International Journal of Dairy Technology

SDT Society of Dairy Technology

doi: 10.1111/1471-0307.12496

ORIGINAL RESEARCH

# Changes in macroelements, trace elements, heavy metal concentrations and chemical composition in milk of Croatian spotted goats during different lactation stages

ZVONKO ANTUNOVIĆ,[1]* IVICA MARIĆ,[2] ZDENKO LONČARIĆ,[1] JOSIP NOVOSELEC,[1] BORO MIOČ[3] and ŽELJKA KLIR[1]

[1]*Faculty of Agriculture in Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia,* [2]*Croatian Agricultural Agency, Ilica 101, 10000 Zagreb, Croatia, and* [3]*Department of Animal Science and Technology, Faculty of Agriculture, University of Zagreb, Svetosimunska cesta 25, 10000 Zagreb, Croatia*

*The research was conducted on days 60, 90, 120 and 150 during the lactation with the aim to determine the effect of lactation stage on the concentration of crucial elements and chemical composition in Croatian spotted goats' milk. Milk yield significantly decreased (from 1.15 to 0.76 kg/day), while the concentration of Ca, Mg, P, Zn and Mo in the milk of the goats significantly increased during lactation. The concentrations of Cr, Cd and Pb in milk were very low, with no significant deviations during lactation. The quality of milk from Croatian spotted goats is adequate compared with other breeds in the surrounding area of Croatia.*

**Keywords** Essential elements, Goats, Lactation, Quality of milk.

## INTRODUCTION

The production of goats' milk worldwide has been increasing over the last few years. The consumption of goats' milk is particularly important in the nutrition of children who develop allergy to cows' milk, in nursing mothers and in the general population as a treatment of peptic disorders (Hejtmankova *et al.* 2002; Haenlein 2004; Kapila *et al.* 2013). Goats' milk contains numerous nutritients necessary for the growth and development of kids (Gaucheron 2011). Concentrations of selected toxic elements, especially of heavy metals in the environment, is generally used as an early indicator of contamination phenomena, both in the programmes of soil quality control and in air quality monitoring (Caggiano *et al.* 2005). Although the concentration of heavy metals in goats' milk is very low, it can be used as an indicator of a contaminated environment (Antunović *et al.* 2012a). The vertical investigation of geochemical information is an essential approach for authenticating the geographical origin of milk/cheese production that has already been attempted for some other products (Osorio *et al.* 2015). Croatian spotted goat, also known as the Balkan goat, is the most abundant goat breed in Croatia (estimated population is approximately 25 000 heads). The goat breed developed in the karst, a scant and inaccessible area of the Velebit, Dinara, Kamešnice and Biokovo mountains, where they are even today mostly reared. Because of their skills and mobility, these goats are reared on the most inaccessible terrain, where there is small or no possibility of rearing other types of livestock, especially cows (Mioč *et al.* 2008a). Croatian spotted goats are very resistant, flexible and durable, having modest requirements for housing, keeping and feeding. The average body weight of an adult goat is 44 kg, height at withers 61.32 cm and body length 69 cm. In the flocks reared for milk production, the lactation length is 150–250 days, during which one goat produces 100–250 L of milk (Mioč *et al.* 2008b). According to the available literature, research on the production properties of Croatian spotted goat is rare and there is no information about the milk quality of this breed. Croatian spotted goat is mostly used for the production of kids' meat; however, in recent years, farmers have opted for the production of valued goat cheese prepared according to traditional recipes, all with the purpose

*Author for correspondence. E-mail: zantunovic@pfos.hr

of enriching what is on offer for tourists (Antunović *et al.* 2012b).

The aim of this study was to determine the milk yield, chemical composition, changes in macroelements, trace elements and heavy metal concentrations in the milk of Croatian spotted goats at different lactation stages.

## MATERIALS AND METHODS

### Experimental design

The research was conducted on a family farm with a long tradition of goat farming in the Dubrovnik–Neretva County. From the flock of 80 adult Croatian spotted goats, 20 goats were selected and monitored during lactation. Apart from uniform body development, good health condition and adequate body condition, the goats were selected according to a uniform age (4 years), parity (3rd lactation), lactation stage (±7 days) and also the same litter size (single). The goats were grazing extensively on the Mediterranean pastures from early morning until 10 a.m., when they returned to a stall because of high temperatures, to be fed with hay and approximately 0.2 kg/day of maize, as well as water and salt. Hand milking was performed. The study was carried out on days 60, 90, 120 and 150 during the lactation. Kids were kept with their mothers and allowed to graze until the age of 90 days. The milk production control was performed using the alternating monthly test (AT method; ICAR, 2003) with a single milking by hand once a month (every 30 days), by measuring the quantity of milk and by taking individual samples of milk for chemical analysis. The milk yield (kg) was calculated by multiplying the quantity of milk shown in litres (L) with the average density of goats' milk 1.030 $kg/m^3$.

