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THE EVALUATION OF LIPIZZANER MARE MILK SELECTED PROPERTIES DURING THE LACTATION PERIOD

Adriana Pavelková, Jana Tkáčová, Miroslav Kročko*

Address(es):
The Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Science, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

*Corresponding author: adriana.pavelkova@uniag.sk

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ABSTRACT

Nowadays growing interest in different types of alternative milks not only because of their nutritional composition and possible use in many aspects of human nutrition. One of them is mare's milk. The analyze the selected properties of Lipizzaner breed mare milk was the aim of this study. The Lipizzaner mares (n=6) were kept under the same conditions and fed the same ration, and they were 5-6 years old. Milk samples were tested for 6 months. The following parameters were evaluated: dry matter, titratable acidity, density, electrical conductivity, pH, and content of fat, calcium, and lactose. On the base of our results, we can state that the average value of titratable acidity was 2.07±0.74 °SH, electrical conductivity 1.61±3.80 mS.cm^{-1}, density 1028.64±3.98 kg.m^{-3}, pH 7.25±0.21, the content of dry matter 9.55±0.22%, fat 0.92±0.26%, lactose 6.71±0.12%, and calcium 105.03±16.14 mg. L^{-1}.

Keywords: milk, mare milk, Lipizzaner breed, physicochemical properties

INTRODUCTION

Milk is a sophisticated food that contains several essential nutrients. The genetic, physiological, and nutritional makeup of milk varies greatly between animals as well as in response to environmental influences **(Malacarne *et al.*, 2002; Weaver *et al.*, 2013).** On the global market, while cow's milk accounts for 82% of all milk production and consumption, the remaining portion is primarily derived from buffaloes, goats, and sheep, with less than 1% coming from other animals like camels, horses/donkeys, and yaks. Less than 0.1% of the world's total production is made up of mare milk **(Faye & Konuspayeva, 2012; FAOSTAT, 2020).** In recent years, there has been an increase in interest in using mare's milk in the human nutrition, cosmetics, and pharmaceutical industries **(Stuparu, Strugariu & Oroian, 2016)**. Depending on the stage of lactation, age, and milk management, milk production, content, and sanitary quality might vary **(Pikul & Wójtowski, 2008; Markiewicz-Kszycka *et al.*, 2013)**. Depending on the breed, daily production values might range from 10 to 30 kg (2 to 3.5 kg of milk per 100 kg of live weight) **(Doreau & Martin-Rosset, 2002).** According to reports, lactation lasts between five and eight months **(Feist & McCullough, 1976).** Due to its nutritional qualities, mare's milk is used as a low-allergenic alternative to cow's milk, as a substitute for human and cow's milk for premature new-borns, and as a treatment for a number of illnesses and disorders. It is beneficial against skin diseases, supports stomach function, the immune system, and overall physical health, maintains the vitality of muscles, joints, and bones, and helps with metabolism issues, liver diseases, cardiovascular diseases, and can also treat several other illnesses and diseases **(Centoducati *et al.*, 2012; Polidori & Vincenzetti, 2013; Stuparu, Strugariu and Oroian, 2016; Jastrzębska *et al.*, 2017).**

The composition and nutritional qualities of milk vary greatly among animals depending on the habitat, evolution, genetics, and dietary management of the animal **(Nikkhah, 2012).** Age, foaling order, mare live weight, nutrition, environmental conditions, and lactation stage, with the latter being the most significant, can all have an impact on the content of an animal's milk. Except for lactose, which increases at the beginning and declines at the conclusion of lactation, the concentration of the major milk ingredients decreases as the lactation period progresses **(Santos *et al.*, 2005).** Additionally, breed and genetics may have an impact on the protein, fat, and lactose content of milk, according to **Costa (2013).**

