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Concentrations of mercury and other elements in ewes' milk: Effect of lactation stage

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HIGHLIGHTS

- Yield and chemical composition of ewes' milk was within lactation curve.
- Hg and Cd decreased in ewes' milk with lactation progress.
- Majority of macro and trace elements in ewes' milk decreased during lactation.
- Hg in Ewes' milk from W Croatia does not pose a health risk for human consumption.

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ABSTRACT

There is an increased production and demand for ewes' milk in the Republic of Croatia, as well as globally. There is also a growing concern about its quality, since milk from farm animals may become contaminated with mercury and other toxic elements. Thus, the aim of this paper is to determine the influence of lactation stage on the ewes' milk quality in western Croatia by considering concentrations of mercury and other elements in ewes' milk. The research was conducted on 36 Travnik pramenka sheep during different lactation stages. The digested milk samples were analysed with continuous flow hydride generation technique by using inductively coupled plasma mass spectrometry. Samples were taken during 40th, 80th and 120th d of lactation. Yield and quality of ewes' milk was within lactation curve. As lactation progressed, significantly lower concentrations of Hg (on 80th d compared to 40th d) and of Cd (120th d compared to 80th d) were noted, and Hg on 120th d was below the detection limit. Concentrations of Ca and Cu were lower on the 120th d compared to 40th d, while P, Mg, Fe, Zn, Mn, and Se were lower on the 80th and 120th d compared to the 40th d. Concentrations of K, Mo, and Cr differed among all stages of lactation. Regarding toxic elements, the observed low concentrations of Hg, Co, Cd and As suggest that ewes' milk in western Croatia is safe for human or animal consumption.

1. Introduction

There is an increased production (35.9%) and demand for ewes' milk both globally and in the Republic of Croatia (FAOSTAT, 2020). Milk and dairy products have been recognized all over the world as good quality products regarding sensory properties and their beneficial influence on human health (Steijns, 2001). Ewes' milk is of high value, as it contains around 200 active substances (Horák et al., 2004), among which it may contain higher amounts of vitamins A, B and E, calcium, phosphorus, potassium, and magnesium in comparison with cow milk, as determined by Zervas and Tsiplakou (2011). Importance of ewes' milk quality is highlighted also in the research by Claeys et al. (2014), who compared the quality of horse, donkey and ewes' milk with human milk and concluded that ewes' milk can be suitable alternative to breast milk

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and infant formula. Milk is a good source of elements, such as Cu, Fe, Zn and Se, which are known to be essential for normal growth, yet they can be toxic in high concentrations (Kochare and Tamir, 2015). There are not many researches that deal with the concentration of macro and trace elements (Coni et al., 1996; Antunović et al., 2016, 2017). In industrial areas, Hg, As, Cd, and Co may be found in milk. According to Duffus (2002), any classification of the metallic elements used for scientific purposes must be based on the periodic table, while the term "heavy metal" or "toxic metal" is meaningless and imprecise. There have been only a few studies on the monitoring of Hg, As, Cd, and Co concentrations in ewes' milk during lactation (Coni et al., 1996; Antunović et al., 2005; Ivanova et al., 2009). Studies on the monitoring of these elements in milk may also indicate environmental conservation (Caggiano et al., 2005; Antunović et al., 2017) regarding extensive sheep farming based on sheep grazing on natural pastures. Tunegova et al. (2016) reported that milk from animals might become contaminated with Hg, Cd and Pb because of feeding with contaminated forage and water, as well as grazing in contaminated areas. Determination of the contamination level with the mentioned elements is important in terms of maintaining an adequate health safety of human food chain components, especially in areas that are significantly contaminated by risk elements, such as heavy metals (Stanovič et al., 2016). There are limited data on Hg residues in milk. Mercury is one of the most toxic elements in the Earth. Since Hg is being utilized in industry and agriculture, it presents a threat to human health because it is associated with many adverse effects on health (Castro-González and Méndez-Armenta, 2008). Mercury is transformed by microbial action in soils into methylmercury, which is one of the most harmful bioavailable forms of mercury (Vollset et al., 2019). It is a neurotoxic compound responsible for microtubule destruction, mitochondrial damage, lipid peroxidation and accumulation of neurotoxic molecules (Patrick, 2002). Bilandžić et al. (2011) researched Hg in raw cattle milk in Croatia to determine that Hg levels in the southern region were significantly higher than in the northern region.

