UDK: 637.352

# Analysis of 18 elements in Alpine goat milk in the first third of lactation

DOI: 10.15567/mljekarstvo.2024.0302

# Zvonko Antunović<sup>1</sup>, Željka Klir Šalavardić<sup>1</sup>\*, Boro Mioč<sup>2</sup>, Josip Novoselec<sup>1</sup>

<sup>1</sup>Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Department for Animal Production and Biotechnology, V. Preloga 1, 31000 Osijek, Croatia <sup>2</sup>University of Zagreb, Faculty of Agriculture, Department of Animal Science and Technology, Svetošimunska cesta 25, 10000 Zagreb, Croatia

Received: 03.11.2023. Accepted: 15.06.2024.

\*Corresponding author: zklir@fazos.hr

# Abstract

The research objective was to examine the influence of the first third of the lactation stage on 18 mineral elements in Alpine goat milk. The research was carried out on 20 goats of the French-Alpine breed, which were on average 5 years old and in the fourth lactation. The goats were monitored on the 30<sup>th</sup> and the 90<sup>th</sup> day of lactation. Their milk was analysed for chemical composition by the infrared spectroscopy. By means of inductively coupled plasma (ICP), a total of 18 minerals were analysed (Ca, P, K, Na, K, Mg, Fe, Zn, Cu, Se, Mn, Mo, Co, Cr, Ni, Cd, Pb, As and Hg). The period of milk sampling in the first third of lactation significantly influenced the changes in concentrations of Na, Se, Mo, Cr, Ni and As. Significantly higher concentrations of Na and As were determined in milk sampled on the 90<sup>th</sup> day, while lower concentrations of Se, Mo, Cr and Ni were determined in milk sampled on the 30<sup>th</sup> day of the first third of lactation. Analysis of the correlation coefficients between the investigated trace elements and the selected toxic elements in Alpine goat milk showed a significantly positive correlation between Ca:Mg, Ca:P. Ca:Co, Ca:Ni, Ca:No; Mg:P, Fe:As, Pb:Cd and P:Ni. In the first third of lactation, Alpine goat milk contained a very low concentration of heavy metals. The described changes indicate that Alpine goat milk is rich in essential elements, while the concentration of toxic elements is very low in the first third of lactation.

Keywords: Alpine goats; lactation; milk; elements

### Introduction

Goat milk is nowadays in high demand, reaching the third place on the list of the most consumed types of milk (Klir et al., 2015; Guo et al., 2021). There are over one billion head of goats in the world, while its milk amounts to about 18.6 million tons, of which 15.14 % in Europe (2.8 million tons from over 15 million dairy goats) (FAO, 2024). The total number of goats in the Republic of Croatia was 65.227, while delivered quantities of goat milk were 3.556 tons in year 2022 (HAPIH, 2023). Contemporary research is focused on determining goat milk guality in dependence on not only goats' feeding regime, but also on other influencing factors, such as genotype/breed, lactation specifics (stage, order), etc. Milk and its products are rich sources of nutrients because of availability of almost all essential elements, especially for children (Ismail et al., 2017). Milk contains significant amounts of elements, especially essential macro elements (Ca, Mg, Na, K), trace elements (Zn, Co, Mo, Se, Cr and Ni), but also small amounts of toxic elements (heavy metals: Cd, Pb, As and Hg) (Llobet et al., 2003; Rey-Crespo et al., 2013; Fayet et al., 2013). In an animal organism, elements have numerous roles, which usually intertwine and significantly affect the growth and development, as well as the animal's production capability (Marguès et al., 2022, Sutlle, 2010). Toxic elements (Pb, Cd, As and Hg) do not have physiological functions in the body, and can cause numerous disorders even at low concentrations. Toxic elements in milk may origin from milk containers or contaminated water as well as through livestock feed and environment in which dairy animals are reared (Hussain et al., 2013; Briffa et al., 2020). In comparison to mammalian milk, goat milk has higher alkalinity, better digestibility, buffering capacity and therapeutic values (Park et al., 2007). Compared to cow milk, goat milk has higher contents of potassium, calcium, chloride, phosphorus, selenium, zinc and copper (Krstanović et al., 2010). Almašiova et al (2023) confirmed that goat milk is rich in essential elements, mainly Ca, Mg, K and Na, but contains toxic elements in very low amounts, often under the limit of detection. Many researches assessed the content of mineral elements in goat milk (Zhou et al., 2017; Antunović et al., 2018; Curró et al., 2019; Chen et al., 2020; Homayonibezi et al., 2020; Abba et al., 2021; Guo et al., 2021; Pan et al., 2023;). Also, many published studies focused on determination of the content of only several elements in the Alpine goat milk, and some researchers investigated the effects of lactation stage (Park and Chukwu et al., 1989; Voutsinas et al., 1990; Antunac et al., 2001; Antunović et al., 2012; Shuvarikov et al., 2021). However, there are no studies that investigated a broad scope of elements in milk of Alpine goats depending on the lactation stage. As lactation, especially in its first third, is a very demanding period for goats, it is expected that the quality of milk would be affected by it, so the authors investigated as many as 18 elements (macro- and micro elements and toxic metals) in Alpine goat milk to determine whether their content was influenced by the first third lactation stage.

