Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE COMPOSITIONAL AND HYGIENIC-SANITARY ANALYSIS OF JENNET MILK

Octavia Maria TAMAS-KRUMPE[1], Andrei STAN[1], Grigore ONACIU[1], Sebastian OGNEAN[2], Arghil ILEA[2], Doru NECULA[1, 3], Aurelia COROIAN[1], Daniel BEREAN[1], Călin LAȚIU[1], Laurenț OGNEAN[1]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372, Mănăştur Street, no. 3-5, Romania
[2]Technical University Cluj-Napoca, 400114, Memorandumului Street, no. 28, Romania
[3]Centre of Mountain Economy "CE-MONT", INCE, Romanian Academy, Petreni Street, no. 49, 725700, Vatra-Dornei, Romania

Corresponding author email: octavia.tamas@usamvcluj.ro

Abstract

Nowadays, the interest in jennet milk has considerably increased worldwide due to its nutritional characteristics. This study aimed to analyse the main physicochemical and hygienic-sanitary parameters of some jennet milk samples, by using current methods of quality monitoring. The following physicochemical (dry matter - DM, non-fat dry matter - NDM, fat, total protein, β-casein, lactose, and cryoscopic point), metabolic (urea, acetone, and β-hydroxybutyrate - BHB) and hygienic parameters (total bacteria count - TBC, somatic cell count - SCC and differential somatic cell count - DSCC) were assessed. Additionally, electrical conductivity (EC), pH, temperature, and density were investigated. The results revealed variations of total DM (9.64-11.11%), which were mainly given by the NDM oscillations (8.62-9.54%) and to a lesser extent by the fat content (0.25-1.66%). The study also emphasized low values of EC (1.75 mS/cm), TBC (22.16-62.15x10^3 CFU/mL), and SCC (3-30x10^3 cells/mL). The good health status was also confirmed by other metabolic indices, such as urea (36.27 mmol/L), acetone (0.06 mmol/L), and BHB (0.14 mmol/L).

Key words*: Fossomatic, jennet milk, Milkoscan, quality testing.*

INTRODUCTION

Jennet milk has been known since Antiquity, especially for its healing effects, being recommended even by Hippocrates in the treatment of joint pain, wounds, or intestinal obstruction. In this context, it is worth mentioning that in the 1880s, a Parisian nursery "Hospice des Enfants'' used to feed newborns, deprived of breast milk with jennet milk (Lauziers, 2011).

Currently, jennet milk is considered a rare and difficult-to-obtain product, and its sampling requires keeping the young near the mother and even resorting to stimulating milk ejection with oxytocin (Salimei & Fantuz, 2012). The jennet lactation lasts between 6-12 months, and the average milk production is 1.5-2.0 L/day; moreover, the quantity and quality of milk are influenced by the breed, the number of lactations, the lactation stage, feeding, season, and other intrinsic or extrinsic factors (Alabiso et al., 2009; Chiavari et al., 2005; Polidori et al., 2010). Regarding the jennet herd distribution, the 2014 statistics placed Ethiopia (7,428,037 animals) at the top of the countries, followed by China (6,033,500) and Pakistan (4,942,000) (Food and Agriculture Organization of the United Nations, 2012). In the absence of available data on jennet milk production in Ethiopia or Pakistan, China ranks first in the world, with about 40,000 tons that were reported annually in the northeastern part of the country (Guo et al., 2007). Regarding the processing capacity, the Chinese company YuKunLun (founded in 2007) stands out, processing annually 20 tons of jennet milk, in the form of freeze-dried powder and 500 tons of fresh milk, intended for human consumption, especially as a substitute for human milk (www.chinadaily.com.cn/cndy/2015-05/18/content_20743564.htm).

The biochemical composition of jennet milk is similar to woman's milk, which makes it the most suitable natural substitute for infants; moreover, jennet milk is very well tolerated, even by infants suffering from cow milk protein allergies (CMA) or intolerance (Cosentino et al., 2012; Molodecky et al., 2012; Swar, 2011; Polidori & Vincenzetti, 2013a). Jennet milk also stands out for its antimicrobial potential, due to the high lysozyme content, which can reach up to 4 g/L (Šarić et al., 2014; Vincenzetti et al., 2008).

