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Research article

CHANGES IN THE CONCENTRATIONS OF HEAVY METALS IN GOAT BLOOD DURING THE FIRST THIRD OF THE LACTATION PERIOD

Zvonko ANTUNOVIĆ[1], Željka KLIR ŠALAVARDIĆ[1*], Boro MIOČ[2], Josip NOVOSELEC[1]

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia; [2]University of Zagreb, Faculty of Agriculture, Department of Animal Science and Technology, Zagreb, Croatia.

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The changes in the concentrations of heavy metals in the goat blood throughout the first three months of lactation were the studied objective of the present paper. The experiment was carried out on 20 French-Alpine goats, aged five years and in their 4th lactation. The tested goats were examined on the 30th and 90th day of lactation. The concentrations of the following heavy metals: Cd, Pb, Cr, Ni, As and Hg, were determined in the feed and serum by inductively coupled plasma mass spectrometry. The GLM procedure was used to analyze the impact of the blood sampling time on the concentration of heavy metals. Morning milk yield of goats was lower (p=0.033) on the 90th day of lactation compared to the 30th day (1.29 vs. 1.69 kg). Compared to the first sampling of blood on the 30th day (As=3.61 and Cd=1.85 μg/kg, p<0.001), this experiment proves a significant decrease in concentrations of As and Cd in goat blood sampled during the second run, on the 90th day (As=1.73 and Cd=0.66 μg/kg, p<0.001). In the blood samples the concentration of Ni has decreased on the 90th day (6.51 vs. 2.39 μg/kg, p=0.013) by 63%. Positive correlations between Cr:Ni (r=0.47, p=0.011) and Ni:Hg (r=0.55, p=0.002) and a negative correlation between As:Pb (-0.40, p=0.028) in the blood were determined in the first third of the lactation period. The concentration of heavy metals in the goat blood measured in the present study were within the reference range, which implies a well-preserved environment in which the goats were reared.

Keywords: blood; elements; environment; goats; lactation

INTRODUCTION

Blood sampling is one of the least-invasive procedures [1] often used as a powerful tool to obtain information on the physiological and nutritional condition of animals and on their health and well-being [2,3]. Giannetto et al. [4] stated that blood biomarkers are

*Corresponding author: e-mail: zklir@fazos.hr

useful biological indicators that allow us to detect a change in the body's homeostasis due to exposure to heavy metals. The so-called heavy metals comprise all transition metals, some metalloids, lanthanides and actinides. In general, heavy metals are all metals with a density greater than 5 g/cm^3 [5]. Kovacik et al. [6] reported that these metals significantly contribute to environmental contamination, among which Cd, Cu, Hg, Pb and Zn are the most notable. Agricultural production that uses fertilizers, pesticides, or field irrigation, as well as intensive industrial development, may cause a significant contamination in the environment since they increase the heavy metal emission [7]. However, relatively high contents of heavy metals in the environment can also occur naturally. Determination of the contamination level with the mentioned heavy metals is important for maintenance of adequate health safety of the human food chain components, especially in areas known to be significantly contaminated by these risk elements [8]. Anthropogenic pollution, like metal industry, mining, heavy industry and transportation, from numerous farms, causes changes in the natural composition of soils and vegetation [9], entering the food chain and affecting animal and human health [10,11]. Toxic metals (Pb, Cd, As, and Hg) do not have a physiological activity in the body and can induce a variety of illnesses. In low amounts, Cr and Ni are essential elements for animals, yet at high concentrations in the blood (for Cr above 0.68 ppm, and for Ni above 0.29 ppm) they are toxic [12-15]. For example, Ni is a cofactor in the building blocks of membranes and metalloenzymes [16]. Moreover, heavy metals in domestic animals have mutagenicity, teratogenicity, and carcinogenicity; they cause poor body condition, reduce reproduction rate, and lead to immunosuppression [17]. The importance of environmental research is increasing due to growing environmental awareness of society and continuous tightening of regulatory requirements [18,19]. The livestock sector is directly impacted by environmental pollution containing heavy metals in terms of both health and economy [20]. Similar mechanisms, such as the production of reactive oxygen species (ROS), the inactivation of enzymes, and the inhibition of antioxidant defenses, are used by heavy metals to cause harmful effects in the body. According to Balali-Mood et al. [21] these processes may result in cellular damage and impaired health. An elevated risk of certain diseases is linked to the cellular response to oxidative stress brought on by heavy metal exposure [22]. Goats are known to be highly resistant to diseases [23], and are more tolerant to Pb and Cd (toxicity dose 400 mg/kg of body weight) exposure than cattle and sheep [19]. Lactation is a very demanding period for female animals during which the metabolism is altered, especially during the first third of the lactation period because of the high milk production. During that period, in addition to the metabolic changes, there are also changes in the concentrations of minerals and heavy metals in animal blood and consequently in the final animal products. Tiwari et al. [24] stated that goat milk is a valuable source of human nutrition, especially in regions where goat farming is well developed. Goat milk, in addition to its nutritional characteristics, has functional properties and health-promoting benefits due to its content of biologically active constituents [25].