## Material and methods

The research was carried out by obeying legal provisions determined by the Animal Protection Act (Republic of Croatia Official Gazette No. 133 (2006), No. 37 (2013), and No. 125 (2013)), and approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences.

#### Animals and diets

The research was carried out on 20 French-Alpine goats kept at the Đurković family farm in the Croatian Osijek-Baranja County. On average, goats were 5 years old and all were in their fourth lactation. The investigated goats were selected from a herd of 50 goats, and all of the selected ones were healthy and in good physical condition. The goats were monitored on the 30<sup>th</sup> and 90<sup>th</sup> day of lactation (sampling period) during morning milking. They were kept in pens together with goat kids. Machine milking was done in a separate facility in the morning and evening, during feeding.

Before taking milk samples, goats were separated from the goat kids for 24 hours. Goats' diet was based on a feeding mixture with 16 % crude protein in the amount of 1.50 kg/day and alfalfa hay, which was offered to goats *ad libitum*. The ratio of voluminous and concentrated part of the diet was 60:40. Water was also available *ad libitum*.

## Milk and feed analysis

Goat milk (morning milking) was sampled in two bottles (30 mL/bottle), which were cooled to 4 °C. One bottle of milk was used for analysis of chemical composition by infrared spectroscopy according to HRN ISO 9622:2017, in the MilkoScan FT 6000 analyser (Foss Electric, Hillerød, Denmark). The other bottle of milk sample was frozen at -80 °C. After defrosting, the sample was tested for concentrations of macro and micro elements (Ca, P, K, Na, K, Mg, Fe, Zn and Cu), values of which were expressed as mg kg<sup>-1</sup>, and concentrations of Se, Mn, Mo, Co, Cr, Ni were presented as µg/kg. Content of selected toxic elements (Cd, Pb, As and Hg) was expressed also as µg/kg. Feed and goat milk samples were dissolved with 10 mL mixture of 5:1 HNO<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> at 180 °C over 60 min in a microwave oven (CEM Mars 6). Digestion of feed and milk samples of goats was carried out as described by Belete et al. (2014). Dilution of digest was carried out to 25 mL with deionized water. The concentrations of elements in goat milk, and in feed and water were determined by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500a, Agilent Technologies Inc., Santa Clara, CA, USA) using continuous flow hydride generation technique. All samples were double analysed. According to Bosnak et al. (2004) digested samples for analyses of As were subjected to the pre-reduction step when 20 mL of sample was set in auto sampler tube (50 mL) and mixed with of KI and ascorbic acid solution (2 mL, 5 %). In mixture, 6 mL of HCl was

Parameters	F	eed	Water	DIE, mg/feed mixture DM		
Faiameters	Feed mixture	Hay	- Water			
Ca, mg/kg DM	11094.50	5554.79	99.09	14811.16		
P, mg/kg DM	4409.00	2657.00	0.0009	5886.02		
K, mg/kg DM	8808.16	15230.05	2.76	11758.89		
Na, mg/kg DM	2628.02	192.56	88.89	3508.41		
Mg, mg/kg DM	1742.74	1811.27	26.52	2326.56		
Fe, mg/kg DM	162.00	445.70	0.000003	216.27		
Zn, mg/kg DM	61.73	20.99	0.03	82.41		
Cu, mg/kg DM	9.29	5.62	0.03	12.40		
Co, µg/kg DM	106.96	157.43	0.05	142.79		
Mn, µg/kg DM	40260.00	12960.00	0.29	53747.1		
Mo, µg/kg DM	474.20	1327.00	0.34	633.06		
Se, µg/kg DM	98.27	299.00	0.18	131.19		
Cr, µg/kg DM	1177.00	645.30	12.76	1571.30		
Ni, µg/kg DM	1397.00	896.70	0.57	1865.00		
Cd, µg/kg DM	54.77	29.72	< LD	73.12		
Pb, µg/kg DM	372.50	632.40	< LD	497.29		
As, µg/kg DM	164.20	140.90	3.01	219.21		
Hg, µg/kg DM	< LD	1.12	0.006	-		

Table 1. Elements content in feed and water of Alpine goat in the first third of lactation

DM - dry matter; LD - instrumental limit detection; DIE - daily intake of elements through feed mixture.

added and left at least 20 min. Deionized water was used to dilute sample until the 50 mL mark. Then samples were ready to run on ICP-MS. For the Se and Hg pre-reduction, a 20 mL of sample was placed in 125 mL beaker and 20 mL of HCl was added. This solution was transferred into 50 mL polypropylene autosampler tube and diluted with deionized water to 50 mL. The limit of detection (mg/ kg) was as follows: Ca 0.01306; P 0.040048; K 0.401634; Na 0.047576; Mg 0.023112; Fe 0.00073; Zn 0.00143; Cu 0.00322; Co 0.0112; Mn 0.03967; Mo 0.04005; Se 1.31133; Cr 0.57387; Ni 0.28553; Cd 0.04344; Pb 0.01147; As 0.438, Hr 0,01216. The concentrations of elements in the feed and water of Alpine goats as well as daily intake of elements through feed mixture are presented in the Table 1.