In terms of nutritional content, the gross composition of mare's, human, and cow's milk (Table 1) reveals striking quantitative variances. Compared to human and cow's milk, mare's milk has significantly less fat (around 12 vs. 36 and 36 g.kg^{-1}, respectively), but its lactose level (about 64 g.kg^{-1}) is comparable to that of human milk and higher than that of cow's milk (67 and 50 g.kg^{-1}, respectively). Comparing human and mare milk to cow milk, however, reveals that both have lower levels of protein and basic minerals **(Potonik *et al.*, 2011, Hanuš *et al.*, 2018, Barreto *et al.*, 2020, Pietrzak-Fieko & Kamelska-Sadowska, 2020)**. The mineral content of mare's milk is almost twice lower than that of cow's milk and is closer to the mineral content of breast milk **(Uniacke-Lowe, Huppertz & Fox, 2010; Potocnik *et al.*, 2011; Pieszka *et al.*, 2016).**

Table 1 Gross composition of mare milk in comparison to human and cow milk **(Malacarne *et al.*, 2002)**

	Mare	Human	Cow
Fat (g.kg^{-1})	12.1 (5 – 20)	36.4 (35 – 40)	36.1 (35 – 39)
Crude protein (g.kg^{-1})	21.4 (15 – 28)	14.2 (9 – 17)	32.5 (31 – 38)
Lactose (g.kg^{-1})	63.7 (58 – 70)	67.0 (63 – 70)	48.8 (44 – 49)
Ash (g.kg^{-1})	4.2 (3 – 5)	2.2 (2 – 3)	7.6 (7 – 8)
Gross energy (kcal.kg^{-1})	480 (390 – 550)	677 (650 – 700)	674 (650 – 720)

In comparison to cow's milk, mare's milk contains a much lower concentration of short- and long-chain saturated fatty acids, a similar concentration of monounsaturated fatty acids, especially palmitoleic (C16:1) and oleic (C18:1) acids, and a much higher concentration of polyunsaturated fatty acids, especially linoleic (C18:2) and linolenic (C18:3) acids. The ratio of unsaturated to saturated fatty acids in mares' milk fat is ca. 1.3 compared to 0.45 in cows' milk fat **(Solaroli, Pagliarini & Peri, 1993a; Barreto *et al.*, 2019).** Triglycerides from human, cow, and mare's milk make up around 80% of the total lipids; phospholipids and sterols make up 5%; and free fatty acids make up 10% of the total lipids **(Marangoni & Narine, 2002).** Mare's milk has higher content of sterols than human and cow's milk, and the sterol fractions of all three contain some cholesterol (Jensen et al., 1990). The range of cholesterol in mare's milk is 50 to 88 mg.L^{-1} **(Malacarne *et al.*, 2002; Barello *et al.*, 2008; Pilarczyk *et al.*, 2015).**

In comparison to human milk, which is like cow's milk in terms of energy supply, mare's milk contains less energy **(Doreau & Boulot, 1989; Mariani, Martuzzi & Catalano, 1993; Salimei, 1999)** (Table 1). Mare's milk has more vitamin C and less fat-soluble vitamins (A, D_3, and E) than cow's milk, but far less vitamin C than human milk **(Salamon *et al.*, 2009).** Mare's milk has a higher concentration of vitamins soluble in fat than cow's milk because to its approximately one-third lower fat content **(Csapo *et al.*, 1995).** Mare's milk also contains a significant amount of vitamin C (428 times more than in cows' milk) **(Solaroli, Pagliarini & Peri, 1993a)**. Higher concentrations of functional substances, hormones, immunoglobulins, and nitrogen compounds with antibacterial activity, such as lysozyme and lactoferrin, are found in the mare's milk **(Chifalo, Drogoul & Salimei, 2006; Vincenzetti *et al.*, 2008)**. These qualities lead to the prevalent

belief that mare milk is better for human nourishment than cows' milk **(Marconi & Panfili, 1998; Malacarne *et al*., 2002).**
Mare's milk differs from cow's milk in terms by organoleptically. It is clear, whitish, and sweeter milk, making it like human milk **(Curadi *et al*., 2000; Potocnik *et al*., 2011)**. Mare milk smells like hay and has a distinctive coconut flavour **(Heger, 1988; Di Cagno *et al*., 2004).** In terms of the physical properties, the density, osmotic pressure, and freezing point of mare and cow milk are comparable. Mares' milk is less viscous, titratable acidic, and electrically conductible than cows' milk **(Solaroli, Pagliarini & Peri, 1993b).** The pH, ca. 7, is slightly higher than that of cows' milk, human milk **(Solaroli, Pagliarini & Peri, 1993b).**
The aim of present study was to determine the selected physicochemical properties of Lipizzaner mare's milk during the lactation period.