Milk samples were collected in 200-mL plastic bottles, cooled in a mobile refrigerator at +4 °C and transferred to the laboratory within 24 h for analysis. Nonfat dry matter, fat, lactose and urea content, the somatic cells count (SCC) and total bacterial count were determined in the milk samples analysed. The analyses were performed in the Central Laboratory for Milk Control in Križevci. The analyses of milk for fat, protein, lactose and urea content were conducted with infrared spectrometry (HRN EN ISO 9622:2001) on the MilkoScan FT 6000 (Foss Electric, Hillerød, Denmark) analyzer within the Comby system. The number of somatic cells was determined by a fluoro-opto-electronic method (HRN EN ISO 13366-2/Ispr. 1. 2007) with a Fossomatic 5000 (Foss Electric, Hillerød, Denmark) analyzer, while the total bacterial count was determined with an epifluorescent flow cytometry method (IDF 161A:1995). To obtain normal distribution of SCC and total bacterial count, the values were transformed to the logarithmic scale ($log_{10}$).

### Soil and plant analyses

The soil layer at a depth of 0–30 cm and composite samples (20–25 subsamples) were sampled at each location. Soil samples were prepared for chemical analyses according to the procedure for the pretreatment of samples for physico-chemical analyses (ISO, 1994). Soil samples were ground using a heavy metal-free grinder (Retsch RM 200, Retsch GmbH, Haan, Germany) and sieved through 2-mm sieves. The concentrations of mineral elements in soil samples were extracted by *aqua regia* (ISO 1995), and this fraction was considered as soil total content. The soil samples were digested at 210 °C for 60 min in microwave oven (Mars 6; CEM, Matthews, NC, USA).

The concentrations of mineral elements in the extracts were determined by ICP-OES (Optima 2100 DV; PerkinElmer, Massachusetts USA). Each batch of soil samples run on the inductively coupled plasma was analysed with an internal pooled plasma control and with the reference material prepared in the same way as the soil samples extracted by *aqua regia*. All samples were analysed in duplicate, and all soil samples from the same year were analysed within the same analytic run. The instrumental detection and quantification limits for the determination of essential minerals and selected toxic elements in soil, goats' milk and feed (mg/kg) are presented in Table 1.

All plant samples (corn, hay and green forage from pastures) were dried and ground into a fine powder using a heavy metal-free ultracentrifugal mill (Retsch ZM 200) or knife mill (GM 200). All plant samples were digested with 10 mL of a 5:1 mixture of $HNO_3$ and $H_2O_2$ at 180 °C for 60 min in a microwave oven (Mars 6; CEM). The concentrations of mineral elements in solutions of digested plant samples were determined by inductively coupled plasma

**Table 1** Instrumental detection and quantification limits for the determination of essential minerals and selected toxic elements in soil, goat milk and feed (mg/kg).

| *Mineral/ Heavy metal* | *IDL* | | *IQL* | |
|---|---|---|---|---|
| | *Milk* | *Soil and feed* | *Milk* | *Soil and feed* |
| Ca | 0.06606 | 0.6606 | 0.202 | 2.02 |
| Mg | 0.01098 | 0.1098 | 0.0366 | 0.366 |
| K | 0.54 | 5.40 | 1.81 | 18.1 |
| Cu | 0.005422 | 0.054219 | 0.018073 | 0.18073 |
| Fe | 0.005433 | 0.05433 | 0.01811 | 0.1811 |
| Zn | 0.000934 | 0.009339 | 0.003113 | 0.03113 |
| Mn | 0.000024 | 0.00024 | 0.00008 | 0.0008 |
| Ni | 0.0027 | 0.027 | 0.009045 | 0.09045 |
| Mo | 0.00318 | 0.0318 | 0.01069 | 0.1069 |
| Co | 0.00115 | 0.0115 | 0.003836 | 0.03836 |
| Cr | 0.000941 | 0.009405 | 0.003135 | 0.031350 |
| Cd | 0.00063 | 0.0063 | 0.00212 | 0.0212 |
| Pb | 0.01001 | 0.1001 | 0.0333 | 0.0212 |
| As | 0.00007735 | 0.002466 | 0.0002578 | 0.00822 |

IDL, instrumental detection limit; IQL, instrumental quantification limit.

(ICP; Optima 2100 DV; PerkinElmer). Each batch of plant samples run on the ICP was analysed with an internal pooled plasma control and with the reference material prepared in the same way as other plant samples. All samples were analysed in duplicate. Concentrations of mineral elements and selected toxic elements in the soil and feed of the goats (mg/kg) are presented in Figure 1.

### Milk digestion and preparation for analyses

Before collecting the raw milk, sampling bottles were soaked in 20% $HNO_3$ for 24 h and washed with deionised water to avoid possible contamination. A milk sample of 100 mL was collected during morning milking from each goat, homogenised by vortex (VIBROMIX 10; Tehtnica, Železniki, Slovenia), stored in fridge box at 4 °C and transferred to a deep freeze (−80 °C) until microwave digestion was carried out.