#### Statistical analyses

Mean and standard deviation of chemical composition and elements in milk were processed by the MEANS procedure, while the influence of the sampling period on milk chemical composition and milk elements' concentration was analysed by the GLM procedure and processed by the SAS 9.4°. Following model was used: Yijk =  $\mu + s_i + e_{ij}$ , where  $\mu$  is overall mean,  $s_i$  is fixed effect of sampling period and  $e_{ij}$  is random error variation. Comparison between mean values of different groups were estimated by Tukey's test (p<0.05). Correlations among elements in goats' milk were evaluated by Pearsons' correlation with CORR procedure. The correlations were declared significant if p<0.05.

# **Results and discussion**

In this research, no significant changes were found in most indicators of the basic chemical composition of Alpine goat milk sampled in the first third of lactation that could be related to the milk sampling period, except for a significant decrease in lactose concentrations in milk obtained during the second sampling compared to the first sampling (Fig. 1). Voutsinas et al. (1990), Antunac et al. (2001), Paskaš et al. (2023) and Bendelja Ljoljić et al. (2023) obtained similar results with Alpine goats, as well Antunović et al. (2018) in their research conducted on Croatian spotted goats.

Analysis of average concentrations of 18 elements (macro and micro elements and toxic elements) in Alpine goat milk showed greater standard deviations for less contained elements, which was expected (Table 2). When compared to our results, the research of Shuvarikov et al. (2021) conducted in Russia confirmed higher concentrations of Ca, Fe, Cu and Zn (145.3, 0.7589, 0.4372 and 5.0155 mg/kg, respectively) and lower concentrations of P, K and Mg (844.0, 1521.1 and 0.1251 mg/kg, respectively) in milk of Alpine goat. Chen et al. (2020) examined goat milk produced in China and obtained similar content of Cd (0.425 µg/kg), lower contents of Ca, K, Na, Mg, Mn and Cr (520, 1377, 253, 92.5, 0.156 mg/kg and 11.7 µg/kg, respectively), and higher contents of Cu, Zn, Se, As, Ni and Pb (0.208, 3.11 mg/kg, 1.08, 28.1, 4.27, Ni 38.3 and Pb 7.97 µg/kg, respectively) than the milk samples examined in our research. The research carried out in Austria on goat milk showed similar concentrations for Ca, Mg, Na and K (Mayer and Fiechter, 2012). As confirmed by our research, K (1954.38 mg/kg) was the most dominant macro element, followed by Ca, P and Na (1561.12, 1058 and 369.29 mg/kg,

respectively). Chen et al. (2020) reported similar results. The observed concentrations of elements in goat milk indicate an adequate supply of these elements through food and water (Table 1). The concentration of Hg and Cd were the lowest in the present research, which was expected since this area is rural and without industry pollutants. Similar concluded Bilandžić et al. (2016).

The milk sampling period in the first third of the lactation was significantly influencing the changes in concentrations of Na, Se, Mo, Cr, Ni and As. Significantly higher concentrations of Na and As were determined in milk sampled on the 90<sup>th</sup> day and lower concentrations of Se, Mo, Cr and Ni were determined in milk sampled on the 30<sup>th</sup> day of the first third of lactation (Table 2). The concentration of essential elements in goat milk were within the recommended levels while the concentrations of toxic elements were under the allowed limits.

In Greece, Voutsinas et al. (1990) tested milk of Alpine goats in lactation (from the 8<sup>th</sup> to 35<sup>th</sup> week) to determine the increase in concentrations of Na, P and Mg and the decrease in concentration of K as the lactation progressed. They also reported the increase in the concentration of Ca, which was not determined in this research. Park and Chukwu (1989) reported that milk produced by the French-Alpine goats from 30<sup>th</sup> to 90<sup>th</sup> day of lactation did not exhibit significant changes in concentrations of Mn, Fe and Zn (0.3-0.3, 0.8-0.7 and 5.1-5.2 mg/kg, respectively), yet the concentration of Cu (0.9-1.2 mg/kg) was significantly increased. In the research on milk of Alpine goats raised in Croatia under ecological conditions, Antunović et al. (2012) did not prove significant changes in concentration of heavy metals and reported that their average values



**Figure 1.** Content (%) of fat proteins, lactose and dry matter non fat (DM-NF) in Alpine goat milk in the first third of lactation (\*means significant difference, p<0.05)

were below allowed limits for heavy metal content in milk. In another research by Antunović et al. (2018) carried out on Croatian spotted goats, it was determined that concentrations of Ca, Mg, P, Zn and Mo significantly increased as lactation progressed. The concentrations of Na, Cu, Fe and Pb also increased, and the concentration of K decreased over lactation, while concentrations of Mn and Ni did not vary in dependence on lactation stage. According to Commission Regulation (EU) 2023/915 (2006) the highest allowed concentration of Pb in raw milk is 0.02 mg/kg. This pointed out the very low concentrations of Pb determined in this research and the preservation of the land areas from which the feed for the goats is prepared. The presence of Pb in milk could be due to different factors (fodder contamination, climatic factors, used pesticide