A study on milk physicochemical components in the first four lactations of a jennet revealed constant increases in fat content (from 0.78 to 2.38%) and lactose (from 6.68 to 6.76%), and slight oscillations of the protein content, with the lowest average (1.72%) in the second lactation (Marchiş et al., 2015), respectively. Jennet milk is, therefore, poor in nitrogenous substances, similar to the mare and woman's milk; nitrogenous matter in these species represents only 20% of the dry matter, compared to 80% in cow milk. It is worth mentioning that the rich content of jennet milk in water-soluble and fat-soluble vitamins provides good antioxidant potential and beneficial effects on human health (Tafaro et al., 2007). Thus, cobalamin, ascorbic acid, thiamine, and riboflavin are present in higher concentrations in jennet milk, compared to bovine and woman's milk (Cunsolo et al., 2011; Salimei & Fantuz, 2012). The mineral content of jennet milk is similar to that of human milk, but calcium and phosphorus levels are higher. The essential trace elements, such as Zn, Co, and I exhibit similar concentrations to human milk, while Fe, Cu, and Se are found in lower concentrations. After the first month of lactation, the mineral content of jennet milk decreases significantly, correlating with the reduction of the casein content, because minerals are mainly associated with casein micelles (Aspri et al., 2017; Fantuz et al., 2012).

Regarding the hygienic-sanitary parameters of jennet milk, there are still little data available. However, the study carried out by Marchiș et al. (2015) revealed lower values of TBC (58-75x10^3 CFU/mL) and similar values of SCC in jennet milk, compared to cow's milk (255-340x10^3 cells/mL). Those decreased values of microbial load could be correlated with the increased resistance of the mammary gland to infection and the high antimicrobial potential of jennet milk (Aspri et al., 2017).

MATERIALS AND METHODS

The present research was performed on milk samples collected from a batch of indigenous jennets, raised in an individual household from the Apuseni Mountains, Romania. Regarding the geographical conditions, this area is located on the outskirts of Câmpeni and is rich in mountain pastures and coniferous forests. The animal batch consisted of 12 jennets (10 lactating and 2 pregnant), 3 donkeys, and 10 foals.

The household made the necessary arrangements for raising and maintaining the animals in enclosed shelters, providing common boxes and outdoor paddocks, and lush pastures, respectively. In the summer season, the feeding was predominantly based on natural grazing, supplemented with coarse fodder (2-4 kg/animal) or concentrate mixtures in lactating jennets (1-1.5 kg/animal). In the winter season, the feed ratio was predominantly (80-90%) based on hay and coarse feed, with the addition of feed concentrates, depending on the stage of lactation (10-20%). The milking procedure consisted of separating the lactating jennets from the foals during the night, while in the morning, the jennets were manually milked. Regarding hygiene and milking techniques, the household staff resorted to the usual sanitation measures, including the separate collection of the first 3-4 jets of milk and the washing, disinfection, and light massage of the mammary gland (Ognean et al., 2007).

The study began with a complete clinical examination, based on the general semiotic methods and specific investigations to assess the health status of the mammary gland, including an organoleptic examination of milk, by applying the Contrast test (Ognean et al., 2007). At a preliminary examination, no changes were found regarding the general condition and mammary gland, all the jennets being considered clinically healthy and therefore, subjected to testing. Thus, milk samples were collected from the lactating jennets batch (no=10), in compliance with the hygienic-sanitary requirements (Ognean et al., 2007). The milk samples were initially collected separately and then reunited into average sample/animal, resulting thus, the biological material being tested. The fresh milk samples were stored at 4ºC during transportation, without additional preservatives; moreover, the samples were investigated within 8 hours.