The method was validated using milk powder (NCSZC73015; National Analysis Center for Iron and Steel, Beijing, China) as a reference material. The digestion of milk samples was carried out according to the method described by Belete *et al.* (2014). A 3.0 mL aliquot of each liquid milk sample was transferred to a 60-mL Teflon digestion vessel. Optimised volumes of 6 mL of 70% nitric acid and 1 mL of 30% hydrogen peroxide were added, and the mixture was shaken carefully and left for 10 min before closing the vessel. The samples were subjected to closed microwave digestion at the optimised microwave digestion programme with 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 Mars 6 (CEM) microwave system. After heating, the sample was cooled to room temperature and the digestion vessels were opened carefully in a digester. The digest was diluted to 25 mL with deionised water and used for further analysis.

The digested samples were analysed with continuous flow hydride generation technique, using ICP (Optima 21000 DV; PerkinElmer) for Ca, Mg, K, P, Na, Cu, Fe, Zn, Mn, Ni, Mo, Co, Cr, Cd and Pb concentrations. The digested milk samples provided for As determination were subjected to the prereduction step before the analysis according to Bosnak and Davidowski (2004). For the prereduction 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 millilitres of concentrated HCl were also added, and the mixture was allowed to stand for at least 20 min. The tube was brought to the 50-mL mark with deionised water, and the sample was then ready to run. For the prereduction of Se and Hg, a 20 mL of sample was placed in a cleaned 125-mL beaker and 20 mL of concentrated HCl were slowly added. The solution was then transferred to a 50-mL polypropylene autosampler tube, and diluted to the 50-mL mark with deionised water. The samples were ready to run with inductively coupled plasma (Optima 21000 DV; PerkinElmer).

### Statistical analysis

A total of 80 observations was collected for mineral composition of milk during lactation stage. Data were analysed

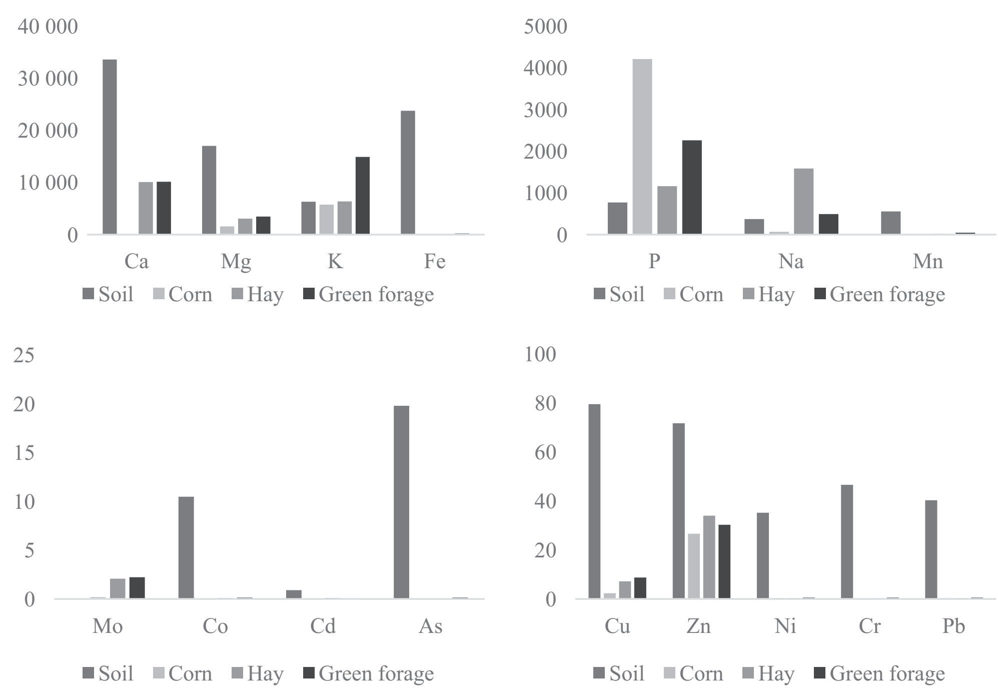


**Figure 1** Concentrations of mineral elements and selected toxic elements in soil and feed of goats (mg/kg). Concentrations of Fe, Mn, Co, Cd, As, Ni, Cr and Pb were very low or below the instrumental quantification limits in corn, hay and green forage.

with the statistical software SAS 9.3® (SAS Institute Inc., Cary, NC, USA). The results are presented as arithmetic means with standard deviation and standard error of mean estimated with MEANS procedure, while Pearson's correlation between essential or toxic elements was estimated with CORR procedure. Data were analysed with GLM procedure using lactation stage as a fixed effect. Means were compared by the LSD test, and differences between lactation stage effect were declared significant at $P < 0.05$ level.

## RESULTS AND DISCUSSION

Table 2 presents the milk yield and indicators of milk quality from the Croatian spotted goats, at different lactation stages.