 Table 2. Effect of lactation stage on the concentration of 18 elements (Mean±SD) in Alpine goat milk during the first third of lactation

	Samplir	ng period	The first third of	P-value			
Parameter, mg/kg	Sampling point I (30 <sup>th</sup> day)	Sampling point II (90 <sup>th</sup> day)	lactation				
Ca	1574.19±178.47	1547.39±199.96	1561.12±187.36	0.653			
Mg	138.15±24.53	149.39±20.96	143.63±23.28	0.124			
K	1965.25±153.31	1942.97±204.49	1954.38±178.16	0.694			
Na	323.74±42.54	417.12±160.61	369.29±124.06	0.014 0.733 0.079			
Cu	0.10±0.03	0.10±0.05	0.10±0.04				
Fe	0.26±0.06	0.37±0.28	0.31±0.21				
Zn	2.97±0.52	2.76±0.54	2.86±0.54	0.213 0.745			
Р	1052.15±97.63	1063.25±118.88	1058±107.29				
Mn, µg/kg	27.23±8.86	28.02±11.61	27.62±10.17	0.808			
Se, µg/kg	22.30±20.10	20.13±3.00	21.24±2.77	0.011			
Mo, µg/kg	15.44±4.47	12.58±4.35	14.04±4.59	0.045			
Cr, µg/kg	59.88±10.17	50.88±8.95	55.49±10.51	0.005			
Co, µg/kg	0.64±0.10	0.61±0.11	0.62±0.10	0.365			
Ni, µg/kg	5.46±0.65	4.91±0.72	5.19±0.74	0.014			
As, µg/kg	1.11±0.29	1.60±0.41	1.35±0.43	<0.001			
Cd, µg/kg	0.50±0.37	0.33±0.25	0.42±0.32	0.100			
Hg, µg/kg	0.06±0.12	0.06±0.05	0.06±0.09	0.797			
Pb, µg/kg	1.75±1.57	3.27±3.24	2.49±2.20	0.051			

SD - standard deviation

compounds, various industrial activities, contaminated water irrigation systems and other (Licata et al., 2004; Antunović et al., 2023). Bilandžić et al. (2016) performed a research on goat milk in Croatia from rural areas during 5 years and observed Pb concentrations from 9.33 to 60.0  $\mu$ g/kg with average values 15.1  $\mu$ g/kg. Hejtmankova et al. (2002) determined an increase in Mg and Fe concentrations during lactation for goat milk produced in Czech Republic. Güler (2007) explained that higher concentrations of Mg in goat milk might be caused by sudden physiological changes, metabolic processes in mammary gland, changes in diet, lactation stage, environmental temperature, and water intake. Kezdzierska-Matvsek et al. (2015) found significant differences as affected by lactation stage in concentrations of K and Na. while a significant Ca and Mg concentrations increased, and opposite trend was determined for concentrations of Zn, Fe and Cu in goats' milk in Poland. Michlova et al. (2016) determined that the content of some elements (Ca, Mg, K, Na, Zn and Cu) in goat milk collected during lactation period from different goat breeds kept at various farms in Czech Republic were very variable. Garba et al. (2018) pointed out that factors such as period of lactation and the amount of feed affected the increase in the levels of metals in cow milk. In goat milk obtained from autochthonous breeds and from the Saanen in Italy, Curró et al. (2019) also determined a significant increase in concentrations of Na in the 4<sup>th</sup> and 8<sup>th</sup> week of lactation when compared to the 24<sup>th</sup> week of lactation. The increase in concentration of Mg and the decrease in Zn in milk of the Pantja goat in India was reported by Chauhan et al. (2019).

Strzalkowska et al. (2008) carried out a research on Polish white improved goats and determined a significant increase in Na and Mg concentration of milk at the beginning of the lactation in comparison to the end lactation (10 months). The same authors determined opposite changes for Zn milk concentration during lactation. The observed changes during lactation were most probably caused by an excess intake of Na if the animals had a free access to salt lick (NRC, 2007). Stocco et al. (2019) determined a significant increase in Mg and Fe concentrations in Italian cow milk during lactation, as well as lower Zn concentration during the first third of lactation, which was in accordance with the results of the present research.

The concentrations of Mg, K, and Na in goat milk in the study carried out in Brazil decreased linearly (p<0.001) throughout the 56 days of lactation (Filho et al., 2024). The reason for absence of significant changes in mineral concentrations in milk of Alpine goats in the present research could be related to effect of different lactation stage when production and chemical composition of milk changed which is also determined in previous research. Similarly concluded Strzalkowska et al. (2008). Pan et al. (2023) determined that concentrations of Ca, Zn, Cr, Cu, Mn and Fe in goat milk were mainly distributed in the casein fraction in comparison to other fractions. According to very low concentration of toxic elements observed, goat milk can be considered as safe and beneficial for human health.

