ORIGINAL SCIENTIFIC PAPER | UDK: 637.112.2 | DOI: 10.15567/MLJEKARSTVO.2019.0204
RECEIVED: 11.09.2018. | ACCEPTED: 08.03.2019.

Microbiological quality of mare's milk and trends in chemical composition by comparison of different analytical methods

Ana Končurat[1], Lidija Kozačinski[2], Nina Bilandžić[3], Željka Cvrtila[2], Tomislav Sukalić[1], Marija Sedak[3], Miroslav Benić[4]*

[1]Croatian veterinary institute Zagreb, Department Križevci, Zakmardijeva 10, 48260 Križevci, Croatia
[2]University of Zagreb, Faculty of veterinary medicine, Department of hygiene and technology of foodstuffs, Heinzelova 55, 10000 Zagreb, Croatia
[3]Croatian veterinary institute Zagreb, Department of veterinary public health, Savska 143, 10000 Zagreb, Croatia
[4]Croatian veterinary institute Zagreb, Department for bacteriology and parasitology, Savska 143, 10000 Zagreb, Croatia
*Corresponding author: E-mail: koncurat.vzk@veinst.hr

Abstract

In this study the quality of Croatian coldblooded mare's milk during six months lactation period was investigated. Samples of milk were collected throughout six month's lactation, from 22 mares and included three consecutive lactations. Physicochemical properties (pH, °SH, density) and chemical composition of raw mare milk have been studied. Fat, lactose, protein and solid non fat contents were analysed by infrared spectrophotometry and by conventional methods. Somatic cell count and microbiological parametres such as the total bacterial count, *Enterobacteriaceae*, coagulase positive staphylococci as well as presence of pathogens, *Salmonella* species and *Listeria monocytogenes* were also researched. Results are presented as comparison of standard and instrumental methods for chemical analysis (fat, protein, solid non fat, lactose). The mean values are presented as trends during lactation. Results were analysed by Stata 10.0. For results obtained by using different methods there were significant differences between methods for milk fat on 10th day, lactose content on 10th and 60th day, and total solids non fat on 60th day of lactation. Values of milk fat, protein, lactose and solids non fat obtained on the 40th, 60th, 120th and 180th days of lactation by IR spectrometry were compared with the value obtained on the 10th day of lactation by the same method (IR spectrometry). Milk of the Croatian coldblooded mares showed stabile chemical composition for all ingredients except lactose (p=0.0001), and high microbiological quality throughout the lactation period.

Key words: **mare milk, composition, quality, method comparison**

Introduction

Previous studies demonstrated favourable composition of mare's milk in diet (Uniacke-Lowe et al., 2010) and confirmed mare's milk as a food with certain health benefits (Ellinger et al., 2002; Salimei and Fantuz, 2012). With respect to many components mare's milk is more similar to human milk than to the milk of ruminants (Potočnik et al.,

2011.). Regardless of the high lactose concentration, it can be consumed either fresh or fermented (Uniacke-Lowe, 2011). Because of its specific chemical and nutritional characteristics, mare milk receives more attention in human nutrition and the demand for mare milk and its products has increased in European countries. Microbiological status of raw milk is one of the indicators of milk hygiene and quality. According to its composition, milk is an extremely valuable foodstuff and suitable medium for microbial growth. Recent research shows data for somatic cell and total bacteria count in raw mare milk, which are generally lower than in conventional dairy species (Danków et al., 2006; Markiewicz-Keszycka et al., 2013), indicating good hygiene of milking. This study provides trend monitoring of chemical composition through the lactation period. Moreover, by comparing the results for the same chemical compound obtained by different methods, the suitability of several instrumental methods were examined. With this study, an expanded microbiological research was conducted to determine the status of raw mare's milk as hygienic foodstuff.