When analysing the results from Table 2, it is evident that most of the estimated indicators increased in the goats' milk during the lactation, except for milk yield and lactose content. Milk yield significantly decreased during lactation and ranged between 1.15 and 0.76 kg/day. A significantly higher content of dry matter without fat, fat content, protein and urea ($P < 0.01$) was determined at the end of lactation (day 150), while the opposite trend was observed for lactose content. The lactose content decreased during lactation, and a higher content was determined on day 90 compared with day 120. Milk yield significantly depended on various factors: age, body weight, litter size, breed, lactation stage, parity, production season, etc. (Antunac 1990; Antunac and Samaržija 2000; Brodziak *et al.* 2014). Milk yield is inversely proportional to the fat and protein content of milk. Likewise, traditional extensive farming of Croatian spotted goat reflects its production traits, while its production results are modest when compared to other breeds of higher genetic potential reared in intensive farming systems. Marković (2003) determined an average daily milk yield of 0.638 L with 3.38% fat, 3.30% proteins and 8.38% dry matter without fat in the Balkan goat in Montenegro. Bogdanović *et al.* (2010) studied milking traits of the Balkan goat in the 'low-input' production systems in Serbia and determined a similar content of milk fat (3.71%). The reason for the lower fat content of Croatian spotted goats' milk on day 60 is probably related to the duration of the suckling period, when goats withhold and store milk for their kids, and it is known that the last jets of milk are the richest in fat. In addition, this period is the peak of lactation, which is related to the lower content of dry matter in milk (Kędzierska-Matysek *et al.* 2015). Similar results for the content of proteins, lactose and dry matter without fat in milk of Alpine goats were determined by Antunac (1990) and Pavliček *et al.* (2006) and by Prpić *et al.* (2015) in the milk of Saanen goats. Mayer and Fiechter (2012) found similar values for the most investigated traits in goats of six breeds in Austria. The SCC determined for the milk of Croatian spotted goats is lower when compared to the higher limit of reference values for goat milk (Regulations on quality of fresh raw milk, 2000). Considering the SCC variability during the lactation, there were no significant differences in the different lactation stages. Similar changes were determined for total bacterial counts, where the trend of increase was evident during the lactation progression. The above-mentioned issue indicated the seasonal changes in the hygienic quality of the milk from Croatian spotted breed. However, it is well known that the SCC in goat milk is variable, as it depends on various factors such as breed, stage of lactation and parity, as well as litter size, reproductive status and season (Raynal-Ljutovac *et al.* 2007). Similar results in the milk of Alpine goats were reported by Pavliček *et al.* (2006).

When analysing Table 3 and Figure 2, there were significant increases in Ca, Mg, P, Zn and Mo concentrations in the milk of Croatian spotted goats as lactation progressed. Furthermore, a trend for Na, Cu, Fe and Pb concentration increase was observed, as well as the decrease in K in the milk of goats during lactation, despite no significant differences. The concentrations of Mn and Ni did not deviate with lactation stage. In addition, there were no significant deviations in heavy metal concentrations in the goats' milk and values for the potential toxic elements such as Cd, Pb

**Table 2** Influence of lactation stage on yield and quality of milk from Croatian spotted goats.

| Indicator | Day in lactation (mean ± SD) 60th | 90th | 120th | 150th | SEM | *P*-value |
|---|---|---|---|---|---|---|
| Milk yield (kg/day) | $1.15^{a} \pm 0.47$ | $0.96 \pm 0.41$ | $0.93 \pm 0.25$ | $0.76^{b} \pm 0.28$ | 0.04 | 0.01 |
| Nonfat dry matter (%) | $8.94^{b} \pm 0.38$ | $9.22^{b} \pm 0.33$ | $9.01^{b} \pm 0.51$ | $10.33^{a} \pm 0.81$ | 0.89 | <0.001 |
| Fat (%) | $3.28^{b} \pm 1.43$ | $3.32^{b} \pm 1.55$ | $3.39^{b} \pm 0.96$ | $5.61^{a} \pm 1.91$ | 0.20 | <0.001 |
| Protein (%) | $3.65^{b} \pm 0.36$ | $3.89^{b} \pm 0.25$ | $4.06^{a,b} \pm 0.48$ | $5.24^{a} \pm 1.04$ | 0.10 | <0.001 |
| Lactose (%) | $4.29^{a} \pm 0.26$ | $4.32^{a} \pm 0.17$ | $3.97^{b} \pm 0.45$ | $4.09^{b} \pm 0.36$ | 0.04 | 0.003 |
| Urea (mg/dL) | $24.98^{c} \pm 4.82$ | $37.91^{b} \pm 10.13$ | $34.39^{b} \pm 9.20$ | $45.16^{a} \pm 9.06$ | 1.25 | <0.001 |
| SSC ($log_{10}$/mL) | $6.01 \pm 0.74$ | $5.53 \pm 0.64$ | $5.67 \pm 0.60$ | $5.91 \pm 0.68$ | 0.08 | 0.105 |
| CFU ($log_{10}$/mL) | $4.66 \pm 1.21$ | $4.86 \pm 0.64$ | $4.65 \pm 0.55$ | $5.24 \pm 0.88$ | 0.10 | 0.102 |

SD, standard deviation, SEM, standard error of mean, CFU, total bacterial count.

Means within a row with different superscripts differ ($P < 0.05$).