In this context, the aim of this paper is to investigate the influence of lactation stage on the quality of ewes' milk in western Croatia by determining mercury concentration and concentrations of other elements.

2. Material and methods

The Bioethics Committee for Research on Animals of the Faculty of Agrobiotechnical Sciences Osijek confirmed that the present research was carried out by obeying all legal provisions according to the Animal Protection Act of the Republic of Croatia (OJ 133/06, OJ 37/13 and OJ 125/13).

2.1. Animals and experimental design

The research was conducted during 2018 on 36 Travnik pramenka ewes during different lactation stages. Sampling was carried out in Croatian region of Western Slavonia (region of Grubišno Polje) from ewes grazing on natural pastures. Selection of ewes was carried out from the herd of 1000 ewes of Travnik pramenka sheep. Ewes were 4 years old on average, healthy, in the 3rd lactation with one lamb in litter. Samples were taken from ewes on the 40th, 80th and 120th day of lactation (±5 d). Ewes were grazing of natural pastures with the following herbal mixture: *Lolium perenne* L., *Lolium italicum* Lam., *Phleum phleoides* (L.) H. Karst., *Trifolium repens* L., *Dactylis glomerate* L. Water and mineral lick were provided *ad libitum* to all ewes during the whole period of research. During research, lambs were kept with the ewes and were allowed to suckle *ad libitum*. Lambs were separated from their mothers one day before milk sampling.

2.2. Milk sampling and analyses

Samples of milk were collected in 100 ml bottles and transferred in mobile coolers. Samples were chilled to 4 °C within 24 h until milk was subjected to chemical composition analysis. Milk was analysed for non-fat dry matter (NFDM), milk fat, protein, lactose, urea, somatic cells count (SCC) and the total number of microorganisms (CFU). Analysis of NFDM was determined by subtracting the milk fat from the dry matter, which was carried out by drying. Analysis of fat, protein, lactose and urea concentrations in milk was carried out by infrared spectrometry (HRN EN ISO 9622: 2001) in the MilkoScan FT 6000 analyzer within the Comby system. The SCC was determined by fluoro-opto-electronic method (HRN EN ISO 13366−2/Correction. 1.2007) with the Fossomatic 5000 analyzer, and the CFU was determined with the epifluorescent method of flow cytometry (IDF 161 A:1995). Another milk sample (100 ml) was collected during morning milking from each ewe, homogenized with vortex (VIBROMIX 10, Tehtnica, Slovenia), stored in fridge box at 4 °C, and transferred in the deep freeze (−80 °C) until microwave digestion was carried out. Sampling bottles were soaked in 20% HNO_3 for 24 h and washed with deionized water to avoid possible contamination. The digestion of milk samples was carried out according to the method described by Belete et al. (2014). Validation of method was carried out with reference material (BCR129). A 3.0 ml of each liquid milk sample was transferred into 60 ml Teflon digestion vessel. Optimized volumes of 6 ml of 70% nitric acid and 1 ml of 30% hydrogen peroxide were added, the mixture was shaken carefully and left for 10 min before closing the vessel. The samples were subjected to closed microwave digestion at the optimized microwave digestion program in the following sequence: 50 W, 165 °C (10 min); 80 W, 190 °C (20 min); and 0 W, 50 °C (10 min), carried out on the Mars 6 (CEM, Matthews, NC, USA) microwave system. The digest was diluted to 25 ml with deionized water and used for further analysis. Instrumental detection and quantification limits for the determination of Hg and other elements in ewes' milk and pasture are presented in Table 1.