Material and methods

Animals and sample collection

Croatian cold blooded, clinically healthy mares (n 22), kept in similar conditions, from five owners of the Zagreb County were monitored. Mares were kept in barns, and some of them, stayed during the summer on common pastures in Lonjsko Polje nature park. Mares were aged 3-12 years, foaled from February to early May. Meal consisted of grains and meadow hay. Foals was weaned at the age of 6-7 months. Mares were not exploited as dairy animals and milking was carried out by the owner of the animal. Samples of milk were collected throughout six month's lactation, on approximately 10th, 40th, 60th, 120th and 180th day. Sampling was carried out through three years and in some animals consecutive lactations were included. The amount of the sampled milk was different, ranging from a minimum of 50 mL to maximum of 750 mL. Usually, 200-250 mL of milk was obtained. Two hours before sampling, foals stayed close to the mares, but on a short yarn, which prevented suckling and provided sufficient quantities of milk for analyses. Before milking, udder and hands were washed with warm water and disinfected with 70 % ethanol. First few jets of milk were rejected. Samples for bacteriological examination were taken in sterile bacteriological test tubes, in the amount of 5-10 mL. For determination of the somatic cell count potassium dichromate has been used as preservative. Samples for microbiological and chemical research were transported on ice and kept at +2 to +8 °C.

Experimental procedures

Microbiological analysis of mare's milk: mare milk was analysed in the laboratory within 12 hours after sampling. Sample preparation, basic and decimal dilutions for microbiological examination were carried out according to HRN EN ISO 6887-5:2011. For determination of the total number of microorganisms, technique of colony count at 30 °C according to HRN EN ISO 4833:2008 has been used. The number of colonies was calculated according to HRN EN ISO 7218:2008 standard. Bacteria belonging to the family *Enterobacteriaceae* were determined by horizontal HRN ISO 21528-2:2008 method. As confirmative biochemical tests, oxidase and glucose fermentation tests were used. Coagulase-positive staphylococci (*Staphylococcus aureus* and other species) were counted according to HRN EN ISO 6888-1:2004. The absence of *Salmonella* was established by HRN EN ISO 6579:2003/Ispr.1:2008 and determination of *Listeria monocytogenes* in accordance with HRN EN ISO 11290-1:1999/A1:2008 standard. To exclude infection of the udder, milk samples were plated on Columbia agar with 5 % defibrinated sheep blood and incubated at 37 °C for 24 h. Biochemical identification was conducted with API system (BioMerieux), morphological characteristics were determined by microscope, and where appropriate, individual enzymes (catalase, oxidase, coagulase) were determined.

The *Somatic cell count* (SCC) was determined in prewarmed samples by using a Fossomatic™ 5000 (FossElectric, Denmark). So far, the instrument was used only for SCC determination in the fresh or preserved samples of cows' milk.

Chemical analyses were performed by MilcoScan 4000 (FossElectric, Denmark) and by conventional methods for same parameters (milk fat, proteins, sugar and total solids non fat): as follows butyrometric Gerber method HRN ISO 2446:2009; internal Kjeldahl method; internal Loof-Schoorl method; drying at 102 °C.

pH value (potentiometric), °SH (titrable acidity according to Soxhlet-Henkel), density and ash at 550 °C were also determined.

Statistical analysis was performed by the program Stata10.0 (STATA Corp. USA). Results are shown as median, minimum and maximum values, or as an arithmetic mean with a standard deviation according to data distribution. Data distribution was checked using Shapiro-Wilk test. The obtained values were compared by the paired t-test, or the paired test for non-parametric values (Wilcoxon sign rank test). For the comparison of more than two groups, the Kruskall-Wallis test or analysis of variance was used, depending on the data distribution (Petrie and Watson, 2013).

Results and discussion

Fat contents determined by two different methods were statistically different only on the 10th day of lactation (Table 1). Fat is a milk component that varies the most in relation to other ingredients, depending on time and the sampling method. Therefore, the observed differences on the 10th day of lactation may be affected by milking interruption before its end because of mare restlessness, or interruption of the milk secretion due to emotional distress which was indicated by other authors (Caroprese et al., 2007). Most of the mares in this study were not accustomed to milking, so even skill and experience of the milker could have affected the results. Navratilova et al. (2018) found that milk fat content is significantly ($p<0.01$) affected by the lactation stage, breed and nutrition. A wide range of results from 2.9 g L^{-1} to 30.1 g L^{-1} and a decreasing value towards the end of the lacatation for milk fat are in agreement with other similar researches performed in Croatia (Ernoić, 1998; Čagalj et al., 2014).