**Table 3** Concentrations of minerals in goats' milk at different stages of lactation.

| Mineral (mg/kg) | Day in lactation (Mean ± SD) 60th | 90th | 120th | 150th | SEM | P-value |
|---|---|---|---|---|---|---|
| Ca | $1108.43^{b,d} \pm 182.06$ | $1226.07^{b,c,d} \pm 109.73$ | $1276.15^{b,c} \pm 223.71$ | $1589.36^{a} \pm 416.88$ | 2.58 | <0.001 |
| Mg | $131.12^{b} \pm 18.73$ | $126.55^{b} \pm 14.12$ | $134.64^{b} \pm 20.15$ | $179.74^{a} \pm 52.29$ | 134.57 | <0.001 |
| K | 1728.28 ± 174.58 | 1816.19 ± 184.27 | 1660.38 ± 339.60 | 1605.60 ± 250.99 | 28.83 | 0.399 |
| P | $687.89^{c} \pm 98.60$ | $895.43^{b} \pm 99.53$ | $785.46^{c} \pm 150.01$ | $1031.70^{a} \pm 241.31$ | 22.49 | <0.001 |
| Na | 294.10 ± 68.72 | 301.09 ± 33.44 | 494.00 ± 346.86 | 523.60 ± 390.54 | 31.77 | 0.279 |
| Cu | 0.07 ± 0.06 | 0.07 ± 0.03 | 0.07 ± 0.036 | 0.11 ± 0.06 | 0.01 | 0.132 |
| Fe | 0.37 ± 0.18 | 1.29 ± 1.03 | 0.53 ± 0.97 | 0.77 ± 0.85 | 0.10 | 0.157 |
| Zn | $2.40^{b} \pm 0.67$ | $3.73^{a} \pm 0.71$ | $3.37^{a} \pm 1.19$ | $3.60^{a} \pm 1.28$ | 0.13 | <0.001 |
| Mn | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.001 | 0.149 |
| Ni | 0.03 ± 0.02 | 0.03 ± 0.01 | 0.03 ± 0.02 | 0.04 ± 0.01 | 0.002 | 0.065 |
| Mo | $0.03^{b} \pm 0.01$ | $0.03^{b} \pm 0.001$ | $0.03^{b} \pm 0.01$ | $0.04^{a} \pm 0.02$ | 0.002 | 0.004 |

SD, standard deviation, SEM, standard error of mean.
Means within a row with different superscripts differ ($P < 0.05$).

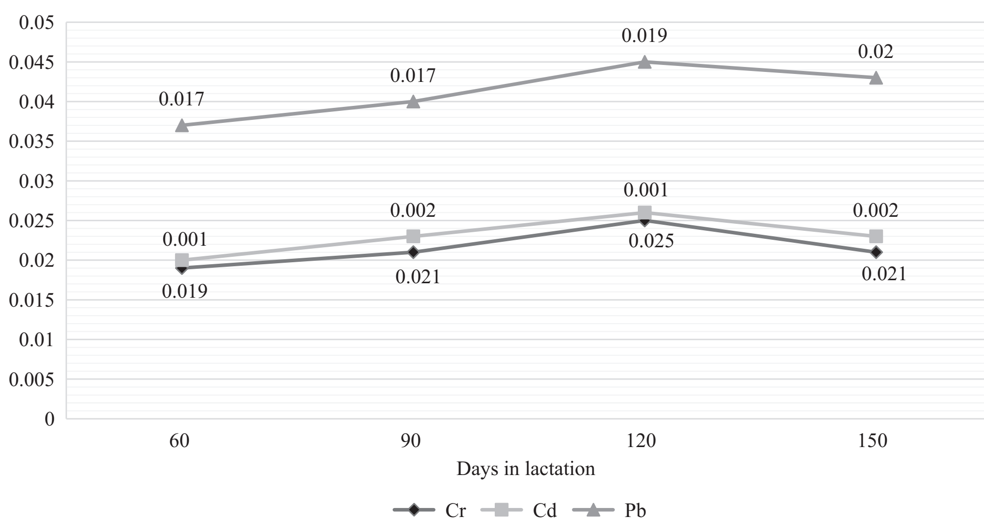


**Figure 2** Concentrations of heavy metals (mg/kg) in goats' milk during different stages of lactation.

and Cr were below the values prescribed by the Commission Regulation directive 333 (2007). Hejtmankova *et al.* (2002) determined an increase in Mg and Fe concentrations during lactation for goats' milk in Czech Republic. Kędzierska-Matysek *et al.* (2015) determined a similar distribution for goats' milk in Poland, but with significant differences depending on lactation stage in concentrations of K and Na, there was a significant increase in Ca and Mg concentrations, as well as an opposite trend for concentrations of Zn, Fe and Cu. The research in Austria with goats' milk showed similar concentrations for Ca, Mg, Na and K (Mayer and Fiechter 2012). Güler (2007) determined higher concentrations of Cd and Pb (0.63 and 0.06 mg/kg) in goats' milk from Turkey. Krelowska-Kulas *et al.* (1999) determined similar concentrations of Pb in goats' milk from Poland (9–20 μg/L) and from Spain; Rodriguez *et al.* (1999) determined lower concentrations of Pb (11.86 μg/L) in goats' milk. Similar results for mineral content of goats' milk in Portugal were obtained by Transoco *et al.* (2010).

