Concentrations of selected toxic elements (cadmium, lead, mercury and arsenic) in ewe milk in dependence on lactation stage

Z. Antunović[1], I. Bogut[2], D. Senčić[1], M. Katić[3], P. Mijić[4]

[1]Department of Animal Science, Faculty of Agriculture, University of J.J. Strossmayer, Osijek, Croatia
[2]Faculty of Agriculture in Mostar, University of Mostar, Mostar, Bosnia and Herzegovina
[3]Department of Physics, Faculty of Education, [4]Department of Livestock Breeding, Faculty of Agriculture, University of J.J. Strossmayer, Osijek, Croatia

ABSTRACT: Biological investigations on ewes of Merinolandschaf breed (n = 10) were conducted by the 60th lactation day during the summer on pasture. Ewe milk sampling was carried out on the 2nd; 10th; 30th and 60th lactation day. An electrothermal technique was used to determine Cd and Pb concentrations in food and milk whereas As and Hg concentrations in food and milk were determined by a hydrid technique on an atomic absorption spectrophotometer. The investigation results indicate that concentrations of selected toxic elements in ewe milk varied in dependence on lactation stage. In colostrum (2nd lactation day) Cd and Pb (0.011 and 0.035 mg/kg) concentrations were significantly higher ($P < 0.01$) whereas As (0.011 mg/kg) concentrations were lower in comparison with milk on the 10th (Cd: 0.004; Pb: 0.022; As: 0.025 mg/kg), 30th (Cd: 0.005; Pb: 0.024; As: 0.028 mg/kg) and 60th (Cd: 0.006; Pb: 0.026; As: 0.029 mg/kg) lactation day. However, no significant differences ($P > 0.05$) were found in milk Hg concentration in relation to lactation stage (from 0.021 to 0.026 mg/kg). The selected toxic elements appeared to be present in ewe milk in very low concentrations.

Keywords: ewe milk; cadmium; lead; mercury; arsenic; lactation stage

In spite of being a favourable product regarding its feeding value, ewe milk cannot be compared by its production volume with either cow or goat milk. Because it is rich in fats, proteins and minerals ewe milk belongs to an outstanding valuable human food (Antunović et al., 2001). It is known that the ewe milk content can vary due to various factors such as lactation stage, feeding, breed, season of the year, etc. (Jelinek et al., 1993). In general, ewe milk contains very low concentrations of heavy metals. Accelerated industrial and agricultural development considerably affects environmental emissions of selected toxic elements: cadmium, lead, mercury and arsenic (Lopez et al., 2002). Their concentrations in animal organisms and their milk concentrations may increase very fast, although their excretion through milk is very low (Miller, 1971; Houpert et al., 1997). Their ecosystem accumulation (water-soil-plant-animal) makes them very toxic and leads to undesirable consequences for live organisms (Bogut et al., 2000; Piskorova et al., 2003). Free-living animals are important indicators of the environmental pollution with heavy metals (Kottferová and Koréneková, 1998). Sheep and cattle reared freely on pasture are also indicators of the environmental pollution (Gallo et al., 1996). Increased concentrations of heavy metals in the body of domestic animals result in low fitness of animals and reproduction problems as well as in immunity decline and occurrence of cancerous and teratogenic diseases (Bires et al., 1995). It is also known that metal (Cd, Pb and Hg) excretion

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is significantly lower in the offspring (Oskarsson et al., 1995). Concentrations of heavy metals in milk were mainly described in cows (Coni et al., 1994; Kottferová and Koréneková, 1995; Tahvonen and Kumpulainen, 1995; Okada et al., 1997; Rosas et al., 1999; Cerkvenik et al., 2000; Simsek et al., 2000; Pilsbacher and Grubhofer, 2002; Sikirić et al., 2003), goats (Lopez et al., 1985; Anke et al., 1990, 1996; Krelowska-Kulas et al., 1999; Milhaud et al., 2000; Hejtmankova et al., 2002) and very scarcely in ewes (Houpert et al., 1997; Mehennaoui et al., 1997, 1999; Milhaud et al., 1998). The objective of this investigation was to determine heavy metal concentrations in ewe milk and their fluctuations depending on lactation stage.