2.3. Herbage and soil sampling and treatment

The sampling of herbage consumed by ewes was carried out on the same day when milk was sampled. In total, 12 samples of plants and soil were taken and presented as three samples, meaning that

Table 1
Instrumental detection and quantification limits for the determination of elements in samples.

mg kg^{-1}	Instrumental detection limits	Instrumental quantification limits
Ca	3.259	32.590
Mg	0.00729	0.07029
K	1.468	14.680
P	0.1638	1.638
Na	0.01002	0.1000
Cu	0.00023	0.00234
Fe	0.0618	0.618
Zn	0.06435	0.6435
Mn	0.001148	0.01148
Ni	0.000197	0.001974
Mo	0.004653	0.015509
Co	0.000297	0.002966
Se	0.0022	0.0217
Cr	0.003399	0.03399
Cd	0.000158	0.001578
As	0.001987	0.01987
Hg	0.000153	0.001531

each sample consisted of 4 subsamples. Concentrations of mercury and other elements in pasture, soil and water samples are presented in Table 2. Validation of method was carried out with reference material as follows: BCR-129 (hay powder, European Commission), CRM027-50G (Sigma Aldrich, USA), PT-SL1 (trace elements in sewage sludge, PHARE) which elements' concentrations are presented in Table 3. The digestion of plant samples was carried out according to the method described by Belete et al. (2014). The samples were subjected to closed microwave digestion at the optimized microwave digestion program in the following sequence: 50 W, 165 °C (10 min); 80 W, 190 °C (20 min) and 0 W, 50 °C (10 min), carried out on the Mars 6 (CEM, Matthews, NC, USA) microwave system. The digest was diluted to 25 ml with deionized water and used for further analysis.

2.4. Analyses of digested samples

The digested samples were analysed with continuous flow hydride generation technique using inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500a, Agilent Technologies Inc., California, USA) for concentrations of Hg, Co, Cd, As, macro elements (Ca, Mg, K, P, Na) and trace elements (Cu, Fe, Zn, Se, Mn, Ni, Mo, Co, Cr). The digested milk samples provided for As determination were subjected to the pre-reduction step prior to the analysis according to Bosnak et al. (2004). For the pre-reduction of As, 20 ml of sample was placed in a 50 ml polypropylene autosampler tube and mixed with 2 ml of 5% solution of KI and ascorbic acid. Six ml of concentrated HCl was also added, and the mixture rested for at least 20 min. The tube was brought to the 50 ml mark with deionized water and the sample was ready to run. For the pre-reduction of Hg and Se, 20 ml of sample was placed in a cleaned 125 ml beaker and 20 ml of concentrated HCl was slowly added. The solution was then transferred to a 50 ml polypropylene autosampler tube, which was diluted to the 50 ml mark with deionized water. The samples were ready to run with ICP-MS (Optima 21000 DV, PerkinElmer, Massachusetts, USA).

2.5. Statistical analyses

Data for Hg and other elements in milk during lactation were obtained with MEANS procedure. The General Linear Model (GLM) was applied to the data for Hg and other elements with lactation stage as fixed effect. Means were compared by using the Tukey test and the differences between the groups were declared significant at $P < 0.05$. Correlations between parameters of ewes' milk were evaluated by Pearson's correlation with CORR procedure. Statistical analysis was performed by using the statistical software SAS 9.4®.

Table 2
Concentrations of mercury and other elements in pasture, mineral lick, water and soil samples.

mg kg^{-1}	Pasture			Mineral lick	Water	Soil
	1	2	3			
Hg	0.013	0.015	0.010	0.352	<LD	0.081
Co	0.108	0.083	0.109	0.003	0.003	9.400
Cd	< LD	< LD	0.037	0.003	<LD	0.157
As	< LD	< LD	< LD	0.020	<LD	9.281
Ca	8870	7261	6986	<LD	121.9	2285
Mg	3395	1869	2755	<LD	56.48	4838
K	44769	39178	49822	600.05	<LD	2580
P	5748	5857	5100	9.35	<LD	877.5
Na	104.4	40.96	205.6	310807	5.50	296.4
Cu	10.29	8.01	7.21	<LD	0.002	14.81
Fe	226.6	207.9	274.3	<LD	<LD	24.49
Zn	33.92	28.07	29.99	<LD	<LD	58.61
Mn	44.25	31.92	44.35	0.083	0.011	597.0
Ni	13.73	16.56	14.79	0.027	0.030	20.91
Mo	1.070	3.031	0.771	ND	ND	ND
Se	0.052	0.047	0.066	0.022	<LD	0.428
Cr	6.267	8.063	9.409	<LD	0.040	34.93

LD- Instrumental detection limits; ND-not determined.