Correlations between macroelements, trace elements and heavy metals in goat milk are presented in Table 4. Numerous significant correlations were determined between various parameters in milk of goats during lactation, which were expected due to their metabolic relationships. Positive correlations between Ca:Mg, Zn:Fe, Zn:Cu, Na:Mg and Fe:Cu, as well as negative correlations between K:Na and K:Ca, were determined. A negative trend was recorded for concentrations of Mn:Cu in goat milk in the study carried out in Poland (Kędzierska-Matysek *et al.* 2015). In cows' milk of Holstein Friesian breed and Simmental breed, a high

 

**Table 4** Correlations between macroelements, trace elements and heavy metal concentrations in Croatian spotted goat milk during investigation.

| | *Ca* | *Mg* | *K* | *Na* | *Cu* | *Fe* | *Mn* | *Zn* | *Ni* | *Mo* | *Co* | *Cr* | *Pb* | *Cd* | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca | 1.000 | 0.567 | −0.066 | 0.108 | 0.535 | 0.141 | 0.502 | 0.377 | 0.470 | 0.582 | 0.160 | 0.105 | 0.211 | 0.132 | 0.842 |
| | | <0.001 | 0.563 | 0.346 | <0.001 | 0.216 | <0.001 | <0.001 | <0.001 | <0.001 | 0.159 | 0.357 | 0.062 | 0.245 | <0.001 |
| Mg | 0.567 | 1.000 | −0.367 | 0.535 | 0.487 | 0.066 | 0.564 | −0.155 | 0.385 | 0.668 | 0.495 | −0.031 | 0.113 | −0.023 | 0.550 |
| | <0.001 | | <0.001 | <0.001 | <0.001 | 0.562 | <0.001 | 0.174 | <0.001 | <0.001 | <0.001 | 0.787 | 0.323 | 0.843 | <0.001 |
| K | −0.066 | −0.367 | 1.000 | −0.711 | −0.361 | −0.046 | −0.079 | 0.314 | 0.036 | −0.159 | −0.354 | 0.019 | −0.010 | 0.177 | 0.175 |
| | 0.564 | <0.001 | | <0.001 | 0.001 | 0.689 | 0.490 | 0.005 | 0.752 | 0.162 | 0.001 | 0.871 | 0.933 | 0.118 | 0.122 |
| Na | 0.108 | 0.535 | −0.711 | 1.000 | 0.321 | 0.083 | 0.159 | −0.303 | −0.006 | 0.177 | 0.421 | 0.082 | −0.070 | −0.194 | 0.051 |
| | 0.346 | <0.001 | <0.001 | | 0.004 | 0.468 | 0.161 | 0.006 | 0.960 | 0.117 | 0.001 | 0.473 | 0.538 | 0.086 | 0.656 |
| Cu | 0.535 | 0.487 | −0.361 | 0.321 | 1.000 | −0.013 | 0.356 | 0.083 | 0.223 | 0.414 | 0.112 | −0.094 | 0.071 | −0.037 | 0.399 |
| | <0.001 | <0.001 | 0.001 | 0.004 | | 0.909 | 0.001 | 0.467 | 0.048 | <0.001 | 0.327 | 0.410 | 0.533 | 0.740 | <0.001 |
| Fe | 0.141 | 0.066 | −0.046 | 0.083 | −0.013 | 1.000 | 0.327 | −0.086 | 0.420 | 0.350 | −0.042 | 0.464 | 0.154 | 0.130 | 0.164 |
| | 0.216 | 0.562 | 0.689 | 0.468 | 0.910 | | 0.003 | 0.449 | <0.001 | 0.002 | 0.713 | <0.001 | 0.176 | 0.255 | 0.150 |
| Mn | 0.502 | 0.564 | −0.079 | 0.159 | 0.356 | 0.327 | 1.000 | 0.047 | 0.456 | 0.534 | 0.162 | 0.217 | 0.236 | 0.014 | 0.574 |
| | <0.001 | <0.001 | 0.490 | 0.161 | 0.001 | 0.003 | | 0.681 | <0.001 | <0.001 | 0.155 | 0.055 | 0.037 | 0.902 | <0.001 |
| Zn | 0.377 | −0.155 | 0.314 | −0.303 | 0.083 | −0.086 | 0.047 | 1.000 | 0.173 | −0.030 | −0.305 | −0.024 | 0.081 | 0.391 | 0.516 |
| | <0.001 | 0.174 | 0.005 | 0.007 | 0.467 | 0.449 | 0.680 | | 0.128 | 0.796 | 0.006 | 0.831 | 0.480 | <0.001 | <0.001 |
| Ni | 0.470 | 0.385 | 0.036 | −0.006 | 0.223 | 0.420 | 0.456 | 0.173 | 1.000 | 0.787 | −0.153 | 0.334 | 0.525 | 0.442 | 0.497 |
| | <0.001 | <0.001 | 0.752 | 0.960 | 0.048 | <0.001 | <0.001 | 0.128 | | <0.001 | 0.179 | 0.003 | <0.001 | <0.001 | <0.001 |
| Mo | 0.582 | 0.668 | −0.159 | 0.177 | 0.414 | 0.351 | 0.534 | −0.030 | 0.787 | 1.000 | 0.140 | 0.286 | 0.357 | 0.222 | 0.589 |
| | <0.001 | <0.001 | 0.162 | 0.118 | <0.001 | 0.002 | <0.001 | 0.800 | <0.001 | | 0.218 | 0.011 | 0.001 | 0.049 | <0.001 |
| Co | 0.160 | 0.495 | −0.354 | 0.421 | 0.112 | −0.042 | 0.162 | −0.305 | −0.153 | 0.140 | 1.000 | −0.086 | −0.149 | −0.354 | 0.140 |
| | 0.160 | <0.001 | 0.001 | 0.001 | 0.327 | 0.713 | 0.155 | 0.006 | 0.179 | 0.218 | | 0.452 | 0.190 | 0.001 | 0.220 |
| Cr | 0.105 | −0.031 | 0.019 | 0.082 | −0.094 | 0.464 | 0.217 | −0.024 | 0.334 | 0.286 | −0.086 | 1.000 | 0.157 | 0.037 | 0.097 |
| | 0.357 | 0.787 | 0.871 | 0.473 | 0.410 | <0.001 | 0.055 | 0.831 | 0.003 | 0.011 | 0.452 | | 0.166 | 0.743 | 0.395 |
| Pb | 0.211 | 0.112 | −0.010 | −0.070 | 0.071 | 0.154 | 0.236 | 0.081 | 0.525 | 0.357 | −0.149 | 0.157 | 1.000 | 0.224 | 0.158 |
| | 0.062 | 0.324 | 0.933 | 0.539 | 0.533 | 0.176 | 0.037 | 0.480 | <0.001 | 0.001 | 0.190 | 0.166 | | 0.047 | 0.166 |
| Cd | 0.132 | −0.023 | 0.177 | −0.194 | −0.038 | 0.130 | 0.014 | 0.391 | 0.442 | 0.222 | −0.354 | 0.037 | 0.224 | 1.000 | 0.275 |
| | 0.245 | 0.843 | 0.118 | 0.086 | 0.740 | 0.255 | 0.902 | <0.001 | <0.001 | 0.049 | 0.001 | 0.743 | 0.047 | | 0.014 |
| P | 0.842 | 0.550 | 0.175 | 0.051 | 0.399 | 0.164 | 0.574 | 0.516 | 0.497 | 0.589 | 0.140 | 0.097 | 0.158 | 0.275 | 1.000 |
| | <0.001 | <0.001 | 0.123 | 0.656 | <0.001 | 0.150 | <0.001 | <0.001 | <0.001 | <0.001 | 0.220 | 0.395 | 0.166 | 0.014 | |