The observed differences for protein content determined by the two different methods were not statistically significant (Table 2). Similarly to the findings of Martuzzi et al. (1995), this research has revealed that the protein content in mare's milk during the whole lactation period was gradually falling, whereas the initial content of 23 g L^{-1} at the beginning of lactation decreased to 16.2 gL^{-1} on the 180th day of lactation.

As well as other indicators of mare's milk quality, lactose content was analysed and the results were compared by two methods throughout the lactation (Table 3). Mare's milk on average contained 62.9 g L^{-1} lactose on the 10th day. Similar results for lactose amount were published by Mariani et al. (2001) for milk from Haflinger mares (63.6-68.8 g L^{-1}). Lactose increases towards the end of lactation, what is consistent with other published results (Mariani et al. 2001; Martuzzi et al., 2004). The observed differences in lactose values measured by two methods, were statistically significant on the 10th ($p = 0.0093$) and the 60th day ($p = 0.008$).

TABLE 1. Fat content in mare milk (%) determined by infrared spectrometry (MilkoScan 4000) and by Gerber method

d	method	n	median	min	max	p
10	A	17	0.96	0.38	2.13	0.0012
	B	30	1.525	0.42	3.01	
40	A	25	0.93	0.44	2.76	0.9584
	B	29	1.2	0.45	2.7	
60	A	22	0.595	0.29	1.73	0.7582
	B	28	0.89	0.29	2.3	
120	A	9	0.87	0.32	2.17	0.4631
	B	27	0.66	0.3	2.0	
180	A	8	0.57	0.3	0.89	0.2733
	B	26	0.505	0.33	1.9	

A: infrared spectrometry; B: Gerber method; n: number of samples; d: day of lactation; p: p-value

TABLE 2. Protein content in mare milk (%) determined by infrared spectrometry (MilkoScan 4000) and by Kjeldahl method

d	method	n	average	sd	p
10	A	17	2.21	0.54	0.7553
	B	26	2.30	0.51	
40	A	25	2.20	0.47	0.1019
	B	19	2.11	0.36	
60	A	21	1.98	0.39	0.1601
	B	15	1.87	0.26	
120	A	9	1.95	0.35	0.2871
	B	20	1.83	0.29	
180	A	8	1.69	0.14	0.4737
	B	18	1.62	0.16	

A: infrared spectrometry; B: Kjeldahl method; n: number of samples; d: day of lactation; sd: standard deviation; p: p-value

TABLE 3. Lactose content in mare milk (%) determined by infrared spectrometry (MilkoScan 4000) and the Loof- Schoorl method

d	method	n	average	sd	p
10	A	17	6.35	0.22	0.0093
	B	19	6.29	0.20	
40	A	23	6.47	0.23	0.2276
	B	14	6.41	0.26	
60	A	24	6.57	0.28	0.0080
	B	11	6.49	0.32	
120	A	9	6.56	0.17	1
	B	18	6.57	0.19	
180	A	8	6.70	0.16	0.4780
	B	13	6.66	0.18	

A: infrared spectrometry; B: Loof-Schoorl method; n: number of samples; d: day of lactation; sd: standard deviation, p: p-value

Since MilkoScan so far was validated for analysis of cow's milk samples with significantly lower lactose content, the difference obtained by the applied methods in this research is not unexpected. Further, the applied Loof-Schoorl method for the sugar extraction is a non-selective volumetric method for evaluation of all reducing sugars, so the observed differences could be related to the method applied.

The total solids non fat content in mare's milk is shown in Table 4. The observed differences in the content of dry matter, measured by refractometer and infrared spectroscopy, were significant on 60th day. Average proportions are in agreement with datapublished within other similar researches performed in Croatia (Ernoić, 1998; Čagalj et al., 2014).