The investigations were performed by using the Combifoss automatic system, consisting of Milkoscan (FT 6000) and Fossomatic devices that operate in compliance with European standards (ISO 9622/IDF 141:2013 and AOAC official method 972.16). From a technical point of view, MilkoScan FT 6000 is represented by a high-capacity automatic spectrophotometer, equipped with IDF and FTIR technologies for milk analysis. Thus, the Combifoss system resorted to the FTIR technique for determining the compositional parameters; furthermore, an automatic BactoScan™ FC equipment was used to determine the hygienic-sanitary parameters of the milk samples. The usage of this complex system allowed the testing of several compositional, metabolic, and hygienic parameters, such as dry matter (DM), non-fat dry matter (NDM), fat, total protein, β-casein, lactose, cryoscopic point (°C), urea (mmol/L), acetone (mmol/L), β-hydroxybutyrate (BHB) (mmol/L), total bacteria count (TBC; x10^3/mL), the somatic cell count (SCC; x10^3/mL) and differential somatic cell count (DSCC; %). Additionally, the electrical conductivity (EC; mS/cm), pH, temperature (°C), and milk density were determined, by using a pH meter/Conductometer (C532) and a lactodensimeter, respectively.

The obtained data were statistically analyzed by using the GraphPad Prism 6, InStat, and Microsoft Excel programs, which allowed the calculation of primary statistical parameters, such as average, standard deviation (St. dev), standard error (St.error), median, minimum (Min.) and maximum (Max.), Coefficient of variation (CV). The results were processed by using the Foss Integrator software platform, with a Process Hazard Analysis (PHA) diagram, which allowed a quick and accurate assessment of the investigated parameters.

RESULTS AND DISCUSSIONS

Evaluation of the physical parameters. The main physical parameters of jennet milk revealed important oscillations of the individual values, which may be included within physiological limits for cow milk and the main ruminant species (Table 1). As it is known, the determination of these parameters, relevant to the testing of milk quality and health in most mammalian species, has been seldom reported in the case of jennet milk (Aspri et al., 2017; Marchiş et al., 2015).

The values recorded when determining the density of jennet milk could be characterized by decreased individual variations (1.030-1.037 g/cm^3), around the average of 1.034 g/cm^3 (Table 1). Based on the obtained data, the jennet milk density varied in intervals close to those of bovine milk, except for a few cases, where values were slightly higher. The results obtained at the pH evaluation also indicated evolutions within the physiological intervals for raw milk in general, with average values of 6.77 and individual values between 6.68 and 6.72 (Table 1). The cryoscopic point highlighted an average value of -0.528°C, considered to be very close to the lower limit of the physiological range for milk in general (Bu et al., 2013). Moreover, the individual values of this parameter were also at the lower physiological limit, with several decreases below its level (-0.50°C and -0.51°C), which excluded, however, the possible addition of water to the milk sample (Table 1). Furthermore, the electrical conductivity pointed out minor differences between the investigated samples, even though they were collected from jennets in different stages of lactation. The distribution of the obtained data suggested an average value of 1.75 mS/cm, with tight oscillations in the range of 1.52-2.01 mS/cm (Table 1).

Evaluation of the biochemical parameters. The results obtained when evaluating the main biochemical indices of jennet milk (Table 1 and Figure 1) emphasized major differences between the composition of jennet and cow milk and other ruminant species. Thus, we recorded a decreased level of total dry matter content, the average value being 10.36%, while the individual values varied between 9.64% and 11.11%, as expected. Additionally, the non-fat dry matter content proved to be unimportant, with individual variations (8.62-9.54%) that suggested limited oscillations around the average value of 9.02%. Particular attention was given to the fat content analysis, as it is known that this index is lower in the case of jennet milk, with a major impact on milk's marketing and processing, in general. In this context, the evolution of the investigated fat content showed wide variations around the average of 0.95% (Table 1). The total protein content emphasized characteristic evolutions, recording an average value of 1.73% and less important individual variations, between 1.37 and 2.09% (Table 1; Figure 1). The lactose content also pointed out valuable features, thus completing the picture of the compositional parameters in jennet milk. Regarding this parameter's evolution, the average values were much higher than those recorded in the case of protein content and especially in lipids, the average level being 6.30%, with mild individual oscillations, between 6.01-6.57% (Table 1; Figure 1).