correlation between Mg:Ca and Zn:Cu (Pilarczyk *et al.* 2013) was determined. In the study conducted in Italy, Miedico *et al.* (2015) determined a positive correlation in goats' milk for concentrations of Fe:Mn, while Singh *et al.* (2015) determined significantly positive correlations between Fe: Zn, Zn:K and Ca:Na in goats' milk.

## CONCLUSION

Based on the results of the present study, it is evident that the values of the chemical composition of milk as well as milk yield in Croatian spotted goat followed a normal lactation curve. The content of crucial elements' concentrations in goats' milk during the study significantly differed when considering the significant concentrations of Ca, Mg, P, Zn and Mo, as well as the nonsignificant decrease in the concentration of K. The concentrations of heavy metals in milk were very low, while the milk quality of Croatian spotted goats did not differ significantly from other Croatian goats and breeds in the surrounding area.

## ACKNOWLEDGEMENTS

The research elaborated in the presented manuscript is a part of the project 079-1780469-0225, funded by the Ministry of Science, Education and Sports of the Republic of Croatia.

## REFERENCES

Antunac N (1990) Alpine and saanen goats milk yield and composition. *Mljekarstvo* **40** 151–158.

Antunac N and Samaržija D (2000) Production, composition and properties of goat's milk. *Mljekarstvo* **50** 53–66.

Antunović Z, Klapec T, Čavar S, Mioč B, Novoselec J and Klir Ž (2012a) Changes of heavy metals concentrations in goats milk during lactation stage in organic breeding. *Bulgarian Journal of Agricultural Science* **18** 166–170.

Antunović Z, Novoselec J and Klir Ž (2012b) Sheep and goat breeding in the Republic of Croatia-present situations and perspectives. *Krmiva* **54** 99–109.

Belete T, Hussen A and Rao V M (2014) Determination of concentrations of selected heavy metals in cow′s milk: Borena Zone, Ethiopia. *Journal of Health Science* **4** 105–112.

Bogdanović V, Perišić P, Đedović R, Popović Z, Mijić P, Baban M and Antunović B (2010) Characteristics of milk production traits of Balkan goats raised under "low-input" production systems. *Mljekarstvo* **60** 30–36.