TABLE 4. Total solids non fat content in mare milk (%) determined by infrared spectrometry (MilkoScan 4000) and by drying

d	method	n	median	min	max	p
10	A	17	9.15	8.77	10.19	0.8658
	B	10	9.415	8.8	10.92	
40	A	25	9.26	8.82	10.15	0.3264
	B	11	9.56	8.8	10.95	
60	A	21	9.2	9.1	9.94	0.0431
	B	6	9.115	9	9.49	
120	A	9	9.31	8.81	9.67	0.1088
	B	11	9.2	8.7	9.41	
180	A	8	9.22	8.81	9.53	0.1088
	B	9	9.9	8.8	9.5	

A: infrared spectrometry; B: drying; n: number of samples; d: day of lactation; p: p-value

TABLE 5. Ash content in mare milk (%)

d	n	average	sd	p
10	6	0.436	0.125	0.1113
40	7	0.408	0.063	
180	5	0.316	0.076	

n: number of samples; d: day of lactation; sd: standard deviation; p: p-value

TABLE 6. Density of mare milk

d	n	average	sd	p
10	7	1.0355	0.0018	0.8222
40	9	1.0325	0.0058	
60	4	1.0341	0.0018	
120	3	1.0340	0.0010	
180	4	1.0288	0.0061	

n: number of samples; d: day of lactation; sd: standard deviation; p: p-value

TABLE 7. Titratable acidity (°SH / 100mL) of mare milk

d	n	average	sd	p
40	7	2.605	0.58	0.9456
60	7	2.573	0.81	
180	6	2.691	0.51	

n: number of samples; d: day of lactation; sd: standard deviation, p: p-value

TABLE 8. pH value of mare milk

d	n	average	sd	p
10	8	6.86	0.07	0.0016
40	5	7.01	0.11	
60	5	7.04	0.11	
120	6	7.07	0.08	

n: number of samples; d: day of lactation; sd: standard deviation, p: p-value

The ash content measured in samples of mare's milk were on average 0.386 %, gradually falling towards the end of lactation and did not differ significantly (Table 5). Result on the 10th day (0.436±0.125) was slightly lower than concentrations of ash at the beginning of lactation. Similar finidngs were reported by Martuzzi et al. (1998) comparing the milk of Bardigiano and Italian saddle horse breeds, which were 0.498±0.096 % and 0.502±0.066 %, respectively.

Mare's milk density at 20 °C varied from 1.0355 to 1.0288 g cm^{-3} during the lactation (Table 6). Analysis of variance showed that the density value did not differ between individual measurements.

The arithmetic mean for titratable acidity according to Soxhlet-Henkel and pH during lactation period with the belonging p values are presented in Tables 7 and 8. Mariani et al. (2001) reported a pH 7.01 on the 40th day of lactation, which is identical to the result on the same lactation day obtained within the present study. Martuzzi et al. (1995) indicated the minimum and maximum pH of mare's milk from 6.58 to 7.8 during lactation, while the pH in this study ranged from 6.81 to 7.2 with significant differences (p=0.0016). The same authors state °SH 3.09±1.06, which is higher than the degree of acidity °SH 2.57-2.69 found in present study.

Comparing the measured values of milk components at day 40, 60, 120 and 180 with a values measured on the 10 th day of lactation (Table 9), lactose is the only parameter which shows statistically significant differences on the 40th and the 180th day of lactation. Other parameters do not differ statistically, so it can be concluded that the composition of mare's milk is stabile with respect to the most basic ingredients.