Table 1. Statistical data obtained of the main physicochemical and hygienic-sanitary parameters of jennet milk

	Parameters	Min.	Max.	Mean	Std. Dev.	Std. Err.	CV%
Physico-chemical parameters	Density (g/cm^3)	1.0300	1.0370	1.0340	0.0021	0.0007	0.20%
	pH	6.7200	6.8300	6.7790	0.0345	0.0109	0.51%
	Cryoscopic point (ºC)	-0.5530	-0.5060	-0.5275	0.0129	0.0041	2.44%
	Conductivity (mS/cm)	1.5200	2.0100	1.7500	0.1514	0.0479	8.65%
	DM (%)	9.6400	11.1100	10.3700	0.5947	0.1881	5.74%
	NDM (%)	8.6200	9.5400	9.0260	0.2499	0.0790	2.77%
	Fat (%)	0.2500	1.6600	0.9560	0.5190	0.1641	54.29%
	Protein (%)	1.3700	2.0900	1.7340	0.2670	0.0844	15.40%
	β-casein (%)	1.1900	1.7300	1.4470	0.1787	0.0565	12.35%
	Lactose (%)	6.0100	6.5700	6.3020	0.1716	0.0543	2.72%
Hygienic-sanitary parameters	TBC (x 10^{3}CFU/mL)	22.1600	62.1500	38.8400	13.9000	4.3950	35.79%
	SCC (x 10^{3}cells/mL)	3.0000	30.0000	8.3000	7.9450	2.5120	95.72%
	DSCC (%)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Metabolic parameters	Urea (mmol/L)	20.5000	55.3000	36.2700	10.1800	3.2210	28.08%
	Acetone (mmol/L)	0.0000	0.2000	0.0560	0.0769	0.0243	137.34%
	BHB (mmol/L)	0.0000	0.3000	0.1400	0.0904	0.0286	64.59%

Legend: St. Dev. – Standard Deviation; St. Err. – Standard Error of Mean; Min. – Minimum; Max. – Maximum; CV% - Coefficient of Variation; Cryoscopic P – Cryoscopic point; EC – Electrical conductivity; DM – Dry matter; NDM – Non-fat dry matter; TBC – Total Bacteria Count; SCC – Somatic cell count; DSCC – Differential somatic cell count; BHB – β-hydroxybutyrate.

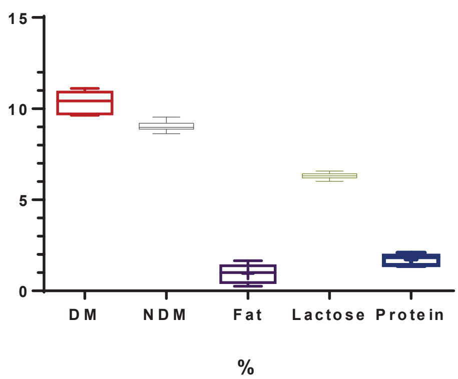

Figure 1. Distribution of the obtained values of the biochemical parameters

Evaluation of the hygienic-sanitary indices. The total bacteria count (TBC) registered much lower values than those provided by the standards required for cow milk (100,000 CFU/mL) (Ognean, 2019). In the animal batch used for testing, the average value of TBC was below this level, 38.83 CFU/mL. The distribution of individual values was within the range of 22.16-62.15x10^3 UFC/mL, being situated below the maximum limits required for cow milk (Figure 2).

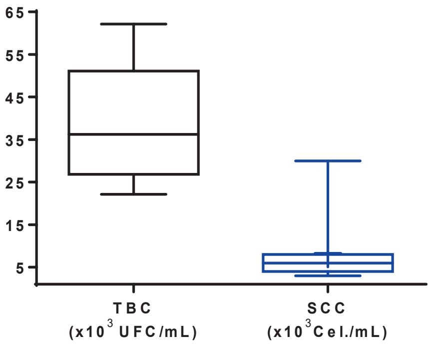

Figure 2. Distribution of the obtained values of the hygienic-sanitary parameters

The somatic cell count (SCC) revealed values at the lower limit of the standards that are required for cow milk, whose maximum level is set at 400.000 cells/mL (Ognean, 2019). Compared to these standards, the recorded data highlighted much lower individual and average values in the case of jennet milk, with decreased oscillations (3-30x10^3 cells/mL), around the average of 8.3x10^3 cells/mL. These values indicated a good milk quality, which was also confirmed by the results obtained in the differential somatic cell count (DSCC). Following the investigations, zero values of DSCC were obtained for all the tested samples, suggesting the lack of polymorphonuclear leukocytes and implicitly, a very good level of mammary gland health and milk quality.