The hypothesis of the present study is that the lactation period of goats will affect the concentration of heavy metals in the blood during the first third period of lactation. Since there are already studies into concentrations of heavy metals presented in the food chain and in goat products carried out in Croatia [26-29], determination of heavy metals in the goat blood during the first third of the lactation period on a selected farm in Slavonia was the aim of the present study.

MATERIAL AND METHODS

This experiment is complying with legal provisions of the Animal Protection Act (Republic of Croatia Official Gazette No. 133 (2006), No. 37 (2013), and No. 125 (2013)), and it is approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek (No. 644-01/22-01/03 issued on 30 June 2022).

Animals and diets

Twenty French-Alpine goats, kept at the Đurković small-scale goat farm in Osijek-Baranja County in Slavonia region (Croatia), were used in the experiment, during the year 2022. The selected research area does not have industrial environmental pollutants. The goats were 60±3 months old on average and in the 4th lactation. They were selected, according to body weight and parity, among 50 goats in the herd. All selected goats were in good health and good physical condition, and had a starting body weight of 53.4±6.0 kg. Body condition score (BCS) of goats was recorded on a 1 – to 5-point scale, as described by Santucci and Maestrini [30], where the scale from 1 (thin) to 5 (obese) was divided into 0.25 intervals.

The experiment involved sampling of goat blood on the 30th and 90th day of lactation during morning milking, since weaning of goat kids was on the 29th day and in this period the peak of lactation occurred. The mentioned samplings were within usual technological processes on a small-scale goat farm. The morning milk yield on the 30th day was 1.69±0.60 kg, and on the 90th day was 1.29±0.51 kg. Goats received 1.50 kg of balanced feed containing 16% crude protein per day, along with unlimited access to alfalfa hay. No refusals of feed mixture were observed during the study period. The diet's proportion of concentrate to forage components was 60:40. There was also unlimited access to water. This experiment is a part of a larger research project implemented on goats [24].

Feed, water and blood collection and analysis

The balanced feed mixture, hay and water samples were taken for further analyses. Blood was sampled after morning milking from the jugular vein of each goat (10 mL) and put into Venoject® sterile vacuum tubes (Sterile Terumo Europe, Leuven, Belgium). The serum was obtained from the blood samples and transferred into sterile vacuum tubes without EDTA by centrifuging the samples for 10 minutes at 1609.92