TABLE 9. Comparison of p-values of mare milk ingredients through lactation with 10th day

Milk ingredient	Day of lactation			
	40	60	120	180
Milk fat	0.9095	0.2719	0.7532	0.0796
Proteins	0.3054	0.3398	0.9344	0.1456
Lactose	0.0309	0.2413	0.0766	0.0001
Solids non fat	0.3786	0.3281	0.4631	0.5002

Very important indicator of udder health are somatic cells, whose type differs, depending on the functional state of the mammary gland and its response to stimuli from the environment. Compared to milk of other dairy animal species, somatic cell count in mare milk is significantly lower (Danków et al., 2006). Similarly to Martuzzi et al. (1998), result for somatic cell count in this research on the tenth day of lactation is 4.04 $\log_{10}$ and 4.53 $\log_{10}$ on the 120th day. Results on 40th, 60th, 120th and 180th day were compared with the value on the 10th day and observed differences were not statistically significant (Table 10). Jacobs (2004) highlights that the significant differences in the number of somatic cells due to the functional state of the udder. Thus, in stage of involution the number could rise to $4x10^5 mL^{-1}$. If the number of somatic cells exceeds $10^5 mL^{-1}$ that is considered to be pathological, or indicates diseases of the mammary gland in mares (Böhm et al. 2009). Somatic cell count may vary significantly, depending on the functional condition of the udder. A relatively small number of somatic cells in mare's milk is the result of frequent emptying and position of the udder, which is hidden in the inguinal region and protected from the lesions and consequently, infection.

The total number of microorganisms identified by horizontal method in mare's milk varied with statistically significant differences (Table 11). According to available data, there is a considerably lower number of microorganisms in the mare's milk, than in the milk of other dairy animal species. Compared with the results of other studies, values found in this research were lower. Thus, Markiewicz-Keszycka et al. (2013) reported mean value of $10,4x10^3$ cfu mL^{-1} and the maximum total bacteria count of $7,2x10^4$ cfu mL^{-1}, measured by flow cytometry. The maximum value obtained on Plate Count Agar (PCA) in this study was $3x10^4$ cfu mL^{-1} at the 120th day of lactation. The number of microorganisms in the fresh mare's milk presented by Zoege von Manteuffel (1989) was 741 cfu mL^{-1}, which is in agreement with this research. The established low values of the total number of colonies per milliliter of milk, indicate the good udder health, as well as a satisfactory hygiene during milk sampling.

TABLE 10. Somatic cell count ($x10^3$) per mL in mare milk during lactation

d	n	median	min	max	p
10	17	11	6	29	-
40	25	16	2	50	0.6082
60	22	14	5	36	0.3460
120	9	34	8	232	0.0747
180	8	10.5	2	45	0.5862

n: number of samples; d: day of lactation; p: p-value (Wilcoxon matched pair signed rank test)

TABLE 11. Total bacterial count (cfu mL-1) in mare milk during lactation

d	n	median	min	max	p
10	9	150	20	1200	0.0127
40	12	450	9	5200	
60	15	1026	51	9005	
120	11	1800	100	30009	
180	14	37	7	15003	

n: number of samples; d: day of lactation; p: p-value (Kruskall Wallis)

TABLE 12. Number of *Enterobacteriaceae* (cfu mL^{-1}) in mare milk during lactation

d	n	median	min	max	p
10	9	1	0	33	0.0334
40	12	1		45	
60	15	12		108	
120	11	12		314	
180	14	1		16	

n: number of samples; d: day of lactation; p: p-value (Kruskall Wallis)

According to previous data of microbiological quality of mare's milk, the obtained results could be compared with Furlič (2011). The number of coliforms in that paper was only 13 cfu mL^{-1}, while the median of *Enterobacteriaceae* in the present study was 12 cfu mL^{-1}. Czyzak-Runowska et al. (2018) did not detect any coliforms on Chromocult®Coliform Agar in fresh mare milk samples. The maximum number of *Enterobacteriaceae* (314 cfu mL^{-1}) was not unexpected considering the stage of lactation (day 120), the summer season and the sampling on common pasture. Since the analysed samples were fresh raw milk, that number is still very low (Table 12).

Mare milk analysis on coagulase-positive staphylococci, *Salmonella* spp. and *Listeria* spp. ended with negative results in total of 25 investigated samples.

