

## **CHANGES OF HEAVY METAL CONCENTRATIONS IN GOATS MILK DURING LACTATION STAGE IN ORGANIC BREEDING**

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### **Abstract**

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The investigations were carried out with 10 French Alpine goats. Investigation was done during winter-feeding season in organic breeding. All animals were in the fourth lactation, average age of four years, healthy and in good condition. They were fed with cereal mixture (oat, corn and barley) 1000 g/day and meadow hay (*ad libitum*). Milk samplings were carried out on 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> and 120<sup>th</sup> lactation day. Electrothermal technique was used to determine Cd and Pb concentrations whereas Hg concentration was determined with a hydride technique. There was no significant difference ( $P>0.05$ ) between determined Cd, Pb and Hg concentrations with regard to the lactation period in goat milk. However, it should be stressed that Cd, Pb and Hg concentrations decreased in goat milk along with the increase of lactation (Cd: from 0.71 until 0.56; Pb: from 8.67 until 7.41; Hg: from 0.012 until 0.010  $\mu\text{g}/\text{kg}$  DM). Concentration of heavy metals in goat milk showed connection with fodder samples. The presence of heavy metals in goat milk in organic breeding appeared to be in a very low concentration in these investigations and did not present any risk to human health.

*Key words:* heavy metals, goat, milk, organic breeding, lactation stage

### **Introduction**

Goat milk belongs to the group of casein milks and their production has increased for the last few years. The usage of goat milk is particularly important in the nutrition of children who are allergic to cow milk and for the general population at treatment of peptic disorders (Hejtmankova et

al., 2002). Some technological improvements in agronomy (e.g. the use of fertilizers, pesticide and irrigations of fields) may cause significant contamination in our environment by intensive industrial development as well as the increase of heavy metal emission (Tajkarimi et al., 2008). Heavy metal concentration monitoring in environment should be done systematically and carefully. Increased con-

centrations of heavy metals in domestic animals body result in low animal fitness and reproduction problems as well as in immunity decline and occurrence of cancerous and teratogenous diseases (Bires et al., 1995). At the same time, contamination of milk by heavy metals can potentially favour bio-accumulative toxic hazards in dairy production systems (Pulina et al., 2006). The way of entering heavy metals in human body by consumption of animal products should not be ignored. Although heavy metal concentrations in milk are relatively low (Unnikrishnan et al., 2005), they can be used as an indicator of contamination of environment (Licata et al., 2004). By developing organic agriculture, it is possible to reduce the negative influence of agricultural technology on environment and to improve the quality of obtained products, because the use of pesticides, organic compounds, and stimulators of growth, antibiotics and veterinary drugs is restricted in organic agriculture (Zagorska et al., 2008). There is a deficiency in investigations regarding the variants of heavy metal concentrations in milk during lactation period in organic goat breeding and small investigation about cow milk from organic breeding (Zagoska and Ciprovica, 2005; Gabryszuk et al., 2008; Zagorska et al., 2008). A small number of investigations have been done on heavy metal concentrations in goat milk (Lopez 1985; Krelowska-Kulas et al., 1999; Milhaud et al., 2000; Hejtmankova et al., 2002; Güler, 2007).

It is, therefore, the purpose of this paper to establish the change of heavy metal concentrations (Cd- cadmium, Pb- lead and Hg-mercury) in goat milk during various stages of lactation (30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> and 120<sup>th</sup> day of lactation) in organic breeding.

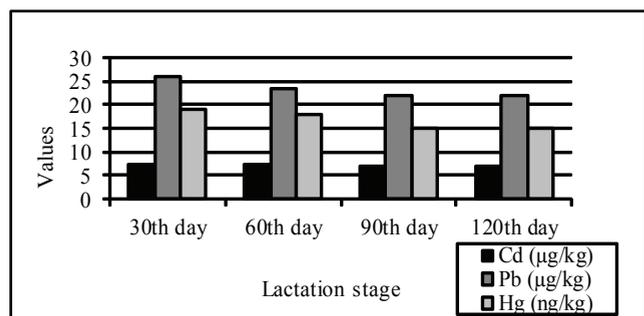
## Materials and Methods

Investigations were carried out with 10 French Alpine goats from 30<sup>th</sup> until the 120<sup>th</sup> lactation day in family organic goat farm in region of Slavonia.