Evaluation of the metabolic indices. Currently, a particular relevance is attributed to lactic indices in the evaluation of the metabolic profile of lactating females, which comes down to the detection of subclinical ketosis, a carbohydrate dysmetabolism found only in highly productive lactating cows (Ognean, 2019). The obtained results emphasized very low values of urea, acetone, and BHB (36.27 mmol/L, 0.06 mmol/L, and 0.14 mmol/L, respectively) (Table 1), indicating the absence of ketosis and other dysmetabolic syndromes in the jennet batch subjected to testing.

The analysis of the physical parameters of jennet milk revealed evolutionary trends more or less similar to those recorded by other researchers in the field. Thus, the density of jennet milk showed wide oscillations, within the physiological intervals for raw milk (Ognean et al., 2007), whereas unlike the findings of some researchers (Aspri et al., 2017), the level of alkalinity was not superior to that of cow milk. Regarding the electrical conductivity (EC), we only mention that this highly topical physical parameter in biomedical tests is given by the increase of the ionic content of milk (Na^+, K^+) to the detriment of the lactose concentration (Ognean, 2019). These components present important oscillations, which in turn generate variations in the milk's electrical conductivity. Among these, the most important ones are considered to be the EC increases, following physiological or pathological conditions of the mammary gland, such as the beginning and the end of lactation and the onset and evolution of mastitis, respectively (Lin et al., 2006; Ognean et al., 2007). In the case of lactating cows, these changes are regarded as being extremely accurate, which explains the special extension of the tests based on determining the milk's electrical conductivity to monitor the mammary gland health status and even to detect the oestrus period (Ognean et al., 2007). Moreover, the oscillations of the fat percentage confirm that lipids represent one of the most variable components in jennet milk. In this regard, available data reported a low average fat content in jennet milk (0.38%) and it was thought that this aspect was due to the increased individual variability (Salimei et al., 2004). Furthermore, these researchers analyzed the lipid fractions of jennet milk and detected high levels of linoleic and linolenic acids. It is also unanimously accepted the major impact of milk fat on the physical, organoleptic, and sensory characteristics of dairy products, these influences being less studied in the case of jennet milk (Bu et al., 2013). The available data on the protein content of jennet milk is also sporadic and controversial, leading to a considerable intensification of current concerns in this area (Hussain et al., 2012). Regarding the protein profile of jennet milk, the following distribution of protein fractions was reported:

lactoferrin (4.48%), serum albumin (6.18%), β-lactoglobulins (29.85%), lysozyme (21.03%) and α-lactalbumin (22.56%) (Salimei et al., 2004). Overall, the results were in accordance with another research, which confirmed a low content of dry matter (8.19%), low protein content (1.34%), a high amount of lactose (6.07%), and low-fat content (0.16%) (Malacarne et al., 2019).

Following the correlation of the data obtained in the present study with those synthesized from literature, a brief characterization of the nutritional and biologically active components of jennet milk could be done, as follows:

- Electrical conductivity (1.52-2.01 mS/cm) close to that of bovine milk and slightly higher alkalinity (pH 7.0-7.2);
- High and variable lactose content (6-7%), which gives it good palatability and it facilitates intestinal absorption of calcium and phosphorus, and the transfer of minerals to bones and helps to prevent osteoporosis, respectively (Heaney, 2012; Salimei & Fantuz, 2012);
- Very low-fat content (0.29-1.82%) and a lipid profile rich in saturated and polyunsaturated fatty acids, linolenic and linoleic acids (Alabiso et al., 2009; Chiofalo et al., 2011);
- Very low protein content (1.3-1.8%), the nitrogenous matter being reduced to 20% of DM (compared to 80% in bovine milk), with a soluble protein/casein ratio close to 1 (compared to 0.2 in bovine milk, and 2 in human milk, respectively). Jennet milk, therefore, has a lower proportion of caseins (dominated by β-casein) (40-50%) and a higher proportion of soluble proteins (α-lactalbumin, β-lactoglobulin, and lysozyme) compared to human and bovine milk (18-66%). This protein profile, characterized by decreased levels of caseins and very high levels of lysozyme (4 g/L or 15% of total protein, throughout the 150 days of lactation), confers an increased degree of digestibility and a particular antimicrobial potential to jennet milk (Carminati et al., 2017; Guo et al., 2007; Polidori and Vincenzetti, 2013b; Šarić et al., 2014);
- Richer content of water-soluble vitamins (cobalamin, ascorbic acid, thiamine, riboflavin, and other B-complex vitamins) and lower content of fat-soluble vitamins (A, E) compared to bovine and human milk (Claeys et al., 2014);
- Mineral content is close to that of human milk, with higher levels of calcium and phosphorus, similar concentrations of Zn, Co, and I, and lower levels of trace elements, such as Fe, Cu, and Se, respectively (Aspri et al., 2017; Fantuz et al., 2012).