g. The serum was stored at – 80 °C, afterwards. Following defrosting, the sample was examined to measure the following heavy metals contents, which are given as µg/kg: Cd= cadmium, Pb= lead, Cr= chromium, Ni= nickel, As= arsenic, and Hg= mercury. In a microwave oven set at 180 °C for 60 minutes, feed and blood samples were dissolved in a 10 ml mixture of 5:1 HNO_3 and H_2O_2 (CEM Mars 6). Goat blood samples and feed were digested in accordance with Belete et al. [31]. A 25 mL dilution of the digest was made by using deionized water. Next, using a continuous flow hydride generation technique, an inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a, Agilent Technologies Inc., Santa Clara, CA, USA) was used to determine the concentrations of heavy elements in the goat blood, feed, and water. In each set of samples, the certified reference material NIST 1547B (NIST, Gaithersburg, USA), and BCR 129 (Joint Research Center, Brussels, Belgium) was digested. Every sample underwent two analyses. The pre-reduction step was applied to the digested samples for As analysis, also indicated in the research by Bosnak et al. [32]. During this phase, 20 mL of the sample was placed in an auto sampler tube (50 mL) and combined with KI and a solution of ascorbic acid (2 mL, 5%). The mixture was added to 6 mL of hydrochloric acid and left for at least 20 min. The sample was than diluted with deionized water until it reached the 50 mL level. The samples were then prepared for ICP-MS analysis. 20 mL of the sample and 20 mL of HCl combined in a 125 mL beaker for the Hg pre-reduction. After being placed into a 50 mL polypropylene autosampler tube, the solution was diluted with 50 mL of deionized water. The limits of detection for the following metals (mg/kg) were: Pb 0.01147, Cr 0.57387, Ni 0.28553, As 0.438, Cd 0.04344, and Hg 0.01216. The limits of detection were calculated by multiplying the standard deviation of the blank by 3, while the limit of quantification was calculated by multiplying the standard deviation of the blank by 10. Every 20 samples, a calibration standard of 10 ppb was measured with all the specified concentrations in the samples to control the stability of the device. Commercial standards were used to calibrate the device. Each set of digested samples contains a "reagent blank" containing the acids we used to digest the samples. Before each measurement, the concentration of the blank was read. Table 1 displays the levels of heavy metals in the goats' food and water, together with the dietary components they consume each day.

Table 1. Concentration of heavy metals in feed and water in the first third of lactation

Heavy metals (µg/kg DM)	Feed		Water
	Hay	Feed mixture	
Cd	29.72	<LD	44.77
Pb	632.40	<LD	372.50
Cr	645.30	12.76	1177.00
Ni	896.70	0.58	1397.00
As	140.90	3.00	164.20
Hg	1.12	0.006	<LD

DM – dry matter; **LD** – instrumental limit detection

Statistical analyses

The MEANS procedure was used to calculate the mean and standard deviation of the chemical composition and elements in blood, while GLM procedure was used to analyze the impact of the sampling time on the concentration of blood elements. $Y_{ijk} = \mu + s_i + e_{ij}$ is the model used, in which μ represents the overall mean, s_i denotes the fixed influence of the sampling time, and e_{ij} represents random error variation. Pearson's correlation with the CORR procedure is used to assess the correlations between the heavy metals in goat blood. If $p<0.05$, the correlations are deemed significant. All data were analyzed in SAS 9.4® [33]

RESULTS

In Table 2 the milking performance of goats is presented. A lower (p=0.033) morning milk yield was determined on the 90th day of lactation.

Table 2. Performance of goats during the first third of lactation

Parameter	Sampling time		SEM	P-value
	I (30th day)	II (90th day)		
Morning milk yield (kg)	1.69	1.29	0.09	0.033
Body weight (kg)	49.06	49.10	1.05	0.984
Body condition score	2.67	2.39	0.08	0.065
Feed mixture intake (kg)	1.50	1.50	-	-
Average daily weight gain (g)	0.714		-	-

Table 3 overviews descriptive statistics referring to the examined heavy metals (Cd, Pb, Cr, Ni, As and Hg) in the blood of goats during the first third of their lactation period. It is evident that Cr was the most abundant heavy metal in the goat blood but in very low concentrations, while Hg had the lowest determined concentration.

Table 3. Descriptive statistics for heavy metals in goat blood during the first third of lactation

Elements (μg/kg)	Mean	sd	Minimum	Maximum	Reference values (μg/kg)
Cd	1.09	0.89	0.05	3.44	< 20[2]
Pb	13.46	12.41	0.33	46.44	< 20 mg/kg[2]
Cr	48.94	24.11	25.11	170.10	70 – 330[1]
Ni	5.19	4.22	0.24	16.93	0.29 mg/kg[2]
As	2.70	1.55	0.45	6.11	2.92[3]
Hg	0.54	0.46	0.13	2.14	< 10 mg/kg[2]

Mean – mean of the 30th and 90th day of lactation; **sd** – standard deviation; [1]Ubwa et al. [34]; [2]Puls [35] [3]Lopez-Alonso et al. [36]

Table 4 contains information on the effect of sampling time in the first third of the lactation period on the examined heavy metals. On the second sampling of goat blood (90th day), significantly lower concentrations of As ($p<0.001$), Cd ($p<0.001$) and Ni ($p=0.013$) were found compared to the first sampling (30th day). Contrarily, the concentration of Hg ($p=0.016$) and Pb ($p=0.024$) significantly increased from the first (30th day) to the second (90th day) blood sampling time.