Table 3
Concentrations of mercury and other elements in blank and reference material samples.

mg kg^{-1}	Blank sample	Reference material		
		BCR129	CRM027-50G	PT-SL1
Hg	<LD	0.038	4.66	1.75
Co	<LD	ND	4.147	5.32
Cd	<LD	0.123	13.34	1.69
As	<QL	ND	12.73	3.32
Ca	<LD	6099	6076	50090
Mg	<LD	1425	3280	6184
K	5.87	33601	2412	1586
P	<LD	1.40	496.8	13354
Na	<LD	3469	175.9	2086
Cu	<LD	9.90	8.76	295.8
Fe	<LD	109.3	13460	19370
Zn	<LD	31.81	52.75	1253
Mn	<LD	71.39	258.0	427.6
Ni	0.015	4.08	10.32	459.3
Mo	0.016	0.993	ND	ND
Se	<LD	0.025	15.29	1.053
Cr	<LD	2.329	33.06	604.4

LD-Instrumental detection limits, QL-Instrumental quantification limits, ND-not determined.

3. Results and discussion

While analysing the yield and chemical composition of ewes' milk, as well as SCC and CFU in milk of Travnik pramenka sheep, significant differences were determined as affected by the lactation stage (Table 4). Significant decrease was determined in milk yield and CFU, as well as increase of milk fat and protein content in milk with lactation progress. Antunac et al. (2002) determined that lactation stage of Travnik pramenka sheep had significant influence both on overall quantity of milk and on the number of somatic cells. At the beginning of lactation (60th d), average daily milk yield was 581.16 ml/day, and at the end of lactation (from 181th to 240th d), it was 480 ml d^{-1}. Komprej et al. (2012) determined in Bovec and Improved Bovec sheep in Slovenia during lactation (45–150 d) a decrease of milk yield by 70% and 69%, respectively. In Istrian sheep during lactation (20–180 d), milk yield decreased by 41% (Vrdoljak et al., 2012). A decrease of milk production along with lactation progress could be explained by progressive weakening of homeorhetic mechanisms involved in galactopoietic as lactation progressed together with the rise in ambient temperature during mid-

Table 4
Milk yield, chemical composition, number of somatic cells and total number of microorganisms (CFU) in ewes' milk during different lactation stages.

Parameters	Lactation stage, d (mean)			SEM	P-
	40th	80th	120th	values	
Milk yield (g d^{-1})	0.76^{a}	0.75^{a}	0.49^{b}	0.03	0.004
Dry matter without fat, %	11.28	11.07	11.10	0.05	0.051
Milk fat, %	7.12^{a}	7.42^{ab}	8.04^{b}	0.10	0.004
Protein, %	5.53^{a}	5.84^{b}	5.72^{ab}	0.06	0.044
Lactose, %	4.35	4.39	4.34	0.03	0.710
$\log_{10}$ (SCC), mL	5.13	5.20	5.36	0.07	0.487
$\log_{10}$ (CFU), mL	4.23^{a}	3.96^{b}	4.16^{ab}	0.05	0.025

SEM–standard error of mean; ^{a,b}data within the same row with different superscripts differ significantly ($P < 0.05$).

lactation, which probably led to the decrease in milk yield and consequently to the increase of milk fat and protein content. Similar conclusion was derived by Sevi et al. (2004). However, milk yield and chemical composition are also influenced by ewes' feeding (Nudda et al., 2020; Bocquier and Caja, 2001).