Mastitis in mares occurs rarely and clinical mastitis is manifested with pain, swelling, fever, ventral edema and dullness. Detection of pathogens is possible even in clinically healthy mares (Jacobs, 2004), so our finding of potentially pathogenic microorganisms (*Streptococcus dysgalactiae equi, Staphylococcus capitis, Staphylococcus equorum, Staphylococcus aureus*) in few individual samples of milk wasn't recognized as mastitis.

By following the entire lactation it is possible to determine changes in milk composition. Mariani et al. (2001) reported significant changes in the composition of mare's milk from the 4th to the 180th day of lactation. Naert et al. (2013) estimated the heterogeneity of mare's milk composition in Flanders, with significant between-farm and within-farm variations.

First analysis in this research were conducted at the 10th day when colostrum period with most drastic changes ended and milk composition is stabilized, so the first measured values were taken as basic values for comparison.

By comparing the statistical p-values of the individual components of milk with a p-value of the 10th day, results suggest statistically significant differences in lactose content. So, the stability of mare's milk chemical composition during the whole lactation was demonstrated considering the content of milk fat, proteins and solids non fat.

Conclusions

The most significant Croatian cold-blooded mare's milk characteristics, with respect to cow's milk, are the low content of fat and protein as well as the high lactose content. Most milk ingredients were constant during the six month lactation peroid, but lactose varied significantly towards the end of lactation.

Fresh mare's milk is characterized by a low total bacterial count and low somatic cell count throughout the lactation. The results indicated that fresh mare's milk is of the high quality and a hygienic foodstuff.

Comparing the results of the main chemical composition of mare's milk performed by standard methods and an alternative method (MilkoScan), some values for fat, lactose and dry matter, in some stages of lactations are significantly different for two methods. The use of instrumental methods in mare's milk research is possible with previous validation of instrument.

Praćenje mikrobiološke kvalitete i promjena kemijskog sastava mlijeka kobila primjenom različitih analitičkih metoda

Sažetak

U ovom je radu istražena kvaliteta mlijeka hrvatskih hladnokrvnih kobila tijekom šestmjesečne laktacije. Uzorci mlijeka prikupljeni su od 22 kobile kroz tri uzastopne laktacije. Proučavane su fizikalno-kemijske osobine (pH, SH, gustoća) i kemijski sastav sirovog kobiljeg mlijeka. Mliječna mast, laktoza, bjelančevine i bezmasna suha tvar analizirani su infracrvenom spektrofotometrijom i konvencionalnim metodama. Utvrđen je broj somatskih stanica te ukupni broj bakterija. Od mikrobioloških parametara pretražene su *Enterobacteriaceae*, koagulazni pozitivni stafilokoki i utvrđeno je odsustvo patogena (*Salmonella* vrste i *Listeria monocytogenes*). Rezultati su prikazani kao usporedba standardnih i instrumentalnih metoda za kemijske analize (masti, bjelančevine, bezmasna suha tvar, laktoza). Srednje vrijednosti prikazane su kao trendovi tijekom laktacije. Rezultati su statistički analizirani pomoću programa Stata 10.0. Za rezultate dobivene primjenom različitih metoda zabilježene su statistički značajne razlike između metoda u udjelu mliječne masti 10. dana laktacije, sadržaj laktoze 10. i 60. dana te ukupne suhe tvari 60. dana laktacije. Vrijednosti mliječne masti, bjelančevina, laktoze i bezmasne suhe tvari dobivene 40., 60., 120. i 180. dana laktacije IR spektrometrijom uspoređene su s vrijednošću dobivenom 10. dana laktacije

istom metodom (IR spektrometrija). Mlijeko hrvatskih hladnokrvnih kobila pokazalo je stabilan kemijski sastav za sve sastojke osim laktoze (p=0.0001) i visoku mikrobiološku kvalitetu tijekom cijelog laktacijskog razdoblja.