Analysis of the correlation coefficients between the

investigated elements in Alpine goat milk, showed that there was significantly positive correlation between Ca:Mg, Ca:P, Ca:Co, Ca:Ni, Ca:Mo; Mg:P, Fe:As, Pb:Cd and P:Ni (Table 3). Numerous significant correlations were estimated between various parameters in goats' milk during lactation, which were expected, because of their metabolic interrelations as well as differences in metabolic background of the animals. Similar concluded Singh et al. (2015) in Beetal goat breed in India. In Greece, Voutsinas et al. (1990) tested Alpine goat milk to determine a strong positive correlations between Ca:P (0.947), Ca:Mg (0.710), and Mg:P (0.630), as well as a high negative correlation between Na:K (0.789), which was of the same direction but without significance in this research. In milk of goats raised in China. Chen et al. (2020) determined significant correlations between Cd:Pb (r=0.723), Cd:Cr (r=0.774) and Cd:As (r= 0666) and explained that they were probably related to environmental factors. In the research conducted in Poland, Kędzierska-Matysek et al. (2015) also determined a strong positive correlation between Ca:Mg (0.65\*\*\*) in goat milk.

	Pb																		1.00
	Hg																	1.00	0.32 0.047
	Cd																1.00	0.18 0.288	0.59 <0.001
	Mo															1.00	-0.29 0.070	0.24 0.151	-0.34 0.029
	Se														1.00	0.47 0.002	-0.30 0.067	-0.24 0.151	-0.47 0.002
-	As													1.00	-0.12 0.451	0.04 0.813	-0.10 0.56	0.34 0.034	0.05 0.766
-	Ņ												1.00	0.01 0.908	0.35 0.027	0.54 0.0003	0.04 0.816	0.04 0.805	-0.24 0.127
4	C											1.00	0.47 0.00	0.27 0.083	0.16 0.322	0.32 0.043	0.04 0.831	0.15 0.353	-0.26 0.097
flactatio	Mn										1.00	0.21 0.186	0.07 0.655	0.24 0.125	0.25 0.116	0.28 0.072	-0.23 0.153	0.19 0.261	-0.29 0.067
st third o	cr									1.00	0.08 0.637	0.27 0.087	0.44 0.004	-0.23 0.155	0.20 0.210	0.15 0.353	0.22 0.181	-0.21 0.215	-0.27 0.084
ing the fir	٩								1.00	0.21 0.194	0.39 0.012	0.34 0.028	0.54 0.0003	0.310 0.049	0.33 0.034	0.40 0.009	-0.13 0.431	0.29 0.082	-0.14 0.396
t milk dur	Zn							1.00	0.18 0.272	0.36 0.019	0.05 0.779	-0.07 0.674	0.10 0.519	-0.32 0.044	-0.09 0.594	-0.3 0.027	0.34 0.032	-0.10 0.566	0.04 0.814
pine goat	Fe						1.00	-0.03 0.869	0.08 0.616	-0.08 0.623	0.002 0.989	0.15 0.354	0.07 0.667	0.65 <0.001	-0.29 0.062	-0.12 0.471	-0.04 0.816	0.177 0.288	0.05 0.778
ents in Al	Cu					1.00	0.07 0.657	0.14 0.371	0.34 0.031	0.27 0.091	0.09 0.570	0.18 0.250	0.16 0.328	0.03 0.849	0.13 0.413	0.14 0.374	0.42 0.008	0.23 0.167	0.45 0.004
18 elem	Na				1.00	-0.04 0.799	0.01 0.927	-0.05 0.740	0.26 0.104	-0.25 0.121	0.22 0.167	0.10 0.545	-0.09 0.566	0.20 0.201	0.26 0.094	0.14 0.383	-0.46 0.003	-0.08 0.654	-0.27 0.091
ficients of	х			1.00	-0.31 0.048	-0.01 0.934	-0.03 0.859	0.02 0.920	0.29 0.067	-0.13 0.405	0.15 0.364	0.05 0.761	-0.16 0.328	0.09 0.568	0.05 0.732	-0.04 0.811	-0.02 0.895	0.14 0.396	0.06 0.718
ition coef	Mg		1.00	-0.05 0.77	0.27 0.091	0.420 0.006	0.06 0.687	0.06 0.697	0.66 <0.001	0.24 0.131	0.18 0.269	0.40 0.010	0.45 0.003	0.34 0.028	0.06 0.722	0.22 0.158	0.11 0.501	0.45 0.004	0.21 0.190
3. Correla	Ca	1.00	0.69 <0.001	-0.13 0.421	0.22 0.166	0.33 0.036	0.03 0.857	-0.06 0.720	0.73 <0.001	0.27 0.094	0.24 0.137	0.50 0.001	0.764 <0.001	0.23 0.147	0.42 0.006	0.57 <0.001	-0.15 0.365	0.26 0.114	-0.17 0.301
Table .		Ca	Mg	×	Na	Cu	Ъ	Zn	٩	Ċ	Ч	C	īŻ	As	Se	Mo	Cd	Hg	Pb

## Conclusion

According to the obtained research results, it can be concluded that the milk sampling period significantly influenced the content of lactose and the concentrations of Na, Se, Mo, Cr, Ni and As in Alpine goat milk in the first third of lactation. Milk of Alpine goats sampled in the first third of lactation had a very low concentration of heavy metals and significantly positive correlation between Ca:Mg, Ca:P, Ca:Co, Ca:Ni, Ca:Mo; Mg:P, Fe:As, Pb:Cd and P:Ni. The observed changes indicate that Alpine goat milk is rich in essential elements, while the concentration of toxic elements is very low in the first third of lactation.