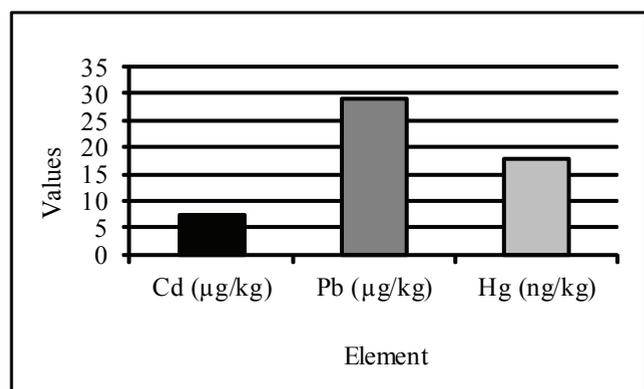
All animals were in the fourth lactation and held the Regulation on organic production (NN, 2002). Animals were at the average age of four years, healthy and in good condition. Investigation was done during winter-feeding season. Winter-feeding season lasted from 1 October and finished on 1 March. Animals were housed in stable with deep litter, in the pens with 10 animals. Animals were fed with cereal mixture (oat, corn and barley) 1000 g/day and meadow hay (*ad libitum*). The animals were supplied with cattle salt and fresh water (*ad libitum*). The farms were located at 95 m of the height above sea level located at 45°43' NL and 17°51' EL with mean annual temperature of 11°C and precipitation amount of 829 mm.

Cereal mixture sampling was accomplished for 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> and 120<sup>th</sup> lactation day prior to milk sampling for analysis. During investigation, we used the same meadow hay. Forage samples were destroyed by a wet procedure (Vukadinovic and Bertic, 1988) and prepared for composition reading. Electrothermal technique was used to determine Cd and Pb concentrations on atomic absorption spectrophotometer Perkin Elmer 4100 ZL whereas Hg concentration was determined with a hydrid technique on atomic absorption spectrophotometer Perkin Elmer 2380 by Mercury-Hydrid Type 10 (Figures 1 and 2).

Milk sampling was accomplished for 30<sup>th</sup>, 60<sup>th</sup>, 90<sup>th</sup> and 120<sup>th</sup> lactation day. The milking was carried out in special small bottles intended for field milk sampling amounting 100 mL of milk per animal during one sampling. Milk samples were digested using a microwave system Star 2 (CEM Corporation, Matthews, North Carolina, USA). Averagely, 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 were concentrated HNO<sub>3</sub> (10 mL) and concentrated H<sub>2</sub>SO<sub>4</sub> (3 mL) as well as milk



**Fig. 1.** Concentration of heavy metals (Cd, Pb and Hg) in cereal mixture



**Fig. 2.** Concentration of heavy metals (Cd, Pb and Hg) in meadow hay

were heated at 120°C. After heating, 1 mL of concentrated HNO<sub>3</sub> for 4 min (total 10 mL) at 240°C was added. The next step was adding 1 mL of 20% H<sub>2</sub>O<sub>2</sub> for 8 min (total 20 mL) at 200°C. Resulting digestate was diluted to 50 mL with demineralised water.

Electrothermal technique was used for determining Cd and Pb concentrations on atomic absorption spectrophotometer Perkin Elmer 4100 ZL whereas Hg concentration was determined with a hydride technique on atomic absorption spectrophotometer Perkin Elmer 2380 by Mercury-Hydride Type 10 (Table 1). Reducing solution for Hg determination were 3% NaBH<sub>4</sub> in 1% NaOH. For Hg determination, we used basic standard 1.000 g Hg/1000 ml and operational standard 1.00 µg Hg/ml. For Hg determination we used aliquots 50;

100; 200 µl i.e. 50; 100 and 200 ng Hg, as well as reagents HNO<sub>3</sub>, 65% Suprapur H<sub>2</sub>SO<sub>4</sub>, 95-97%, p.a. Merck KMnO<sub>4</sub>, 5%.