MATERIAL AND METHODS

Material

The monitored Lipizzaner mares (n=6) came from a private breeder and were fed the same rations per mare/per day during the entire lactation period:
• 3 kg of oats,
• 4 kg of hay,
• 2 kg of bedding straw,
• pasture and water *ad libidum.*
Milk samples were collected at monthly intervals after two weeks from the foaling of the mares. The mare's milk was obtained by one made milking in the morning between seven and eight o'clock into sterile sample bottles in a volume of 500 ml. Subsequently, it was stored in a mobile cooling device (cooled below 10 °C) and transported to the laboratory of the Institute of Food, SUA in Nitra.

Methods

As part of the physicochemical analysis, we determined the following selected physicochemical indicators:
- density by lactodensimeter device at a temperature of + 20 °C. The results were expressed in units $kg.m^{-3}$
- electrical conductivity by conductometer Mini-digi Conductivity Meter OK-113 expressed in units $mS.cm^{-1}$,
- the pH was measured directly in the brine with a Gryf 209L pH meter (Gryf, Czech Republic) equipped.
- the measurement of titratable acidity was according to the method of **Cvak *et al*. (1992).** One hundred ml of milk and 1 ml of 2 % w/v phenolphthalein solution were pipetted into an Erlenmeyer flask. The contents were titrated with 0,25 M NaOH solution to give a faint pink colour which did not disappear for 2 minutes. The acidification of the milk (°SH) is equal to the volume of base consumed for neutralisation.
- calcium content was determined by complexometric titration with EDTA according to the method of **Cvak *et al*. (1992**).
- Fat was determined by butyrometric method according to COSMT 2001,
- dry matter of milk was determined as the proportion after drying the samples at a temperature of 102 °C to a constant weight.
- polarimeter (PolamatA) was used for determination of lactose (optical active substance) in milk. The preparation and calculation were done according to the method of **Cvak *et al.* (1992).**

Statistical analysis

Results of the experiment were evaluated by the statistical program SAS 9.3 using the application EnterpriseGuide 4.2.

RESULTS AND DISCUSSION

The Lipizzaner horse is a native breed and one of the oldest cultured horse breeds, having originated in Lipica, Slovenia, in the 16th century. It is mostly employed as a leisure horse for riding, for sport dressage, for traditional dressage, and for pulling carriages. The majority of Lipizzaners are raised by private breeders in 19 nations and on nine state studs in Central and Eastern Europe, according to Lipizzaner International Federation **(Kai *et al*., 2019)**. According to **Potonik (2012),** milk production and processing might be used to preserve equine breeding while also increasing its economic value.
The results for selected physicochemical properties of Lipizzaner mare's milk are shown in Table 2.

Table 2 Physicochemical properties of Lipizzaner mare's milk during lactation period (n=6)

	M1	M2	M3	M4	M5	M6	Average
TA (°SH)	3.07±0.33	2.97±0.15	2.07±0.10	1.67±0.10	1.43±0.20	1.27±0.10	2.07±0.74
EC ($mS.cm^{-1}$)	1.35±0.72	1.70±0.80	1.44±1.10	1.32±1.14	1.53±2.88	2.29±3.05	1.61±3.80
D ($kg.m^{-3}$)	1029.17±0.68	1031.58±0.49	1031.17±0.68	1031.25±0.88	1030.17±1.17	1027.50±0.84	1028.64±3.98
LC (%)	6.57±0.21	6.55±0.41	6.84±0.36	6.71±0.35	6.75±0.29	6.81±0.77	6.71±0.12
pH	7.16±0.07	7.12±0.08	7.07±0.17	7.22±0.09	7.36±0.21	7.54±0.15	7.25±0.21
DM (%)	9.77±0.18	9.71±0.21	9.73±0.18	9.51±0.14	9.37±0.23	9.24±0.14	9.55±0.22
FC (%)	1.35±0.40	1.06±0.66	0.92±0.04	0.78±0.62	0.62±0.27	0.76±0.32	0.92±0.26
CC ($mg.L^{-1}$)	120.63±6.46	114.33±8.89	104.96±8.46	93.42±7.88	95.30±22.49	101.52±19.39	105.03±16.14