During lactation progress, significantly lower concentrations of Hg and Cd were determined (Table 5). Special attention was paid to Hg, As, Cd and Cr accumulation in milk and their possible transfer to humans via food. Other possible sources of Hg might be related to environment pollution (Zwierzchowski and Ametaj, 2018). Concentrations of Hg, Cd, Cr and As during lactation depend mostly on feed or water intake, and possible consumption of soil (Ghidini et al., 2012). Krupicer (1995) observed that grazing animals accumulated mercury in their organs, mainly in parenchymatous organs when higher values of mercury were contained in pasture. Antunović et al. (2016) observed Hg concentration from 0.0001 mg kg^{-1}in late stage compared to 0.0003 mg kg^{-1}in early and mid-lactation stages in ewes' milk in Croatia. Starska et al. (2011) determined higher values of Hg in milk and milk products obtained on the market and directly from manufacturers throughout Poland in 2006–07, and estimated 0.001 and 0.002 mg $kg^{-1}$of mercury, respectively, with observation that these concentrations did not pose a threat to human health. The results from the present research are bellow these values, representing ewes' milk as of good quality, with the lowest concentration of Hg determined on the 120th d of lactation. Stanovič et al. (2016) determined concentrations of Hg and Cd in 53 samples of ewes' milk obtained from the area of Slovakia that is characterized by historical mining and metalworking activity. The Hg content in milk samples from that area did not exceed the maximum level of 50 μg kg^{-1} set by the Codex Alimentarius of Slovakia. However, Cd concentrations exceeded limited value for Cd (10 μg kg^{-1}) in 25 samples. In their meta-analytical study, Zwierzchowski and Ametaj (2018) determined concentrations of mercury in cows' milk obtained from commercial system to be 0.01 μM L^{-1}, in conventional farming 0.02 μM $L^{-1}$and in organic farming <0.01 μM L^{-1}, with all values lower than oral minimal risk level of 0.70 μM L^{-1}. Anastasio et al. (2006) observed 0.002 ± 0.005 μg g^{-1} d.w. of Hg in ewes' milk in Southern Italy, although higher concentration was estimated in fresh cheese (0.008 ± 0.0005 μg g^{-1} d.w.) compared to milk. Authors explained that chemical and physical properties of the manufacturing process might affect concentrations of metal. Furthermore, in the present research, concentrations of Hg, As, Cd and Co in milk during lactation were lower than permitted concentrations (Commission Regulation, 2006). Very low concentrations indicate low level of pollution and preservation of environment. However, Gallo et al. (1996) reported that sheep and cattle reared freely on pasture are also indicators of the environmental pollution like the wild animals. However, it is known that Cd is air pollutant, as it is emitted as a result of various industrial activities (Tunegova et al., 2016). Concentration of Cd in milk decreased on the 120th d of lactation compared to 80th d. As reported by Rodriguez et al. (1999), Cd concentration increase is associated with protein content in cows' milk. According to Mata et al. (1995), protein content in cows' milk was mainly associated with the protein fraction (casein fraction) obtained by enzymatic coagulation. However, in the present study, content of proteins in milk determined on the 120th d of lactation did not differ from other samplings. Moreover, Miedico et al. (2016) found that ewes' milk contained more Cd when compared to goats' milk (0.934 : 0.650 ng g^{-1}, respectively). Ivanova et al. (2011) found a decrease in As concentrations with lactation in the milk of East Friesian sheep and Awasi sheep. In general, in this study, concentrations of these elements in milk were lower than in other researches (Póti et al., 2012; Najarnezhad et al., 2013; Parsaei et al., 2019).