Table 4. Effect of goat blood sampling on the concentrations of heavy metals in first third of lactation period

Elements (µg/kg)	Sampling time		SEM	P-value
	I (30th day)	II (90th day)		
Cd	1.85	0.66	0.19	<0.001
Pb	9.21	18.19	2.01	0.024
Cr	54.19	43.68	3.81	0.171
Ni	6.51	2.39	0.80	0.013
As	3.61	1.73	0.28	<0.001
Hg	0.37	0.71	0.07	0.016

SEM – standard error of mean

Figure 1 overviews the correlation coefficients and p-values between heavy metals in goat blood during the first third of the lactation period. There are significantly positive correlations determined between Cr:Ni ($r = +0.47$; $p = 0.011$) and between Ni:Hg ($r = +0.55$; $p = 0.002$), as well as a positive Ni:As ($r = +0.40$; $p = 0.051$) trend, and negative correlation between As:Pb ($r = -0.40$; $p = 0.028$).

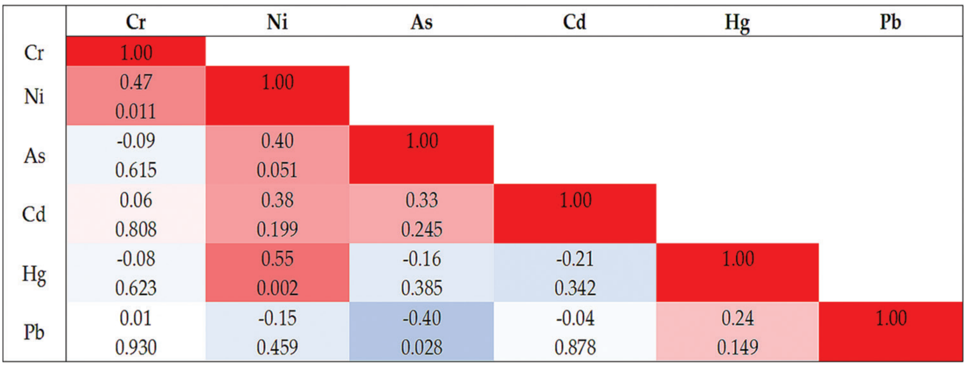

Figure 1. Heatmap correlation coefficients between heavy metals in goat blood in the sampling period, where red means positive correlation, and blue means negative correlation

DISCUSSION

Goats´ performance

In the present study no significant change in goat performance was determined, except for milk yield. In the research by Sutton et al. [37] and Salama et al. [38] the highest milk production in goats is between the 6^{th} and 8^{th} week of lactation when it begins to gradually decrease until the end of lactation. Therefore, in this period up to the 8th week of lactation, body weight loss often occurs because it is a period in which goats undergo negative energy balance [39]. In the present research, there was no loss of body weight in comparison to the sampling time (30^{th} : 90^{th} day; Table 2), which indicates a satisfactory feed intake. This is indicated by the observed BCS of goats during the study. Along with the body weight, BCS is a simple and quick process for assessing the BCS of the goats and indicates the available fat reserves in the body which can be used to determine the nutritional and health status of the goats [40]. In agreement with our research, the results of Atasoglu et al. [41] reported that the body weight of the Saanen goats was not significantly (p=0.159) different during the lactation period. Dunshea and Bell [42] also found no significant variations in the body weight of multiparous Saanen goats, aged 3-6 years during lactation (days 10, 38 and 76 of lactation). In the Norwegian dairy breed of goats grazing cultivated lowland pastures during lactation (from day 11 until day 125 of lactation), a negative energy balance was observed in goats on pasture, which was manifested in decreased adipose tissue by 3.5 kg on average. After three weeks of lowland grazing of better quality, the goats deposited body fat again, and during the next 8 weeks on indoor feeding fat accretion continued [43]. The reason for the decrease in body weight is the impossibility of increasing the capacity of feed intake, because in the research of lactating Saanen goats, the maximum intake capacity is during 97-117 days, which is later (56-98 days) than in the research by Zambom et al. [44]. Similar results were determined by Oliveira et al. [45] in the research with multiparous Alpine goat. In goats, milk yield increased rapidly, reaching its peak between the third and fourth weeks of lactation, but the amount of dry matter intake was insufficient to support the increase in milk yield, so there was a mobilization of body reserves (body weight loss was 365.7 g/day) during the eight lactation weeks. In the present research, a significant decrease in the milk yield on the 90^{th} day (p=0.033) was determined, which was expected (Table 2), since it occurred after the peak of lactation. These findings are consistent with those obtained by Rojo-Rubio et al. [46] for Alpine goat during period of 90 days of lactation. The decline in the daily milk yield after the peak was due to advancement of lactation [47].