Taking into account the results obtained at total bacteria count determination, it might be assumed that the predominantly low values of this parameter could be correlated with the good level of mammary gland health in the investigated jennets. Regarding the potential risks, it is known that milk is extremely exposed to microbial contamination, and various pathogens could reach the mammary gland in an ascending way or contaminate milk in an exogenous way. In addition to this, the natural risk of contamination of the mammary gland and implicitly, of jennet milk is considerably lower than in the case of bovine and other ruminant species milk, due to low production and the rich content of components with antibacterial action.

Regarding the impact of the hygienic-sanitary indices on the evaluation of mammary gland health and of the milk intended for public consumption, it is important to mention that the automatic TBC and SSC monitoring systems have gained remarkable attention in the production and processing field of cow milk, with real possibilities for expansion in terms of quality control in goat, buffalo or sheep milk. In the case of jennet milk, which is barely exploited, the cellular and microbial testing is sporadic and possesses a predominantly scientific character. In this regard, the obtained results highlighted the possibility of using automatic systems for determining TBC and SCC in jennet milk, as well as the need to outline physiological limits for this species. Thus, compared to the present study, other researchers obtained lower values for TBC (24-46x10^3 CFU/mL) (Cavallarin et al., 2015; Coppola et al., 2002; Malissiova et al., 2016; Pilla et al., 2010; Salimei et al., 2004) and higher values for SCC, respectively (254-340x10^3 cells/mL) (Marchiş et al., 2015). As it is already proven, this low microbial load is due to the high antimicrobial potential of jennet milk, conferred by the high content of lysozyme, immunoglobulins, lactoferrin, and lactoperoxidase (Aspri et al., 2017; Brock, 2002). On the other hand, the determination of TBC and SCC contribute to the diagnosis, prophylaxis, and combating of mastitis (Rotaru & Ognean, 1998). Thus, it could be stated that the risk of mastitis in jennets is considerably lower than in cows, goats, or sheep. Moreover, it has been observed that among the many pathogens involved in mastitis, strains of *B. cereus* and *Staphylococcus* spp. have been isolated from jennet milk (Cavallarin et al., 2015), but without any mammary gland complications being reported (Verraes et al., 2014). There are also data showing that *Enterococcus faecalis,* a potentially pathogenic, thermotolerant, lysozyme-resistant bacterial strain of public health interest, was isolated from raw jennet milk (Aspri et al., 2017).

Regarding public health regulations, it should be recalled that in accordance with European norms (EC Regulation 853/2004), the clause "other milk-producing species" applies for jennet milk, providing for TBC, values that are lower than 1,500,000 CFU/mL in the case of raw milk (at 30°C) and lower than 500,000 CFU/mL in the case of milk intended for processing, whilst for SCC, values that are below 500,000 cells/mL.