Ključne riječi: **kobilje mlijeko, sastav, kvaliteta, usporedba metoda**

References

1. Böhm K.H., Klug, E., Jacobs, B.J. (2009): Mastitis in the mare - a long-term study on the incidence, clinical symptoms, diagnostics, microbiology, therapy and economic importance, as well as recommendations for veterinary practice. *Praktischer Tierarzt* 90, 842-849.
2. Caroprese, M., Albenzio, M., Marino, R., Muscio, A., Zezza, T., Sevi, A. (2007): Behavior, milk yield and milk composition of machine and hand-milked Murgese mares, *Journal of Dairy Science* 90, 2773-2777. https://doi.org/10.3168/jds.2006-603
3. Czyzak-Runowska G., Wojtowski, J., Niewiadomska, A., Markiewicz-Keszycka, M. (2018): Quality of fresh and stored mares' milk. *Mljekarstvo* 68 (2), 108-115. https://doi.org/10.15567/mljekarstvo.2018.0204
4. Čagalj, M., Brezovački, A., Mikulec, N., Antunac, N. (2014): Composition and properties of mare's milk of Croatian Coldblood horse breed. *Mljekarstvo* 64 (1), 3-11.
5. Danków, R., Wojtowski, J., Pikul, J., Niznikowski, R., Cais-Sokolinska, D. (2006): Effect of lactation on the hygiene quality and some milk physicochemical traits of the Wielkopolska mares. *Archiv Tierzucht* 49, 201-206, Special Issue.
6. Ellinger, S., Linscheid, K.P., Jahnecke, S., Goerlich, R., Enbergs, H. (2002): The effect of mare's milk consumption on functional elements of phagocytosis of human neutrophil granulocytes freom healthy volunteers. *Food and Agricultural Immunology* 14, 191-200. https://doi.org/10.1080/09540100220145000b
7. Ernoić, M. (1998): Proizvodnja kobiljeg mlijeka kao alternativa iskorištavanja konja u Hrvatskoj. Sveučilište u Zagrebu, Agronomski fakultet. Magistarski rad. Rukopis, 91 str.
8. Furlič, M. (2011): Sestava in posebnosti kobiljega mleka, diplomsko delo, Ljubljana, Biotehniška fakulteta, 65 str.
9. HRN EN ISO (1999/A1:2008): Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za dokazivanje prisutnosti i određivanje broja Listeria monocytogenes - 1. dio: Metoda dokazivanja - Amandman 1: Modifikacija podloge za izolaciju, test hemolize i uključivanje podataka o točnosti (Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method - Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data). No 11290-1. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
10. HRN EN ISO (2003/Ispr.1:2008): Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za dokazivanje prisutnosti Salmonella spp (Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp). No. 6579. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
11. HRN EN ISO (2004): Mikrobiologija hrane i stočne hrane - Horizontalni postupak brojenja koagulaza-pozitivnih stafilokoka (Staphylococcus aureus i druge vrste) - 1. dio: Postupak primjene Baird-Parkerove hranjive podloge na agaru (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphyloccoco (Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium). No. 6888-1. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
12. HRN EN ISO (2008): Mikrobiologija hrane i hrane za životinje - Opći zahtjevi i upute za mikrobiološka ispitivanja (Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations). No 7218. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
13. HRN ISO (2008): Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za dokazivanje prisutnosti i brojenje Enterobacteriaceae - 2. dio: Metoda određivanja broja kolonija (Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method). No 21528-2. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
14. HRN EN ISO (2008): Mikrobiologija hrane i stočne hrane - Horizontalna metoda za brojenje mikroorganizama - tehnika brojenja kolonija na 30 °C (Microbiology of food and animal feeding stuffs - Horizonthal method for the enumeration of microorganisms - Colony - count technique at 30 degrees C). No 4833. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
15. HRN EN ISO (2011): Mikrobiologija hrane i hrane za životinje - Priprema ispitnih uzoraka, osnovnih i decimalnih razrjeđenja za mikrobiološko ispitivanje - 5. dio: Posebna pravila za pripremu mlijeka i mliječnih proizvoda (Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5: Specific rules for the preparation of milk and milk products). No 6887 - 5. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.