Heavy metals

Bioaccumulation of heavy metals has toxic effects on body tissues and organs. These metals are not biodegradable; they tend to accumulate in internal organs, causing hemato-biochemical and pathological alterations [48]. Heavy metals disrupt cellular activities, like growth, proliferation, differentiation, damage-repairing processes, and apoptosis. Some toxic metals, such as Cr, Cd, and As can cause genomic instability [21]. Determination of heavy metal contents in the serum is a sign of body exposure to environmental pollutants [49]. Longer-term exposure to heavy metals causes a greater buildup of these metals in several organs, including blood and hair [50]. The level of heavy metal contamination varies depending on exposure routes, environmental conditions, animal feeding, lactation stage, and animal breed [49]. In the presented experiment, the average contents of heavy metals in goat blood are below the limits [35,36], which can be correlated with the low contents of heavy metals in the goats' diet (Table 1). Animal nutrition is a key factor in controlling nutrients flow on livestock farms [51]. Besides, it is important to monitor water quality and evaluate the risk of exposure to heavy metals [52]. The determined values of heavy metals in our research were within the allowed limits, as reported in Table 3. In a research carried out in Serbia, Hg, Cd and Pb were found in animal feedstuffs in a concentration from 0.005 to 7.341 mg/kg [53]. The same heavy metals (Hg, Cd and Pb) were present in the feed in the range of 0.01–0.02, 0.84–1.15, and 0.74–7.34 mg/kg, respectively. The Balkans unique geochemical conditions are correlated with a high As concentration, which is probably caused by pesticide use in agriculture [53]. According to Anke [54] fish meal (fish) had a substantially greater As content (2.6–19 mg/kg DM) than seafood (0.1–1.0 mg/kg), small grains (0.05–0.4 mg/kg), and agricultural pasture feed (0.1–1.0 mg/kg). In their review study on mineral and trace elements in goats, Haenlein and Anke [55] pointed out that deficiency and sufficiency levels in diet were as follows: for As 10 and 350 μg/kg, for Cd 68 and 300 μg/kg, for Cr 0.36 and 1.4 mg/kg, for Ni 0.1 and 4.4 mg/kg, respectively. The reference values for Pb and Cd contents in the blood of dairy cows are 0.5 and 0.05 mg/kg, respectively [56]. Compared to the average values of the present experiment, the research of Castro-González et al. [57] determined higher contents of Pb and Cd (0.38 and 0.016 mg/kg). These authors concluded that heavy metals ingested by cows through the feed were transferred to milk and urine through the blood. In the study of Luna et al. [58] performed on Holstein Friesian lactating cows in the Spanish province of Lugo, the following concentrations of toxic elements in the serum were measured: As – 3.53 μg/L; Cd – 0.169 μg/L; Cr – 5.06 μg/L; Hg – 0.321 μg/L; Ni – 1.88, Pb – 1.19 μg/L. These researches differed not only in the sampling procedure but also in applied methods and in researched media (serum, plasma, dry or wet matter, etc.). One of the least studied aspects of heavy metals ingested by cows is the correlation between the content of metals in the blood and their transfer to milk, feces and urine. Such studies may indicate the extent of negative effects of cow's milk on consumers' health, or extent of environmental contamination through feces and urine [57]. Castro-Gonzalez et al. [57] founded a significantly lower concentration of Cd in the blood than in milk and urine (0.001 g/kg vs. 0.002 g/kg and 0.004 g/kg, respectively) and higher Cr in the blood than in urine (0.034 mg/kg vs. 0.007 mg/kg), and a significantly lower concentration of Pb in the blood than in milk and urine (0.015 mg/kg vs. 0.024 mg/kg and 0.027 mg/kg, respectively). Fazio et al. [59] investigated the bioaccumulation of heavy metals in the blood, serum, and tail and mane hair of horses living nearby the industrialized area of Milazzo, and found that Cd showed higher values in blood samples than other tested biological substrates (0.0031 mg/kg in blood vs. 0.002 mg/kg in mane hair); moreover, they also found a more significant increase of Pb in the whole blood and serum compared to mane and tail hair. It is well known that blood Pb in lactating cows can