CONCLUSIONS

The physicochemical and hygienic-sanitary analysis of the investigated jennet milk samples allowed the characterization of the main compositional indices and of some markers with relevance in monitoring the mammary gland health and the biologically active potential of milk. Thus, the total content of DM revealed low values and less important individual variations, mainly given by NDM, as the fat content was poorly represented. Unlike in ruminant species milk, lactose reached the highest proportions, and the protein percentage, dominated by β-casein, was close to the fat content. The evolution of the indices with a major impact in monitoring the health of the mammary gland and milk was also relevant, materialized by low levels of EC, TBC, and SCC and by zero values of DSCC, respectively. A unique character might be attributed to the evolution of lactic indices with metabolic relevance (urea, acetone, BHB), indicating a good general health status, the lack of susceptibility to mastitis, and the absence of predisposition to ketosis in lactating jennets.

REFERENCES

Alabiso, M., Giosuè, C., Alicata, M. L., Mazza, F., & Iannolino, G. (2009). The effects of different milking intervals and milking times per day in jennet milk production. *Animal, 3*(4), 543–547.

Aspri, M., Economou, N., & Papademas, P. (2017). Donkey milk: An overview on functionality, technology, and future prospects. *Food Rev. Int*., 33(3), 316–333.

Brock, J. H. (2002). The physiology of lactoferrin. *Biochem Cell Biol*., 80(1), 1-6.

Bu, G., Luo, Y., Chen, F., Liu, K., & Zhu, T. (2013). Milk processing as a tool to reduce cow's milk allergenicity: a mini-review. *Dairy Sci. Technol*., *93* (3), 211–223.

Carminati, D., & Tidona, F. (2017). Nutritional Value and Potential Health Benefits of Donkey Milk. In book: *Nutrients in Dairy and their Implications on Health and Disease*, 407-414.

Cavallarin, L., Giribaldi, M., Soto-Del Rio M. de los D., Valle, E., Barbarino, G., Gennero, M.S., & Civera, T. (2015). A survey on the milk chemical and microbiological quality in dairy donkey farms located in NorthWestern Italy. *Food Control*, 50, 230–235.

Chiavari, C., Coloretti, F., Nanni, M., Sorrentino, E., & Grazia, L. (2005). Use of donkey's milk for a fermented beverage with lactobacilli. *Le Lait, INRA Editions, 85*(6), 481–490.

Chiofalo, B., Dugo, P., Bonaccorsi, I., & Mondello, L. (2011). Comparison of major lipid components in human and donkey milk: new perspectives for a hypoallergenic diet in humans. *Journal of Immunopharmacology and Immunotoxicology*, 1(12), 1-13.

Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., Dewettinck, K., & Herman, L. (2014). Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control,* 42, 188-201.

Coppola, R., Salimei, E., Succi, M., Sorrentino, E., Nanni, M., Ranieri, P., Belli Blanes, R., & Grazia, L. (2002). Behaviour of Lactobacillus rhamnosus strains in ass's milk. *Annals of Microbiology*, 52(1), 55-60.

Cosentino, C., Paolino R., Freschi, P., & Calluso A. M. (2012). Short communication: jenny milk production and qualitative characteristics. *J Dairy Sci.,* 95(6), 2910-2915.

Cunsolo, V., Muccilli, V., Fasoli, E., Saletti, R., Righetti, P. G., & Foti, S. (2011). Poppea's bath liquor: The secret proteome of she-donkey's milk. *J. Proteomics*, 74(10), 2083–2099.

Fantuz, F., Ferraro, S., Todini, L., Piloni, R., Mariani, P., & Salimei, E. (2012). Donkey milk concentration of calcium, phosphorus, potassium, sodium and magnesium. *Int. Dairy J*., 24(2), 143–145.

Food and Agriculture Organization of the United Nations (2012). *FAOSTAT DATABASE*, http://faostat3.fao.org/.

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. *J. Dairy Sci*., 90(4), 1635–1643.

Heaney, R. P. (2012). Calcium, dairy products, and osteoporosis. *J. Am. Coll. Nutr*., 19 (2), 83S-99S.

Hussain, R., Javed, M., & Khan, A. (2012). Changes in Some Biochemical Parameters and Somatic Cell Counts in the Milk of Buffalo and Cattle Suffering from Mastitis. *Pakistan veterinary journal*, 32(3), 418-421.

Lauziers, V. (2011). *L'alimentation de l'ânesse en lactation.* Thèse d'exercice, Ecole Nationale Vétérinaire de Toulouse – ENVT.