## Acknowledgments

The study was carried out within the research team Innovative breeding and technological processes in animal production (No. 1126) at Faculty of Agrobiotechnical Sciences Osijek.

# Analiza 18 elemenata u mlijeku alpina koza u prvoj trećini laktacije

#### Sažetak

Cilj ovoga rada bio je utvrditi utjecaj stadija laktacije u prvoj trećini laktacije na 18 elemenata sadržanih u mlijeku alpina koza. Istraživanje je provedeno na 20 koza pasmine francuska alpina, prosječne dobi 5 godina i u četvrtoj laktaciji, koje su praćene 30. i 90. dana laktacije. U uzorcima mlijeka je određen osnovni kemijski sastav infracrvenom spektrofotometrijom te koncentracija 18 elemenata (Ca, P, K, Na, K, Mg, Fe, Zn, Cu, Se, Mn, Mo, Co, Cr, Ni, Cd, Pb, As i Hg) primjenom induktivno spregnute plazme (ICP). Vrijeme uzorkovanja mlijeka u prvoj trećini laktacije značajno je utjecalo na promjene koncentracije Na, Se, Mo, Cr, Ni i As. Utvrđene su značajno više koncentracije Na i As u mlijeku 90. dana uzorkovanja te niže Se, Mo, Cr i Ni 30. dana uzorkovanja tijekom prve trećine laktacije. Analizom koeficijenata korelacije između istraživanih elemenata u tragovima i određenih toksičnih elemenata u mlijeku alpina koza utvrđena je značajno pozitivna korelacija između Ca:Mg, Ca:P, Ca:Co, Ca:Ni, Ca:Mo; Mg:P, Fe:As, Pb:Cd i P:Ni. Utvrđena je vrlo niska koncentracija teških metala u mlijeku alpina koza u prvoj trećini laktacije. Navedene promjene ukazuju da je mlijeko Alpina koza bogat izvor esencijalnih elemenata, dok je koncentracija toksičnih elemenata bila vrlo niska u prvoj trećini laktacije.

Ključne riječi: alpina koze; laktacija; mlijeko; elementi

#### References

- 1. Abba, B., Petrol, B.B., Ali, H.A., Chamba, G., Sanda, F.S., Modu, S. (2021): Determination of some heavy metals and proximate composition of camel, cow, goat and sheep milk. *Chem Search Journal* 12 (2), 50-55.
- Almašiova, S., Toman, R., Pšenkova, M., Tančin, V., Miklaš, Š., Jančo, I. (2023): Chemical elements content in goat milk, whey, cheese and yogurt from an ecological and conventional farm in Slovakia. *Journal of Central European Agriculture* 24 (1), 43-52. https://doi.org/10.5513/JCEA01/24.1.3733
- 3. Antunac, N., Lukač Havranek, J., Samaržija, D. (2001): Effect of breed on chemical composition of goat milk. *Czech Journal of Animal Science* 6, 268-264.
- Antunović, Z., Klapec, T., Čavar, S., Mioč, B., Novoselec, J., Klir, Ž. (2012): Changes of heavy metals concentrations in goats milk during lactation stage in organic breeding. *Bulgarian Journal of Agricultural Science* 18, 166-170.
- Antunović, Z., Mioč, B., Novoselec, J., Širić, I., Držaić, V., Klir Šalavardić, Ž. (2023): Essential trace and toxic element content in Lacaune sheep milk during lactation. *Foods* 12, 4291. https://doi.org/10.3390/foods12234291