be transferred into milk [60]. In this experiment, the measured average content of Pb (13.46 μg/kg) in goat blood was below the limit set for animals (0.025 mg/L), according to Puls [35]. Zadnik [61] argued that in the area of up to 10 km around lead smelters, Pb contents in cattle blood could be taken as a good bioindicator of environmental contamination with Pb. Average content of Cd in the goat blood in this experiment (1.09 μg/kg) is lower if compared with the reference values (< 20 mg/kg; [35]). When compared to the reported experiment results, the higher content of Cd in ruminants blood was detected by Tahir et al. [62] (0.007 mg/L) and Swarup et al. [63] (0.03 mg/L). Environmental contaminants have a negative impact on human and animal health, particularly heavy metals like Pb and Cd [21]. Compared to this experiment, Kovacik et al. [6] determined a higher content of Cd (1.46 mg/kg) in sheep blood. Gowda et al. [64] determined a concentration of Cd of 0.065 ppm in the blood plasma of dairy animals. In Slovenia, Zadnik [61] reported that average Pb content in cow blood was 0.047 mg/kg. Content of Pb in whole blood of ruminants is usually below 0.05-0.250 mg/kg, and poisoned animals have usually higher contents of Pb, 0.350 mg/kg [65]. In comparison to those values, significantly lower values are obtained in this experiment. Cr is an essential component for animals to use proteins, lipids, and carbohydrates as insulin activators. Average content of Cr in goat serum in this experiment (48.94 μg/kg) is lower when compared with results obtained on Kilis goats by Paksoy et al. [66]. They reported a value of 427.7±43.6 μg/L, which was within the tolerable limits, in goat serum it was 0.68 mg/kg [67], in sheep serum it was 0.12 mg/kg (Diyarbakir Province and Districts in Turkey). In Nigeria, mean contents of Cr (mg/L) in the blood of cows, native goats and non-native goats were ranging from 0.07 to 0.39, 0.07 to 0.33, and 0.03 to 0.20, respectively [34]. In this experiment, the average content of As in goat blood (2.70 μg/kg) is also lower when compared to the result reported by Popovic et al. [53] (2.0 mg/L), Tyutikov and Ermakov [68] 0.208 mg/L, and Aluc and Ekici [69] 0.086 mg/L. Sheep kept in the Kurdistan province in Iran, in risky areas with severe pollution with As, had 10.024 mg/kg mean content of As in the blood, which differed significantly from the reference values [70]. Content of As in cattle blood was 2.92 μg/l [36], which was higher than average concentration of As determined in this experiment. As is contained in different insecticides and fungicides, and it is commonly associated with poisoning. In sheep blood, Popovic et al. [53] determined the following values: As 0.9 mg/kg, Ni 0.6 mg/kg, and Cr 1.1 mg/kg, and in cows those values were: As 2.0 mg/kg, Ni 0.6 mg/kg and Cr 0.5 mg/kg. The enzymes urease, hydrogenase, and carbon monoxide dehydrogenase are dependent on Ni, one of the important trace elements for animals [51]. At low concentrations, Ni is an essential element, yet its essentiality for animals is still being scientifically discussed [72], as it can be toxic at higher levels [14]. However, Ni is not a cumulative element [73]. In the present study, the average concentration of Ni is 5.19 μg/kg, which corresponds to the results of Durak et al. [14], who researched sheep in Turkey (Diyarbakır districts) to determine similar average concentrations of Ni (5.9 μg/kg; from 3.9 μg/kg to 6.9 μg/kg). However, compared to our results, significantly higher concentrations of Ni in the serum of Kilis goats in Turkey were reported by Paksoy et al. [66] 83.9±6.35 μg/L), and in goats kept in rural areas in India by Shukla et al. [15] (0.190 mg/kg). Contents of Hg in cattle reported by Puls [35] were far under the recommended values, and values of Pb were comparable with values reported by other authors [61] implying that there was no danger for cattle being poisoned by Hg or Pb. In the research of Popovic et al. [53] performed in Serbia, the contents of heavy metals in the blood of homebred cows and sheep were within the reference values, in accordance with the available literature and with the results of previous studies run in that area. Apart of Cd, there were no signs of pollution by heavy metals in the feedstuffs.