MATERIAL AND METHODS

Selection, keeping and feeding of ewes

Biological investigations on 10 ewes of Merinolandschaf breed were carried out by the 60th lactation day during summer in "Jasenje" sheep farm (Slavonian region). All ewes were in the fourth lactation. The ewes were at the average age of four years, healthy and in good condition. In the summer they were exposed to rotation pasture (*Lolium perenne, Lolium italicum, Phleum phleoides, Trifolium repens, Dactylis glomerata*). The ewes received salt lick (1 kg of salt lick contains 900.00 g NaCl, 1.00 g Mg, 3.20 g Fe, 0.25 g Cu, 0.50 g Mn, 0.048 g Co, 0.032 g I and 0.16 g Zn) and fresh water *ad libitum*. An average annual temperature of this region is 17.7°C during the summer season whereas a long-time average amount of total precipitation is 466.7 mm/m^2.

Forage sampling and analysis

Forage (pasture and meadow hay) samples were taken on the 2nd, 10th, 30th and 60th lactation day prior to milk sampling for chemical analyses. Forage samples were digested by a wet procedure (Vukadinović and Bertić, 1988) and prepared for the composition reading. An electrothermal technique was used to determine Cd and Pb concentrations on an atomic absorption spectrophotometer Perkin Elmer 4100 ZL whereas As and Hg concentrations were determined by a hydrid technique on an atomic absorption spectrophotometer Perkin Elmer 2380, Mercury-Hydrid Type 10.

Table 1. Parameters for Cd and Pb determination on AAS – electrothermal technique

Parameters	Cd	Pb
Lamp type	Hollow cathode	Hollow cathode
Lamp intensity (W)	10	10
Slit width (nm)	0.7	0.7
Spectral lines (nm)	228.8	283.3
Background correction	yes	yes
Absorption signal (s)	3	3

Step	Phase	Temperature (°C)		Time (s)	*R* (s)	Argon flow (ml/min)
		Initial	Final			
Thermal programme by Cd						
1	drying	110	110	50	1 MM	250
2	ashing	400	700	20	20 MM	250
3	atomization	1 400	1 400	5	–	30
4	cleaning	2 400	2 400	2	1	250
Thermal programme by Pb						
1	drying	110	110	50	1	250
2	ashing	500	500	30	5	250
3	atomization	1 900	1 900	5	–	30
4	cleaning	2 400	2 400	2	1	250

MM – matrix modifier (mg) = 0.05 $NH_4H_2PO_4$ + 0.003 $Mg(NO_3)_2$

Table 2. Parameters for Hg and As determination on AAS – hydrid technique by Mercury-Hydrid Type 10

Parameters	As	Hg
Lamp type	EDL	EDL
Lamp intensity (W)	8	5
Slit width (nm)	0.7	0.7
Spectral lines (nm)	193.9	254.2
Background correction	–	–
Temperature (°C)	900	room temperature
Reducing solution	3% $NaBH_4$ in 1% NaOH	3% $NaBH_4$ in 1% NaOH
Flame	acetylene-air	–
Basic standard	1.000 g As/l	1.000 g Hg/1 000 ml
Operational standard	1.00 µg As/ml	1.00 µg Hg/ml
Aliquots	10; 20; 50; 100 µl i.e. 10; 20; 50 and 100 ng As	50; 100; 200 µl i.e. 50; 100 and 200 ng Hg
Reagents	dilution solution: 1.5% HCl	HNO_3 65% Suprapur H_2SO_4 95–97% p.a. Merck $KMnO_4$, 5%

EDL – Electrode discharge lamp

Milk sampling and its analysis

Ewe milk samples were taken on the 2nd, 10th, 30th and 60th lactation day. The milking was carried out into special small bottles intended for field milk sampling; an amount of 100 ml milk per ewe was collected during one sampling. Milk samples were digested using a microwave system Star 2 (CEM Corporation, Matthews, North Carolina, USA). On average, 5 ml of milk was pipetted into the 200 ml cylindrical flask which was then connected to the instrument. The digestion programme is an adaptation of the programme for milk digestion found in the manual supplied with the instrument. Initial reagents HNO_3 (10 ml) and H_2SO_4 (3 ml) as well as milk were heated at 120°C. After heating 1 ml of concentrated HNO_3 for 4 minutes (total 10 ml) at 240°C was added. In the next step 1 ml of 20% H_2O_2 heated for 8 minutes (total 20 ml) at 200°C was added. The final digestate was diluted to 50 ml with demineralised water.