Bosnak C P and Davidowski L (2004) Continuous flow hydride generation using the optima ICP. Field application report. *PerkinElmer Life and Analytical Sciences*.

Brodziak A, Król J, Barłowska J and Litwińczuk Z (2014) Effect of production season on protein fraction content in milk of various breeds of goats in Poland. *International Journal of Dairy Technology* **67** 410–419.

Caggiano R, Sabia S, D'Emilio M, Macchiato M, Anastasio A, Ragosta M and Paimo S (2005) Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of Southern Italy. *Environmental Research* **99** 48–75.

EU Commision (2007) Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, and 3-MCPD in foodstuffs. *Official Journal of the European Union* L364/5-L364724 L88, 29–38.

Gaucheron F (2011) Milk and dairy products. A unique micronutrient combination. *Journal of the American College of Nutrition* **30** 400–409.

Güler Z (2007) Levels of 24 minerals in local goat milk, its strained yoghurt and salted youghurt (tuzlu yoğurt). *Small Ruminant Research* **71** 130–137.

Haenlein G F W (2004) Goat milk in human nutrition. *Small Ruminant Research.* **51** 155–163.

Hejtmankova A, Kučerova 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.

ICAR (2003) International agreement of recording practices (Approved by General Assembly held in Interlaken. Switzerland, 30th May, 1992.)

International Dairy Federation (1995) Standard 161A, IDF, Brussels, Belgium

ISO 11464: 1994 (1994) Soil quality – Pretreatment of samples for physico-chemical analyses. *Geneva, Switzerland: International Organisation for Standardization* (ISO).

ISO 11466: 1995(E) (1995) Soil quality - Extraction of trace elements soluble in aqua regia. Geneva, Switzerland: International Organisation for Standardization (ISO).

Kapila R, Kavadi P K and Kapila S (2013) Comparative evaluation of allergic sensitization to milk protein of cow, buffalo and goat. *Small Ruminant Research* **112** 191–198.

Kędzierska-Matysek M, Barłowska J, Litwińczuk Z and Koperska N (2015) Content of macro-and microelements in goat milk in relation to the lactation stage and region of production. *Journal of Elementology* **20** 107–114.

Krelowska-Kulas M, Kedizor W and Popek S (1999) Content of some metals in goat's milk from southern Poland. *Nahrung-food* **43** 317–319.

Marković B (2003) Milk traits and polymorphism of alfa S1 casein in Balkan breed of goats. [Internet document] URL http://agris.fao.org/agris-search/search.do?recordID=CS2004000635.

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.

Miedico O, Tarallo M, Pompa C and Chiaravalle E (2015) Trace elements in sheep and goat milk samples from Apulia and Basilicata regions (Italy). Valuation by multivariate data analysis. *Small Ruminant Research* **135** 60–65. https://doi.org/10.1016/j.smallrumres.2015.12.019.

Mioč B, Prpić Z, Vnučec I, Sušić V, Antunović Z, Barać Z and Pavić V (2008a) Exterior characteristics of different categories of Croatian coloured goat. *Stočarstvo* **62** 439–447.

 

Mioč B, Barać Z, Prpić Z, Vnučec I and Pavić V (2008b) Exterior characteristics of Croatian coloured goat. Proceedings 43rd Croatian and 3rd Symposium of Agriculture, Opatija, 827–829.

Osorio T M, Koidis A and Papademas P (2015) Major and trace elements in milk and Halloumi cheese as markers for authentication of goat feeding regimes and geographical origin. *International Journal of Dairy Technology* **67** 1–9.

Pavliček J, Antunović Z, Senčić Đ and Šperanda M (2006) Production and goat milk content depending on number and stage of lactation. *Poljoprivreda* **12** 52–57.

Pilarczyk R, Wojcik J, Czerniak P, Sablik P, Pilarczyk B and Tomsza-Marciniak A (2013) Concentration of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. *Environmental Monitoring and Assessment* **185** 8383–8392.

Prpić Z, Žamper P, Grgić Z and Mioč B (2015) Dairy and reproductive traits of German fawn goats in Mediterranean breeding conditions. *Mljekarstvo* **65** 251–258.

Raynal-Ljutovac K, Pirisi A, Cremoux R and Gonzalo C (2007) Somatic cells of goat and sheep milk: analytical, sanitary, productive and technological aspects. *Small Ruminant Research* **68** 126–144.

Regulations on quality of fresh raw milk (2000). Official Gazette No. 102/2000.

Rodriguez E M R, Uretra E D and Romero C D (1999) Concentrations of cadmium and lead in different types of milk. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung A-Food Research and Technology* **208** 162–168.

Singh M, Yadav P, Garg V K, Sharma A, Singh B and Sharma H (2015) Quantification of minerals and trace elements in raw caprine milk using flame atomic absorption spectrophotometry and flame photometry. *Journal of Food Science and Technology* **52** 5299–5304.

Transoco I M, Transoco M A, Martins A P and Roseiro L B (2010) Chemical composition and mineral content of goat milk from four indigenous Portuguese breeds in relation to one foreign breed. *International Journal of Dairy Technology* **63** 516–522.

 