Lin, M. J., & Lewis, M. J. (2006). Measurement of ionic calcium in milk. *International Journal of Dairy Technology*, 59(3), 192-199.

Malacarne, M., Criscione, A., Franceschi, 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(12): 1161. DOI: 10.3390/ani9121161.

Malissiova, E., Arsenos, G., Papademas, P., Fletouris, D., Manouras, A., Aspri, M., Nikolopoulou, A., Giannopoulou, A., & Arvanitoyannis, I. S. (2016). Assessment of donkey milk chemical, microbiological and sensory attributes in Greece and Cyprus. *Int. J. Dairy Technol*, 69(1), 143–146.

Marchiş, Z., Negrea, O., Stan, A., Coroian, A., & Coroian, C. O. (2015). The influence of lactation on SCC and TNG of the donkey milk. *ABAH Bioflux*, 7(2), 208-212.

Molodecky, N. A., Soon, I. S., Rabi, D. M,, Ghali, W. A., Ferris, M., Chernoff, G., Benchimol, E. I., Panaccione, R., Ghosh, S., Barkema, H. W., & Kaplan, G. G. (2012). Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases With Time, Based on Systematic Review. *Gastroenterology*, 142(1), 46- 54.e42.

Ognean, L. (2019). *Veterinary physiology*. Cluj-Napoca, RO: Colorama Publishing House.

Ognean, L., Drăgan, P., & Moga, Silvia, Tripon (2007). Electrical conductibility of milk, an alternative for lactation and mammary health supervision of cows. *Rev.Med.Vet*., 18(2), 29-36.

Pilla, R., Daprà, V., Zecconi, A., & Piccinini, R. (2010). Hygienic and health characteristics of donkey milk during a follow-up study. *J. Dairy Res*., 77(4), 392–397.

Polidori, P., Beghelli, D., Mariani, P., & Vincenzetti, S. (2010). Donkey milk production: State of the art. Ital. *J. Anim. Sci*., 8, 677-683.

Polidori, P., & Vincenzetti, S. (2013a). Use of Donkey Milk in Children with Cow's Milk Protein Allergy. *Foods,* 2 (2), 151–159.

Polidori, P., & Vincenzetti, S. (2013b). Effects of thermal treatments on donkey milk nutritional characteristics. Recent Pat. *Food Nutr. Agric*., 5 (3), 182–187.

Rotaru, O., & Ognean, L. (1998*). Morphology and physiology of the milk cell population.* Cluj – Napoca, RO: Casa Cărţii de Ştiinţă Publishing House.

Salimei, E., & Fantuz, F. (2012). Equid milk for human consumption. *Int. Dairy J*., 24(2), 130–142.

Salimei, E., Fantuz, F., Coppola, R., Chiofalo, B., Polidori, P., & Varisco, G. (2004). Composition and characteristics of ass's milk. *Anim. Res*., 53(1), 67–78.

Šarić, L. Ć., Šarić, B. M., Kravić, S. Ž., Plavšić, D. V., Milovanović, I. L., Gubić, J. M., & Nedeljković, N. M. (2014). Antibacterial activity of Domestic Balkan donkey milk toward *Listeria monocytogenes* and *Staphylococcus aureus. Food and Feed Res*., 41(1), 47–54.

Swar, O. M. (2011). Donkey milk-based formula: A substitute for patients with cow's milk protein allergy. *Sudan. J. Paediatr*., 11(2), 21–24.

Tafaro, A., Magrone, T., Jirillo, F., Martemucci, G., D'Alessandro, A.G., Amati, L., & Jirillo, E. (2007). Immunological properties of donkey's milk: its potential use in the prevention of atherosclerosis. *Curr. Pharm. Des*., 13(36), 3711–3717.

Verraes, C., Claeys, W., Cardoen, S., Daube, G., De Zutter, L., & Imberechts, H. (2014). A review of the microbiological hazards of raw milk from animal species other than cows. *International Dairy Journal*, 39(1), 121-130.

Vincenzetti, S., Polidori, P., Mariani, P., Cammertoni, N., Fantuz, F., & Vita, A. (2008). Donkey's milk protein fractions characterization. *Food Chem*., 106, 640–649.