The computer program Statistica (Stat Soft Inc. 2008) performed statistical analysis of data. The results were statistically evaluated by using LSD test. Differences were considered significant at the level of 0.05 or less.

## Results and Discussion

There was no significant difference between determined Cd, Pb and Hg concentrations with regard to the lactation period in goat milk in organic breeding. However, it should be stressed that Cd, Pb and Hg concentrations decreased in goat milk along with the increase of lactation (Table 2).

The contents of Cd, Pb and Hg in goat milk in organic breeding have not published in the Republic of Croatia yet. In the literature, we did not find studies related to the determination of heavy metals in goat milk from organic breeding. Therefore, we obtained results comparable with the results of

**Table 1**  
Parameters for determination of Cd, Pb and Hg

Parameters	Element	
	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 (sec)	3	3
Thermal programme by Cd and Pb		
Step	Phase	Temperature (°C)
		Initial
1	drying	110
2	ashing	400
3	atomization	1400
4	cleaning	2400

Electrode discharge lamp, \*\*mm (matrix modifier) = 0.05mg NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> + 0.003 mg Mg(NO<sub>3</sub>)<sub>2</sub>

the organic cow milk and goat milk from conventional breeding. In organic cow milk, Zagorska et al. (2008) determined 0.007 mg/kg Cd and 0.020 mg/kg Pb. In investigation, Zagorska and Ciprovic (2005) in organic cow milk determined 0.024 mg/kg Pb wet matter and very low Cd content. In investigation Gabryszuk et al. (2008) in cow milk on two organic farms determined higher contents of Cd (1.06 and 1.00 µg/kg) and lower Pb (4.13 and 5.30 µg/kg) in comparison with this results. Hejtmankova et al. (2002) determined similar Cd and Pb content in goat milk during lactation in conventional breeding. In goat milk from conventional breeding, Krelowska-Kulas et al. (1999) and Rodriguez et al. (1999) obtained higher Pb content (from 9 till 20 µg/kg and 11.86 µg/L) and Cd content (from 2 till 6 µg/kg and 7.81 µg/L). In investigation with goat milk in Turkey in conventional breeding Güler (2007) determined higher Cd and Pb levels (0.63 and 0.06 ppm) compared with this investigation. In ewe milk fed on pasture during summer season Antunovic et al. (2005) found higher Pb, Cd and Hg contents. Lead is well absorbed from milk (Morisson and Quarterman, 1987) and the risk to the suckling or other consumer may arise. The presence of Pb in milk could be due to different factors (fodder contamination, climatic factors, used pesticide compounds and other). Decline of goat milk Pb and Hejtmankova et al. (2002) determined Cd concentrations in the course of lactation measured from April to October with goats fed pasture in conventional breeding. Concentration of heavy metals in goat milk showed connection with fodder samples (Fig. 1 and 2). Rodriguez et al. (1999) found that milk Cd and Pb source might have been cow dietary constituent. The percentage of decrease of Cd concentration in cattle feed is followed by this decrease in milk (Vidovic et al., 2005). Mercury absorbed in organic forms is avidly retained, little appearing in milk (0.17%) and most (78%) in muscle (Neathery et al., 1974). Investigations on cow milk in con-

**Table 2**

**Content of heavy metals (Cd, Pb and Hg) in goat milk dependence on lactation stage in organic breeding**

Elements (µg/kg DM)		Lactation stage (n = 10)			
		30 <sup>th</sup> day	60 <sup>th</sup> day	90 <sup>th</sup> day	120 <sup>th</sup> day
Cd	<i>Mean</i>	0.71	0.67	0.61	0.56
	<i>s</i>	0.18	0.16	0.24	0.17
Pb	<i>Mean</i>	8.67	8.29	7.43	7.41
	<i>s</i>	3.12	4.09	3.39	3.63
Hg	<i>Mean</i>	0.012	0.010	0.008	0.010
	<i>s</i>	0.005	0.004	0.004	0.006

DM- dry matter; s- standard deviation

ventional breeding conducted by Mata et al. (1997) were characterized by correlation of Hg concentration with proteins (casein and beta-lactoglobulin). The above mentioned could be the case in this paper as well as in connection with fodder samples. Simsek et al. (2000) determined higher Hg content in cow milk (0.005 mg/kg) from conventional breeding in comparison with our results. The said changes could be also connected with the environment, which can have a negative impact on toxic elements levels in feed for flock breeding. Finally, heavy metals concentrations in goat milk samples from organic breeding much lower than the MAC in Croatia (Ministry of Health, 1994) and by EU Regulation (466/2001) as well as did not present any risk to human health.