Legend: Values shown as mean ± *SD* (standard deviation); M1 - M2 – the month of the lactation period, TA – titratable acidity, EC – electrical conductivity, D – density, LC – lactose content, DM – dry matter, FC – fat content, CC – calcium content

In our study, the average value of titratable acidity of Lipizzaner mare milk ranged during the lactation period from 3.07 °SH (M1) to 1.27 °SH (M6). **Mariani *et al.* (2001)** stated titratable acidity in Haflinger nurse mare milk throughout 6 lactation months from 6.08 °SH to 2.01 °SH. The Average titratable acidity 2.51 °SH mare milk of Croatian Coldblood horse breed was observed by **Čagalj *et al.* (2014),** and 2.65 °SH of Polish Coldblood horse breed in mid-lactation **Cais-Sokolińska *et al.* (2018**). The average pH value of 7.25 was determined. The publications of some authors emphasized the significant pH fluctuations in mare's milk. **Mariani *et al.* (2001)** stated that, the pH of mare's milk steadily increased throughout lactation, rising from 6.6 (right after delivery) to 6.9 (on the 20th day), or up to 7.1. (on the 180th day).
While **Pagliarini *et al. (*1993)** suggested a pH value of 7.2, **Kücükcetin *et al.* (2003)** stated that the average pH of mare's milk is 7.0. The pH value of colostrum, which was 6.69 according to **Cosentino *et al*. (2017),** fluctuated dramatically throughout lactation; the value seen at 15 days (6.85) was noticeably lower than those seen at 90 and 150 days. **Tomczynski, Roman & Szybinska (1999)** stated the average pH value of milk from mares of the Wielkopolska and Konik Polski breeds during lactation was 6.7 and 6.4, respectively. The pH, ca. 7, is slightly higher than that of cows' milk, like human milk **(Solaroli, Pagliarini & Peri, 1993b).** The variable pH values are probably the result of different contents of protein and salt concentrations **(Čagalj *et al.*, 2014)**.
Due to the presence of salts and other charged substances, milk exhibits conductive qualities **(Fox & McSweeney, 1998).** Milk from infected quarters has higher concentration of sodium and chloride ion concentrations, which leads to increase the electrical conductivity of the milk **(Kitchen, 1981).** The stage of lactation, the time of year, and the feed are just a few of the variables that can impact electrical conductivity. Additionally, elements like milk's pH, temperature, and fat content might affect how electrical conductivity is measured. According to **Danków *et al.* (2006)**, the mean electrical conductivity of mare milk during lactation was 4.0 $mS.cm^{-1}$, whereas according to **Kuy (1998)**, the electrical conductivity of mare's milk ranged from 1.9 to 4.3 $mS.cm^{-1}$. During lactation, the average electrical conductivity of tested mare milk samples was 1.60 $mS.cm^{-1}$.
The density of the mares' and cows' milk is comparable **(Solaroli, Pagliarini & Peri, 1993b).** Tested milk samples from Lipizzaner mares had an average density of 1028.64 $kg.m^{-3}$ throughout lactation. According to **Uniacke-Lowe (2011)**, the much higher protein content of mare's colostrum accounts for its higher density (1028 to 1035 $kg.m^{-3}$) compared to mare's milk. In the first 12 hours after birth, density is at its maximum and rapidly declines. According to **Cais-Sokoliska *et al*. (2018),** Polish Coldblood mares had a milk density of 1 037 $kg.m^{-3}$. The density of Haflinger mare milk at 15 °C ranged from 1.0377 to 1.0245 $g.cm^{-3}$, according to **Mariani et al. (2001).** In a study **Nurliyani, Aryuanti & Indratiningsih (2015)** mare milk had a density of 1.033 $g.cm^{-3}$. **Summer *et al.* (2000)** stated similar findings, the density of mare milk at 15°C in the 20–180-day lactation stage ranged from 1.0345 to 1.0361 $g.cm^{-3}$. With rising temperatures, density of milk decreases, milk loses density primarily due to water expansion. Fat, protein, lactose, and other solids compositions were the most important elements in density estimation **(Ueda, 1999).**
Mare's milk has a significantly different major component composition from the milk of other domesticated animals. Mare milk may be blended with goat, sheep, and, most importantly, cow milk for a variety of practical reasons (health, functional, technological, and marketing) **(Kckcetin *et al*., 2003; Cais-Sokoliska, Wójtowski & Pikul, 2016a,b; Cais-Sokoliska *et al*., 2017)**. The first month of lactation had the highest dry matter content (9.77%) and the sixth month of lactation had the lowest (9.24%). Additionally, in the 1st month of lactation, milk samples contained, on the average more total solids in relation to the rest of lactation. According to **Gibbs *et al.* (1982), Schryver *et al.* (1986b), and Salimei, Varisco, and Rosi (2002)**, the dry matter content of mature milk ranges from 10 to 12% and declines over the course of lactation. **Nurliyani, Friska Aryuanti &**