Concentrations of macro minerals and trace elements in milk of Travnik pramenka ewes during lactation are shown in Table 6. There is significant decrease in concentrations of most elements (Ca, P, K, Mg, Fe, Cu, Zn, Mn, Mo, Cr, Ni, Se), except for the Na concentration, which did not differ depending on the lactation stage. In Karagouniki and Serron ewes' milk, Polychroniadou and Vafapoulou (1985) determined decrease of P, K and increase of Mg concentration during lactation. Similar results for Na concentration in milk of Tswana sheep were determined by Aganga et al. (2002). Wohlt et al. (1981) determined the decrease in Ca, P, Na, Mg and Fe concentrations in ewes' milk during lactation. In Dubrovnik sheep milk during lactation, Antunovic et al. (2016) determined increased concentrations of Na, Mg, Mn, Mo, Co and Se, and decreased K and Zn concentrations. In the present study, concentrations of Ca and P, as fundamental elements for growth and bone maintenance that are essential for neonates (Balthazar et al., 2017), were more abundant in early lactation stage. Decrease of Ca concentration during lactation in Comisana ewes was found by Sevi et al. (2004). In milk of Karakachan breed of sheep in Bulgaria, Ivanova (2011) determined the decrease of Cr and Se concentrations, and increase of Zn, Cu, Mn concentrations during lactation. General reduction of elements in ewes' milk during lactation may occur because of the decrease in osmotic pressure in the mammary gland during summer months (hot summer temperatures). Similar results in cows' milk during summer was found by Bahga et al. (1985).

Numerous correlations were determined between elements in ewes' milk during lactation, which were expected due to their metabolic relationships. On the 40th d of lactation, positive correlations were found between Ca:Zn, Ca:Cr, Ca:P, K:Ni, Fe:Mo, Fe:As, Zn:Cr, Zn:P, Mo:Cr, Mo:Se, Cr:P, Se:Hg, and negative correlations

Table 5
Concentration of mercury and other elements in ewes' milk during different lactation stages.

Elements, mg kg^{-1}	Lactation stage, d (mean)			SEM	*P*-values
	40th	80th	120th		
Hg	0.000628^{a}	0.000160^{b}	<LD	0.000065	<0.001
Co	0.001453	0.001419	0.001438	0.000545	0.966
Cd	0.000598^{ab}	0.000773^{a}	0.000391^{b}	0.000036	<0.001
As	0.000342	0.000415	0.000375	0.000025	0.439

SEM–standard error of mean; LD- Instrumental detection limits; ^{a,b}data within the same row with different superscripts differ significantly ($P < 0.05$).

Table 6
Concentration of macro minerals and trace elements in ewes' milk during different lactation stages.

Elements, mg kg^{-1}	Lactation stage, d (mean)			SEM	*P*-values
	40th	80th	120th		
Ca	1993.36^{a}	1895.46^{ab}	1838.38^{b}	19.85	0.008
P	1851.15^{a}	1565.40^{b}	1480.50^{b}	20.63	<0.001
K	1435.39^{a}	1345.20^{b}	1221.50^{c}	17.54	<0.001
Na	567.52	573.36	571.41	19.97	0.991
Mg	200.58^{a}	180.05^{b}	179.33^{b}	2.53	<0.001
Fe	0.614^{a}	0.329^{b}	0.288^{b}	0.025	<0.001
Cu	0.048^{a}	0.0395^{ab}	0.029^{b}	0.002	0.004
Zn	7.38^{a}	5.05^{b}	4.47^{b}	0.18	<0.001
Mn	0.090^{a}	0.045^{b}	0.037^{b}	0.0033	<0.001
Mo	0.044^{a}	0.031^{b}	0.024^{c}	0.001	<0.001
Cr	0.052^{a}	0.044^{b}	0.037^{c}	0.0007	<0.001
Ni	0.014^{a}	0.013^{a}	0.002^{b}	0.0008	<0.001
Se	0.039^{a}	0.016^{b}	0.014^{b}	0.0023	<0.001

SEM–standard error of mean ^{a,b}data within the same row with different superscripts differ significantly ($P < 0.05$).