- Antunović, Z., Marić, I., Lončarić, Z., Novoselec, J., Mioč, B., Klir, Ž. (2018): Changes in macroelements, trave elements, heavy metal concentrations and chemical composition in milk of Croatian spotted goats during different lactation stages. *International Journal of Dairy Technology* 71, 3, 621-628. https://doi.org/10.1111/1471-0307.12496
- Belete, T., Hussen, A., Rao, V.M. (2014): Determination of concentrations of selected heavy metals in cow's milk: Borena Zone, Ethiopia. *Journal of Health Science* 4 (5), 105-112. https://doi.org/10.5923/j.health.20140405.01
- Bendelja Ljoljić, D., Prpić, Z., Mašek, T., Vnučec, I., Kostelić, A., Benić, M., Antunac, N. (2023): Milk urea concentration as a tool for optimising crude protein content in dairy goat diets: a path to sustainable milk production. *Mljekarstvo*, 73 (2), 85-94. https://doi.org/10.15567/mljekarstvo.2023.0202
- Bilandžić, N., Šedak, M., Čalopek, B., Božić Luburić, D., Solomun Kolanović, B., Varenina, I., Đokić, M., Kmetoć, I., Murati, T. (2016): Lead concentrations in raw cow and goat milk collected in rural areas of Croatia from 2010 to 2014. *The Bulletin of Environmental Contamination and Toxicology*. https://doi.org/10.1007/s00128-016-1749-z
- 10. Bosnak, C.P., Davidowski, L., Life, P. (2004): Continuous flow hydride generation using the optima ICP. Shelton: Perkin Elmer Field Application Report. 1-4.
- 11. Briffa, J., Sinagra, E., Blundell, R. (2020): Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon* 6, e04691. https://doi.org/10.1016/i.heliyon.2020.e04691
- Chauhan, S.P.S., Kumar, S., Jeena, A., Verma, R., Panwar, V.A.R., Patel, P., Budhalakoti, M. (2019): Study the effect of different lactation stages on minerals of Pantja goat milk. *International Journal of Current Microbiology and Applied Sciences* 8 (6), 1598-1602.
  - https://doi.org/10.20546/ijcmas.2019.806.192
- Chen, L., Li, X., Li, Z., Deng, L. (2020): Analysis of 17 elements in cow, goat, buffalo, yak, and camel milk by inductively coupled plasma mass spectrometry (ICP-MS). *The Royal Society of Chemistry* 10, 6736. https://doi.org/10.1039/d0ra00390e
- 14. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance).
  - http://data.europa.eu/eli/reg/2023/915/oj
- Curró, S., de Marchi M., Claps, S., Salzano, A., de Paolo, P., Manuelian, C.L., Neglia, G. (2019): Differences in the detailed milk mineral composition of Italian local and Saanen goat breeds. *Animals* 9 (7), 412. https://doi.org/10.3390/ani9070412
- 16. FAO (2024). Available online: http://www.fao.org/faostat/en/#data/QP (accessed on 11 January 2021).
- 17. Fayet, F., Ridges L.A., Wright, J.K., Petocz, P. (2013): Australian children who drink milk (plain or flavored) have higher milk and micronutrient intakes but similar body mass index to those who do not drink milk. *Nutrition Research* 33 (2), 95-102. https://doi.org/10.1016/j.nutres.2012.12.005
- Filho, E.J.L.M., Aniceto, E.S., da Silva. I.N., Júnior, V.R.R., Rodrigues, M.T., de Oliveira, T.S. (2024): Body content and mineral requirements of dairy goats in early lactation. *Small Ruminant Research* 230, 107153. https://doi.org/10.1016/j.smallrumres.2023.107153
- 19. Garba, S., Abdullahi, S., Abdullahi, M. (2018): Heavy metal content of cow's milk from Maiduguri Metropolis and its environs, Borno state Nigeria. *America Journal of Engineering Research* 7, 63-73.
- 20. Güler, Z. (2007): Levels of 24 minerals in local goat milk, its strained yoghurt and salted youghurt (tuzlu yogurt). *Small Ruminant Research* 71, 130-137.
- Guo, X., Liu, H., Zhao, Q., Qin, Y., Zhang, J. (2021): Discrimination of goat, buffalo, and yak milk from different livestock, regions, and lactation using microelement contents. *Journal of Food Science* 86, 1283-1295. https://doi.org/10.1111/1750-3841.15685