Indratiningsih (2015) reported that their study's average total solid of mare milk was 10%. The results of our study are comparable with the study by **Cagalj *et al.* (2014)** that the total solid of Croatian Coldblood horse breed mare milk at 3 – 11 months of lactation is around 8.91 – 10.86% and according to **Summer *et al.* (2000),** the total solid of mare milk at 20 – 180-day lactation stage was around 9.21 – 10.39%.

The most changeable component of milk is milk fat. Compared to cow's milk (3.61%) and human milk (3.64%), mare's milk has less fat (1.21%) **(Malacarne *et al*., 2002).** In our study, was an average milk fat content of 0.92%, ranging from 0.62% (M5) to 1.35% (M1). In contrast to **Kai *et al.* (2019),** who claimed that the average milk fat content of Lipizzaner mares was 4.0%, **Gregi *et al.* (2022)** reported that the average milk fat content was 1.18%. The average amount of fat in mares' milk is between 1 and 1.5% **(Gibbs *et al*., 1982; Csapó *et al*., 1995; Malacarne *et al*., 2002).** Most of the authors reported lower milk fat concentrations in the milk from Arabian mares (1.16%) **(Bornaz *et al*., 2010)**; Halfinger mares (1.04%) **(Salamon *et al*., 2009)**; Hucul mares (0.92%) **(Pieszka *et al.*, 2011)**; and Wielkopolski mares (0.8%) (**Pieszka, Huszczyski & Szeptalin, 2011)**. On the other hand, **Santos & Silvestre (2008**) found that the average fat percentage in Lusitano mares was 5.9%, whereas **Centoducati *et al.* (2012)** stated that the average fat content in Italian Draft mares was 11.07%. In the research conducted by **Doreau & Martuzzi (2006)** the content of milk fat in mare's milk decreased during lactation, from the initial value of 1.5 – 2.5% to 0.5 – 1.5% at the end of lactation. According to **Doreau *et al.* (1992),** compared to milk from mares receiving the more concentrated feed, milk from those feds with fodder that contained a high percentage of hay contained more fat and protein and less lactose. The average values of fat are also lower and higher **(Bouwman & Van der Schee, 1978; Santos & Silvestre, 2008)**, with diets high in concentrate providing the lowest average values. During lactation period, the fat content also changes, with a general decline in fat content from colostrum until the end of lactation **(Gibbs *et al*., 1982; Doreau &Boulot, 1989).**