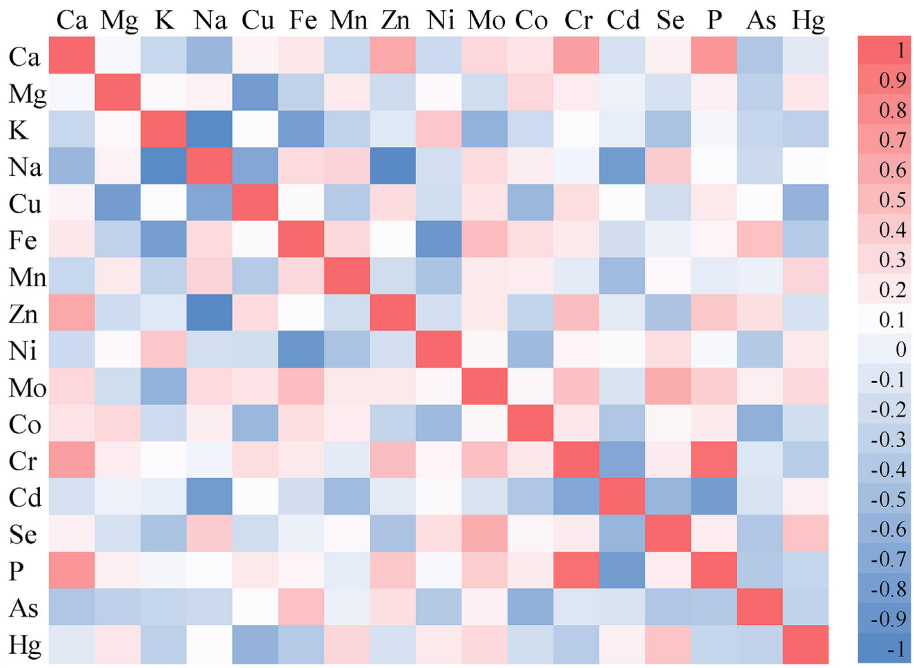

Fig. 1. Heatmap correlations between elements on d 40 in lactation.

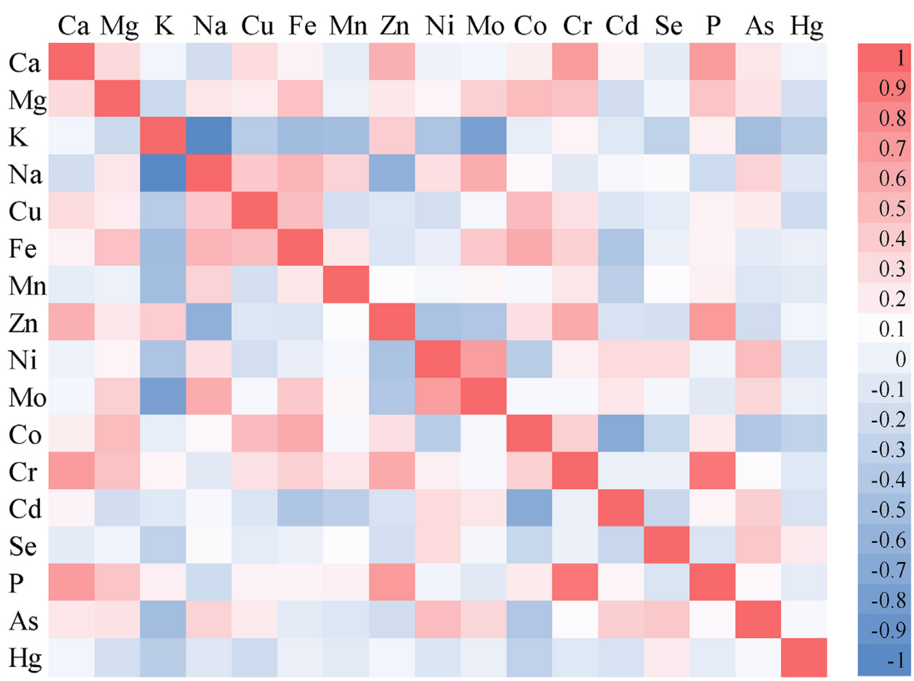

Fig. 2. Heatmap correlations between elements on d 80 in lactation.