Effect of sampling time

Concentrations of heavy metals in animal organisms are greatly depending on exposure routes, environmental conditions, animal species and age, animal feeding, body's physiological state, lactation stage, level of productivity and animal breed, the route taken to enter the body, the chemical's structure, and how it interacts with other metals [49,74]. Heavy metals like Cd and Pb are passed quickly through the food chain and produces highly toxic impacts on humans and animals [75,76] and should be monitored in cattle blood [77]. It is believed that Pb and Cd are transferred from the mother plasma to the mammary gland during breastfeeding and then released in the milk [78]. This further highlights the need of investigating the presence of heavy metals in blood. We were unable to locate any studies on the impact of the lactation stage on the levels of heavy metals in goat blood in the literature. This experiment confirms the significant influence of the sampling time on concentrations of As and Cd in goat blood. On the 90th day the concentrations of As and Cd in goat blood decrease significantly (by 52% and 64%, respectively), whereas the decrease in concentrations of Cr and Ni (by 19% and 63%, respectively), and the increase on concentrations of Hg and Pb (by 91% and 97%, respectively). The above could be under the influence of various factors, first of all the food content, which is satisfactory in this research, but also can be connected with changes in metabolism because of the lowered milk production during the second blood sampling. It is known that the majority of heavy metals do not participate in the metabolism, but due to metabolic deviations, marked storage in the tissues of goats can occur which can also lead to a decrease in the concentrations in the blood of lactating goats. Milam et al. [79] determined a higher mean concentration of heavy metals in the blood of sheep in Nigeria (Cd: 0.042 mg/L; Cr: 0.025 mg/L, Ni: 0.025 mg/L). In the research into the sheep lactation stage, Antunović et al. [80] reported that along with the lactation progress, concentration of Cd in sheep blood was significantly increased (from 0.19 to 0.55 μg/l), and the concentration of As was decreased, but not significantly. The authors assume that if this experiment on goats lasted longer, the changes in the concentrations of Cd in goat blood would be more significant.

Correlations between heavy metals

There are significantly positive correlations determined between Cr:Ni ($r = +0.47$; $p = 0.011$), Ni:Hg ($r = +0.55$; $p = 0.002$), and also a positive correlation between Ni:As ($r = +0.40$; $p = 0.051$) which was at the limit of significance, and negative correlation is established between As:Pb ($r = – 0.40$; $p=0.028$) in the blood of goats during the first third of the lactation period. There are few literature reports on the investigation of correlations between heavy metals in the blood of ruminants in different stages of lactation, while in goats there is no available research on this topic. Investigation on sheep in Slovakia during different seasons (winter and spring) Kovacik et al. [6] determined in the blood during the spring season a similar correlation between Cd:Pb ($r = – 0.15$) and Pb:Hg (+0.14), but also a highly significant correlation ($p<0.001$) between Cd:Hg (+0.86). Tomza-Marciniak et al. [73] in the blood of cows on conventional farms in Poland determined also a significantly positive correlation between Cr:Ni ($r = +0.488$; $p <0.01$) and As:Ni (0.637; $p <0.01$). In blood of buffaloes in Ludhiana, India on areas with levels of heavy metal pollutionthe above the permissible limits, Yeotikar et al. [81] observed significant positive correlations ($p<0.01$) between Cr: Ni ($r =0.828$), Cr:As ($r =0.923$), Cr:Pb ($r =0.826$), Ni:As ($r =0.826$), Ni :Pb ($r= 0.835$) and As:Pb ($r = 0.845$), but also significant changes in blood metabolic profile indicators. The mentioned authors concluded that the metabolic disturbance in metal exposed buffaloes may have been mediated by heavy metal pollution. Low concentrations of toxic elements in blood point out the fact that animals were reared in a well-preserved environment. Similar conclusions were also reached by Antunović et al. [80] during their research on sheep.