Lactose, the primary carbohydrate in milk, has an average amount of 6.26%, which is significantly higher than that of cow's, goat's, or sheep's milk. Mare's milk tastes significantly sweeter than other milk kinds typically designed for human consumption because of its high lactose concentration **(Solaroli, Pagliarini, & Peri, 1993; Curadi *et al*., 2000; Di Cagno *et al*., 2004; Potonik *et al*., 2011; Jastrzbska *et al*., 2017).**

Mare's milk is distinguished by having a lot of lactose (from 58 to 70 $g.kg^{-1}$). The average lactose level of 6.71% in the present study was somewhat higher than the 6.56% average lactose content of Lipizzaner horse milk reported by **Potonik *et al.* (2011).** A mare's milk is known for its high lactose content, which remains consistent during lactation. According to **Kai *et al*. (2019),** the average lactose content was 63.2 $g.kg^{-1}$. Compared to the average lactose content reported by **Smolders, Veen, and Polanem (1990)** for light mares (62.0 $g.kg^{-1}$) and **Santos & Silvestre (2008)** for Lusitano mares (60.8 $g.kg^{-1}$), our study's average lactose content was slightly higher. In a study by **Gregić *et al*. (2022)**, the lactose values ranged from 5.71 to 6.88%. **Czyzak-Runowska *et al.* (2021)** stated that lactose content was 6.44% on average. The amount of lactose throughout lactation may increase or stay the same **(Oftedal *et al*., 1983).** The average percentage lactose value of full mare's milk in the middle of the lactation period was 6.4 – 6.91% **(Oftedal *et al*., 1983; Nikkhah, 2012; Pieszka & Łuszczyński, 2013; Salimei & Fantuz, 2012)**. According to **Teichert *et al*. (2020)**, the lactose concentration of an Arabian mare's milk was 61.5 $g.L^{-1}$, compared to 65 $g.L^{-1}$ for Polish Coldblood mares, 70.2 $g.L^{-1}$ for Quarter Horses, 64.7 $g.L^{-1}$ for Halfinger, and 67.8 $g.L^{-1}$ for Rapid Heavy Draft **(Pelizzola *et al*., 2006).**

Mare milk has roughly four times as much magnesium and calcium as human milk but less than cow's or goat's milk. Though probably lower than that of human milk, the ratio of calcium to phosphorus in mare's milk is significantly higher than that of cow's or goat's milk **(Holmes *et al*., 1947).** The calcium content in samples of Lipizzaner mare milk during the lactation period was ranging from 93.42 $mg.L^{-1}$ (M4) to 120.63 $mg.L^{-1}$ (M1). The mineral content may vary depending on diet. **Doreau *et al.* (1992)** obtained milk containing more calcium and phosphorus from a diet rich in forage than from a diet rich in concentrate. The calcium content in mare milk is 50 – 135 $mg.100ml^{-1}$ **(Claeys et al., 2014). Holmes *et al.* (1947)** reported that mare's milk contains 102 mg of calcium per liter. The average calcium content in the Quarter Horse mare breed during lactation was 787 $mg.kg^{-1}$ **(Anderson, 1991),** Przewalski, Thoroughbred, and Shetland pony mare breed 804 $mg.kg^{-1}$, 811 $mg.kg^{-1}$, 857 $mg.kg^{-1}$, respectively (**Schryver *et al*., 1986b**). **Martuzzi *et al.* (1998)** stated 1220 $mg.kg^{-1}$ of calcium in the Bardigiano mare breed, and **Summer *et al.* (2004)** average content of 802 $mg.kg^{-1}$ in the Haflinger mare breed.

CONCLUSION

With the growing interest in new, ethnic alternative dairy products, a few research teams are dealing with the use of mare's milk. Its nutritional composition, as well as its properties, can predestinate it for human nutrition as well as the production of a certain group of dairy products. However, information on its physicochemical and microbiological properties is needed to apply the quality aspects and standardization of technological processes. Therefore, this work was aimed to determine the selected physicochemical parameters of milk originating from the Lipizzaner mare's breed. However, to obtain more information about the Lipizzaner mare's milk and to define its quality aspects, it is necessary to carry out further studies.

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