16. HRN ISO (2009): Mlijeko - Određivanje udjela masti (Milk - Determination of fat content). No 2446. Hrvatski zavod za norme (Croatian Standards Institute). Zagreb.
17. Jacobs, B.J. (2004): Klinische, mikrobiologische und zytologische Untersuchungen zur Stutenmastitis eine Langzeitstudie. Hannover, Tierärztliche Hochschuke, Dissertation http://elib.tiho-hannover.de/dissertations/jacobsb_ss04.pdf
18. Mariani, P., Summer, A., Martuzzi, F., Formaggioni, P., Sabbioni, A., Catalano, A.L. (2001): Physicochemical properties, gross composition, energy value and nitrogen fractions of Haflinger nursing mare milk throught 6 lactation months. *Animal Research* 50, 415- 425. https://doi.org/10.1051/animres:2001140
19. Markiewicz-Keszycka, M., Wajtowski, J., Kuczyeska, B., Puppel, K., Czyčak-Runowska, G., Bagnicka, E., Strzaekowska, N., Jačwik, A., Krzyčewski, J. (2013): Chemical composition and whey protein fraction of late lactation mares'milk. *International Dairy Journal* 31 (2), 62- 64. https://doi.org/10.1016/j.idairyj.2013.02.006
20. Martuzzi, F., Mariani, P., Zanzucchi, G., Catalano, A.L. (1995): Composizione azotata e minerale e valore energetico del latte di cavalla nel corso dei primi 4 mesi di lattazione. *Rivista Societa Italiana di Ippologia* 1, 15-25.
21. Martuzzi, F., Summer, A., Catalano, A.L., Mariani, P. (1998): Macro- and micro-mineral elements of the milk and sialic acid bound to casein and to whey proteins in nursing mares of Bardigiano horse breed. *Annali della Facolta di Medicina Veterinaria, Universita di Parma* 18, 57-64.
22. Martuzzi, F., Summer, A., Formaggioni, P., Mariani, P. (2004): Milk of Italian Saddle and Haflinger nursing mares: physicochemical characteristics, nitrogen composition and mineral elements at the end of lactation. *Italian Journal of Animal Science* 3, 293- 299. https://doi.org/10.4081/ijas.2004.293
23. Naert, L., Vandevyvere, B., Verhoeven, G., Duchateau, L., De Smet, S., Coopman, F. (2013): Assesing heterogeneity of the composition of mare's milk in Flanders. *Vlaams Diergen Tijds*, 82, 23- 30.
24. Navratilova, P., Pospišil, J., Borkovcova, I., Kaniova, L., Dluhošova, S., Horakova, S. (2018): Contetnt of nutritionally important components in mare milk fat. *Mljekarstvo* 68 (4), 282-294. https://doi.org/10.15567/mljekarstvo.2018.0404
25. Petrie, A., Watson, P. (2013): Statistics for Veterinary and Animal Science. 3rd edition. Willey-Blackvell. Oxford, United Kingdom.
26. Potočnik, K., Gantner, V., Kuterovac, K., Cividini, A. (2011): Mare's milk: composition and protein fraction in comparison with different milk species. *Mljekarstvo 61*, 107-113.
27. Salimei, E., Fantuz, F. (2012): Equid milk for human consumption. *International Dairy Journal* 24, 130-142. https://doi.org/10.1016/j.idairyj.2011.11.008
28. Uniacke-Lowe, T. (2011): Studies on equine milk and comparative studies on equine and bovine milk systems. PhD Thesis, University College Cork. 61-65.
29. Uniacke-Lowe, T., Huppertz T., Fox, P.F. (2010): Equine milk proteins: Chemistry, structure and nutritional significance. *International Dairy Journal* 20, 609-629. https://doi.org/10.1016/j.idairyj.2010.02.007
30. Zoege Von Manteuffel, N. (1989): Anwendungsmöglichkeiten einiger ausgewählter chemischer, physikalischer und mikrobiologischer Untersuchungs-methoden aus dem Bereich der Eutergesundheit des Rindes für die Untersuchung von Stutenmilch, Tierärztliche Hochshule Hannover, Dissertation, 134.