- 22. Hejtmankova, A., Kucerova, J., Mihalova, D., Kolihova, D. and Orsak, M. (2002): Levels of selected macro and micro elements in goat milk from farms in the Czech Republic. *Czech Journal of Animal Science* 47, 253–260.
- Homayonibezi, N., Dobaradaran, S., Arfaeinia, H., Mahmoodi, A., Mohammad Sanati, A., Farzaneh, M.R., Kafaei, R., Afsari, M., Fouladvand, M., Ramavandi, B. (2020): Toxic heavy metals and nutrient concentration in the milk of goat herds in two Iranian industrial and non-industrial zones. *Environmental Science and Pollution Research* 28 (12), 14882-14892. https://doi.org/10.1007/s11356-020-11732-w
- 24. Croatian Agency for Agriculture and Food Hrvatska, HAPIH (2023): Sheep, goats and small animals breeding. Annual Report for 2022. Croatian Agency for Agriculture and Food, Osijek, Croatia, pp. 28 and 79.
- Hussain, A., Alamzeb, S., Begum, S. (2013): Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. *Food Chemistry* 136 (3-4), 1515-1523. https://doi.org/10.1016/j. foodchem.2012.09.058
- Ismail, A., Riaz, M., Akhtar, S., Goodwill, E.J., Sun, J. (2017): Heavy metals in milk: global prevalence and health risk assessment, *Toxin Reviews* 1-12. https://doi.org/10.1080/15569543.2017.1399276
- 27. Kędzierska-Matysek, M., Barłowska, J., Litwińczuk, Z., Koperska, N. (2015): Content of macro- and microelements in goat milk in reation to the lactation stage and region of production. *Journal of Elementology* 20, 107-114.
- Klir, Ž., Potočnik, K., Antunović, Z., Novoselec, J., Barać, Z., Mulc, D., Kompan, D. (2015): Milk production traits from Alpine breed of goats in Croatia and Slovenia. *Bulgarian Journal of Agricultural Science* 21 (5), 1064-1068.
- 29. Krstanović, V., Slačanac, V. Božanić, R., Hardi, J., Rezessyne, J., and Lučan, M. (2010): Nutritional and therapeutic value of fermented caprine milk. *International Journal of Dairy Technology* 63, 171-189.
- Licata, P., Trombetta, D., Cristani, M., Giofre, M., Martino, D., Calo, M., Naccari, F. (2004): Levels of "toxic" and "essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy. *Environment International* 30, 1-6.
- Llobet, J.M., Falco, G., Casas, C., Teixido, A., Domingo, J.L. (2003): Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *Journal* of Agricultural Food Chemistry 51 (3), 838-842.
- Marquès, M., Correig, E., Capdevila, E., Gargallo, E., González, N. Nadal, M., Domingo, J.L. (2022): Essential and non-essential trace elements in milks and plant-based drinks. *Biological Trace Element Research* 200, 4524-4533. https://doi.org/10.1007/s12011-021-03021-5
- 33. Mayer, H.K. and Fiechter, G. (2012): Physicochemical characteristics of goat's milk in Austria-seasonal variations and differences between six breeds. *Dairy Science & Technology* 92, 167-177.
- 34. Michlová, T., Hejtmánková, A., Dragounová, H., Horníčková, Š. (2016): The content of minerals in milk of small ruminants. *Agronomy Research* 14 (2), 1407-1418.
- 35. NRC (2007): Nutrient requirements of small ruminants (sheep, goats, cervids and new world camelides). The National Academies Press. Washington, D.C., p. 362.
- Pan, J., Yu, Z., Jiang, H., Shi, C., Du, Q., Fan, R., Wang, J., Bari, L., Yang, Y., Han, R. (2023): Effect of lactation on the distribution of mineral elements in goat milk. *Journal of Dairy Science* 107 (5), 2774-2784. https://doi.org/10.3168/jds.2023-23877
- 37. Park, Y.W., Chukwu, H.I. (1989): Trace mineral concentrations in goat milk from French-Alpine and Anglo-Nubian breeds during the first 5 months of lactation. *Journal of Food Composition and Analysis* 2 (2), 161-169.
- Park, Y., Juarez, M., Ramos, M., Haenlein, G. (2007): Physico chemical characteristics of goat and sheep milk. *Small Ruminant Research* 68, 88-113. https://doi.org/10.1016/j.smallrumres.2006.09.013

- Paskaš, S., Miočinović, J., Pihler, I., Čobanović, K., Savić, M., Becskei, Z. (2023): The influence of grazing and indoor systems on goat milk, brined cheese and whey quality. *Mljekarstvo* 73 (3), 143-154. https://doi.org/10.15567/mljekarstvo.2023.0301
- Rey-Crespo, F., Miranda, M., López-Alonso, M. (2013): Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. *Food and Chemical Toxicology* 55, 513-518. https://doi.org/10.1016/j.fct.2013.01.040
- 41. SAS® 9.4 (2002-2012). SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA.
- 42. Shuvarikov, A.S., Pastukh, O.N., Zhukova, E.V., Zheltova, O.A. (2021): The quality of milk of goats of Saanen, Alpine and Nubian breeds. *IOP Conference Series: Earth Environmental Science* 640, 032031, 15.
- Singh, M., Yadar, P., Garg, V.K., Sharma, A., Singh, B., Sharma, H. (2015): Quantification of mineral and trace elements in raw caprine milk using flame atomic absorption spectrophotometry and flame photometry. *Journal of Food Science and Technology* 52 (8), 5299-5304. https://doi.org/10.1007/s13197-014-1538-9
- 44. Stocco, G., Summer, A., Malacarne, M., Cecchinato, A., Bittante, G. (2019): Detailed macro- and micromineral profile of milk: Effects of herd productivity, parity, and stage of lactation of cows of 6 dairy and dual-purpose breeds. *Journal of Dairy Science* 102, 9727-9739.

https://doi.org/10.3168/jds.2019-16834

- 45. Strzalkowska, N., Bagnicka, E., Jóźwik, A., Krzyzewski, I. (2008): Macro- and microelements' concentration in goat milk during lactation. *Züchtungskunde* 80 (5), 404-411.
- 46. Suttle, N.F. (2010): Mineral nutrition of livestock, fourth ed. Cabi Publishing, UK
- 47. Voutsinas, L.R, Pappas, C.R, Katsiari, M.C. (1990): The composition of Alpine goat's milk during lactation in Greece. *Journal of Dairy Research* 57, 41-45.
- Zhou, X., Qu, X., Zhao, S., Wang, J., Li, S., Zheng, N. (2017): Analysis of 22 elements in milk, feed, and water of dairy cow, goat, and buffalo from different regions of China. *Biological Trace Element Research* 176, 120-129.