The current study has some limitations, like small sample size and deficiency in analyzing the anthropogenic sources. However, this survey could be a starting point for future research throughout the whole lactation period. Besides, the reason for the low sample size is that the study was done on a small-scale goat farm, which production aims for family and commercial uses. Future studies should focus on understanding heavy metals long-term physiological effects, as well as their impact on animal and human health.

CONCLUSIONS

It is recommended that the model for monitoring the animals exposure to heavy metals incorporate lactation as a crucial parameter in the interpretation of the current findings, given the observed variations in the concentrations of heavy metals in goat blood during the first third of the lactation period. The concentrations of heavy metals in goat blood measured in this experiment did not exceed the reference range, which indicates a well-preserved environment in which goats are kept.

Authors' contributions

ZA carried out the investigation and study design, participated in the methodology, and wrote the original paper. ŽKS performed the statistical analyses and participated in the investigation, formal analysis, editing, and field experiments. JN participated in the investigation, visualization, editing, formal analysis, and field experiments. BM contributed to the methodology and visualization. All authors read and approved the final manuscript.

Declaration of conflicting interests

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ORCID iDs

Zvonko Antunović https://orcid.org/0000-0002-4922-705X
Željka Klir Šalavardić https://orcid.org/0000-0003-4078-6864
Boro Mioč https://orcid.org/0000-0002-0728-8380
Josip Novoselec https://orcid.org/0000-0001-9763-3522

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PROMENE KONCENTRACIJA TEŠKIH METALA U KRVI KOZA TOKOM PRVE TREĆINE PERIODA LAKTACIJE

Zvonko ANTUNOVIĆ, Željka KLIR ŠALAVARDIĆ,
Boro MIOČ, Josip NOVOSELEC

Promene koncentracija teških metala u krvi koza tokom prva tri meseca laktacije bile su predmet izučavanja ovog rada. Ogled je sproveden na 20 francusko-alpskih koza, starosti pet godina, u njihovoj četvrtoj laktaciji. Testirane koze su pregledane 30. i 90. dana laktacije. Koncentracije sledećih teških metala: Cd, Pb, Cr, Ni, As i Hg, određene su u hrani i serumu metodom masene spektrometrije sa induktivno spregnutom plazmom. GLM postupak je korišćen za analizu uticaja vremena uzorkovanja krvi na koncentraciju teških metala. Jutarnja mlečnost koza bila je niža ($p=0,033$) 90. dana laktacije u poređenju sa 30. danom (1,29 naspram 1,69 kg). U poređenju sa prvim uzorkovanjem krvi 30. dana (As=3,61 i Cd=1,85 μg/kg, $p<0,001$), ovaj eksperiment dokazuje značajno smanjenje koncentracija As i Cd u krvi koza uzorkovanoj tokom drugog uzorkovanja, 90. dana (As=1,73 i Cd=0,66 μg/kg, $p<0,001$). U uzorcima krvi koncentracija Ni je smanjena 90. dana (6,51 naspram 2,39 μg/kg, $p=0,013$) za 63%. Pozitivne korelacije između Cr:Ni ($r=0,47$, $p=0,011$) i Ni:Hg ($r=0,55$, $p=0,002$) i negativna korelacija između As:Pb (-0,40, $p=0,028$) u krvi utvrđene su u prvoj trećini perioda laktacije. Koncentracija teških metala u krvi koza izmerena u ovoj studiji bila je u referentnom opsegu, što ukazuje na dobro očuvano okruženje u kojem su koze gajene.