An electrothermal technique was used to determine Cd and Pb concentrations on an atomic absorption spectrophotometer Perkin Elmer 4100 ZL (Table 1) whereas As and Hg concentrations were determined by a hydrid technique on an atomic absorption spectrophotometer Perkin Elmer 2380, Mercury-Hydrid Type 10 (Table 2).

Statistical analysis

Statistical analysis of data was performed by the computer program Statistica (StatSoft, Inc., 2001). The results were statistically evaluated using Student's t-test. Differences were considered significant at the level of 0.05.

RESULTS AND DISCUSSION

Table 3 shows concentrations of selected toxic elements (Cd, Pb, Hg and As) in ewe milk in dependence on lactation stage.

Cd and Pb concentrations were significantly ($P < 0.01$) higher in colostrum compared to milk on the 10th, 30th and 60th lactation day whereas As concentrations were significantly lower ($P < 0.01$) in colostrum compared to milk in later lactation measurements (Table 3). It was stated that Cd increase was associated with protein content in cow milk (Rodriguez et al., 1999). This supports the hypothesis that Cd is mainly associated with the protein fraction (casein fraction) obtained by enzymatic coagulation (Mata et al., 1995). Kirova (1993) stated that ewe milk contained 1.5 times more Cd compared to cow milk. Ewes extract much more Pb via milk relative to cows (Mehennaoui et al.,

Table 3. Content of selected toxic elements (Cd, Pb, Hg and As) in ewe milk in dependence on lactation stage

Elements (mg/kg DM)		Lactation stage			
		2nd day	10th day	30th day	60th day
Cd	$\bar{x}$	0.011**2,3,4	0.004**1	0.005**1	0.006**1
	S	0.003	0.001	0.001	0.001
	CV	25.53	26.61	23.11	19.34
Pb	$\bar{x}$	0.035**2,3,4	0.022**1	0.024**1	0.026**1
	S	0.005	0.002	0.004	0.004
	CV	15.32	9.30	18.24	16.95
Hg	$\bar{x}$	0.026	0.023	0.025	0.021
	S	0.005	0.004	0.005	0.003
	CV	18.88	15.60	20.70	13.69
As	$\bar{x}$	0.011**2,3,4	0.025**1	0.028**1	0.029**1
	s	0.002	0.004	0.004	0.005
	CV	19.04	16.17	14.40	18.62

Depending on lactation stage: [1]1st–2nd day, [2]2nd–10th day, [3]3rd–30th day, [4]4th–60th day; $^{**}P < 0.01$

1997). Coni et al. (1996) determined the highest Cd concentration in ewe milk, lower in goat milk and the lowest in cow milk. Rodriguez et al. (1999) detected lower concentrations of Cd and Pb in cow milk in comparison with our investigations. They also revealed that the Cd and Pb source might have been a dietary constituent. This data clearly support our data (Figures 1 and 2).

Cadmium is a metal that is rightly considered as an "industrial risk" (Massanyi et al., 1995). Kottferová and Koréneková (1995) detected similar concentrations of Cd (0.006 mg/kg) and higher concentrations Pb (0.048 mg/kg) in milk of cows from the control and from the exposed area, but the difference between the two areas was not statistically significant. Considerably higher Cd concentrations in cow colostrum compared to cow milk were determined by Smith et al. (1991) and Zomborszky-Kovacs et al. (2000). A decrease in goat milk Pb and Cd concentrations in the course of lactation measured from April to October with goats fed pasture was determined by Heitmankova et al. (2002). Simsek et al. (2000) determined average Pb concentration of 0.018 mg/kg whereas Okada et al. (1997) found Pb concentrations of 0.04 mg/l and Cd below 0.02 mg/l in cow milk.