were found between K:Na, Na:Zn and Fe:Ni (Fig. 1). In milk of early lactating Dubrovnik sheep, Antunovic et al. (2016) determined significant positive correlations between Ca:Zn, Ca:P, Na:Fe, Na:Mo and Fe:Mn. In the same research, milk of sheep in mid lactation exhibited significant positive correlations between Ca:Cr, Mo:Cd, Ca:P, Mg:Cr, Mo:Cd, and negative correlations between Ca:Na. On the 80th d of lactation, positive correlations were determined between Ca:Zn, Ca:Cr, Ca:P, Mg:Fe, Mg:Co, Mg:Cr, Mg:P, Na:Cu, Na:Fe, Na:Mo, Cu:Fe, Cu:Co, Fe:Mo, Fe:Co, Zn:Cr, Zn:P, Ni:Mo, Ni:As, Cr:P, Se:As, and negative ones between K:Na, K:Mo, Na:Zn and Co:Cd (Fig. 2). Polychroniadou and Vafopoulou (1985) considered correlation between K and Na in milk of Karagouniki sheep, which was strongly negative ($r = -0,566$), however, in Serron sheep it was weak ($r = -0.235$). In the late lactation (120th d), positive correlations were determined between Ca:Se, Mn:Se, Zn:Cr, Ni:Cd, Cr:Zn, Cr:P, Cd:Ni, and negative correlations were established for Ca:Mg, K:Fe, Na:Zn, Cu:Zn, Mo:Hg (Fig. 3). In Holstein cows' milk, Nozad et al. (2012) determined similar positive correlation between Na:Mg ($r = 0.28$) and Na:K ($r = 0.74$).

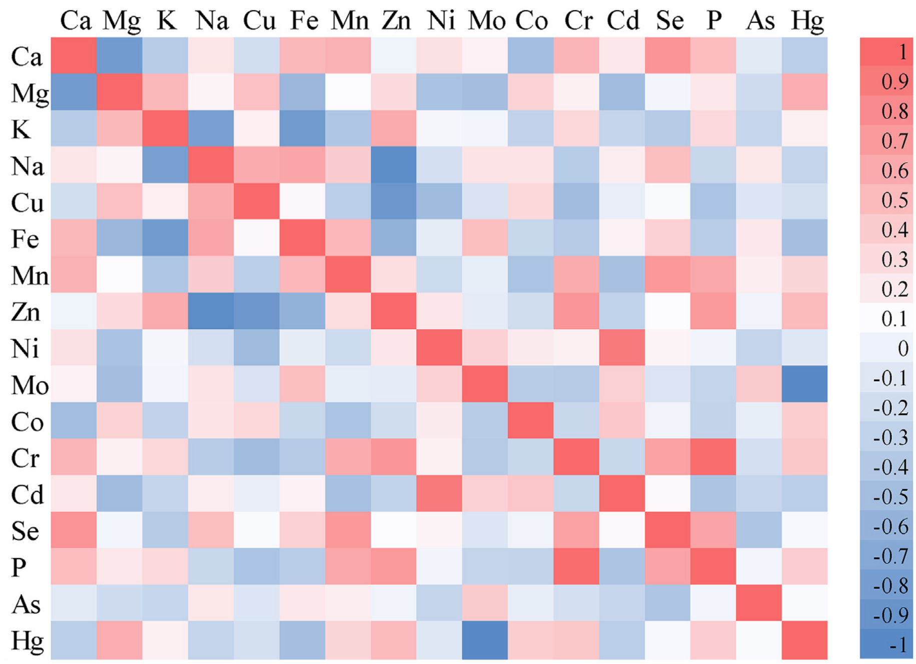

Fig. 3. Heatmap correlations between elements on d 120 in lactation.

4. Conclusion

According to the determined concentrations of mercury and other elements in ewes' milk, as well as concentrations of micro and macro elements during lactation, it can be concluded that lactation stage influenced ewes' milk quality. Besides, the yield and quality of ewes' milk was within the lactation curve. The low concentrations of Hg, Co, Cd and As detected in this study suggest that the consumption of ewes' milk from Western Croatia does not present a health risk for either human or animal consumers.

Authorship Contribution Statement

Z. Antunović, Conceptualization, Writing - original draft, Supervision. B. Mioč, Conceptualization, Investigation. Ž. Klir, Investigation, Writing - review & editing. I. Širić, Formal analysis, Visualization. V. Držaić, Investigation. Z. Lončarić, Methodology, Formal analysis. G. Bukvić, Formal analysis. J. Novoselec, Formal analysis, Investigation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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