. (2020) found that donkey milk improved viability of damaged pancreatic β cells but did not stimulate insulin secretion from damaged β cells and that α-lactalbumin increased target organ insulin sensitivity. Donkey milk reduces the level of glycosylated hemoglobin and acts positively in the treatment of diabetes by inhibiting the expression of phosphoenolpyruvate carboxykinase 1 and glucose-6-phosphatase, which are key enzymes in hepatic gluconeogenesis.

Conclusion

Nowadays, donkey milk is considered a medicinal food because of its nutritional and functional properties and it has a composition similar to mother's milk. Consumption of donkey milk products (with higher peptide content) by the elderly should be encouraged because of its excellent source of bioavailable calcium, low caloric intake and its ability to modulate the immune system of the elderly, including the immune gut mucosal response.

Although there is an increase in consumer interest in donkey milk, it is still a "niche product". The food industry should try to increase the production and market availability of donkey milk; this will raise awareness and promote this sector by highlighting nutritional applications with multiple health benefits not only for newborns but also for people of all ages.

However, despite the increasing research on donkey milk in recent years, it is still necessary to do a full and thorough analysis of the impact of consumption on certain types of diseases such as diabetes, cancer, and why not Covid-19.

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