Although relatively higher Hg concentrations were determined in colostrum, the data do not significantly differ ($P > 0.05$) from the subsequent measurements (Table 3). Investigations on cow

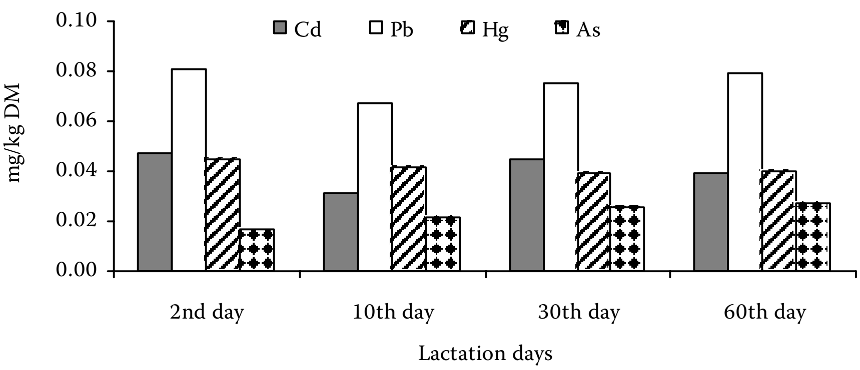

Figure 1. Concentration of selected toxic elements (Cd, Pb, Hg and As) in pasture

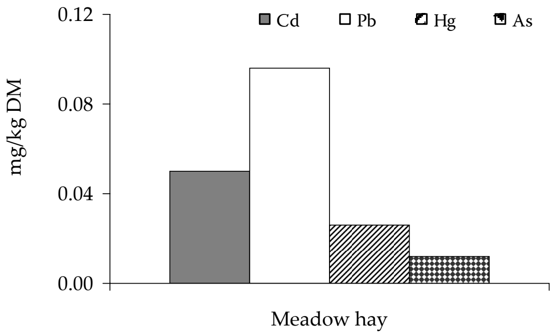

Figure 2. Concentration of selected toxic elements (Cd, Pb, Hg and As) in meadow hay

milk conducted by Mata et al. (1997) showed correlations of Hg concentration with proteins (casein and beta-lactoglobulin). This data supports our results. Krupicer (1995) reported that with higher values of mercury in pasture, grass-grazing animals cumulated mercury in their organisms, mainly in parenchymatous organs.

Concentrations of As were significantly lower ($P < 0.01$) in colostrum compared to milk in later lactation measurements. In similar investigations, Anke et al. (1996) determined significantly lower As concentrations in goat colostrum compared to goat milk (from 0.01 to 0.024 mg/kg). Toxic effects of As on the human health are well known (Mandal and Suzuki, 2002). Because As concentrations in ewe milk are very low, we can conclude that this product is not a source of this toxic element. Rosas et al. (1999) reported that As concentration in cow milk ranged from 0.0009 to 0.028 mg/kg. The investigations of As concentrations aimed at arsenic transfer into sheep tissues and excreta conducted by Beresford et al. (2001) showed that arsenic concentrations in tissues rapidly reached an equilibrium with the dietary intake level. However, the investigations of As in human milk (Grandjean et al., 1995) showed a low As excretion from the organism via milk. Higher food concentrations (Figures 1 and 2) could cause increased As concentrations in milk.

CONCLUSION

As for colostrum (2nd lactation day) Cd and Pb concentrations were significantly ($P < 0.01$) higher whereas As concentrations were lower in comparison with milk on the 10th, 30th and 60th lactation day. However, no significant differences ($P > 0.05$) were found in Hg concentration in milk in relation to lactation stage (from 0.021 to 0.026 mg/kg). Our results indicate that concentrations of As and heavy metals in ewe milk vary in dependence on lactation stage. Selected toxic elements appeared to be present in ewe milk in very low concentrations.

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Corresponding Author

PhD. Zvonko Antunović, University of J.J. Strossmayer in Osijek, Faculty of Agriculture, P.O. Box 117, Trg sv. Trojstva 3, 31 000 Osijek, Croatia
Tel. +385 31 224 220, fax +385 31 207 017, e-mail: zantun@pfos.hr