## Conclusions

There was no significant difference ( $P > 0.05$ ) between determined Cd, Pb and Hg concentrations with regard to the lactation period in goat milk in organic breeding. However, it should be stressed that Cd, Pb and Hg concentrations decreased in goat milk along with the increase of lactation. Concentration of heavy metals in goat milk showed connection with fodder samples. The presence of heavy metals in goat milk in organic breeding ap-

peared to be in a very low concentration in these investigations.

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### References

- Antunovic, Z., I. Bogut, D. Seneic, M. Katic and P. Mijic, 2005. Concentration of selected toxic elements (cadmium, lead, mercury and arsenic) in ewe milk in dependence on lactation stage. *Czech J. Anim. Sci.*, **50**: 377-383.
- Bires, J., J. Dianovsky, P. Bartko and Z. Juhasova, 1995. Effects on enzymes and the genetic apparatus of sheep after administration of samples from industrial emissions. *Bio Metals*, **8**: 53-58.
- EU Regulation, 466/2001. Official Journal of the European Communities, *L 313*, 30 November 2001.
- Gabryszuk, M., K. Sloniewski and T. Sakowski, 2008. Macro- and microelements in milk and hair of cows from conventional vs organic farms. *Animal Sci.Rep*, **26**: 199-209.
- Güler, Z., 2007. Levels of 24 minerals in local goat milk, its strained yoghurt and salted youghurt (tuzlu yođurt). *Small Rum. Res.*, **71**: 130-137.
- Hejtmankova, A., J. Kučerova, D. Mihalova, D. Koliňova and M. Orsak, 2002. Levels of selected macro and micro elements in goat milk from farms in the Czech Republic. *Czech J. Anim. Sci.*, **47**: 253-260.
- Krelowska-Kulas, M., W. Kedizor and S. Popek, 1999. Content of some metals in goat's milk from southern Poland. *Nahrung-food*, **43**: 317-319.
- Licata, P., D. Trombetta, M. Cristani, M. Giofre, D. Martino, M. Calo and F. Naccari, 2004. Levels of „toxic“and „essential“metals in samples of bovine milk from various dairy farms in Calabria, Italy. *Environ. Int.*, **30**: 1-6.
- Lopez, A., W. F. Collins and H. L. Williams, 1985. Essential elements, cadmium and lead in raw and pasteurized cow and goat milk. *J. Dairy Sci.*, **68**: 1878-1886.
- Mata, L., L. Sanchez and M. Calvo, 1997. Interaction of mercury with human and bovine milk. *Biosci. Biotechnol. Biochem.*, **61**: 1641-1645.
- Milhaud, G. E., A. Delacroix-Buchet, M. Han, S. Mehennaoui, A. Duche, B. Enriquez and M. Kolf-Clauw, 2000. Transfer of cadmium from goat milk to cream and to rennet and lactic curds. *Lait*, **80**: 277-288.
- Ministry of Health Republic of Croatia, 1994. By-law on the allowed concentrations of pesticides, toxins, mycotoxins, metals, and histamine and related substances in food and on other safety conditions of food and necessities in large-scale use. *Off. Gaz.*, **46**: 30.
- Morrison, J. N. and J. Quarterman, 1987. The relation between iron status and lead absorption in rats. *Biol. Trace Elem. Res.*, **14**: 115-126.
- Neathery, M. W., W. J. Mills, R. P. Gentry, P. E. Stake and D. M. Blackman, 1974. Cadmium<sup>109</sup> and methyl mercury-203 metabolism, tissue distribution and secretion into milk in dairy cows. *J. Dairy Sci.*, **57**: 1177-1184.
- N:N., 2002. Regulation on organic production of animal products, 13,02.
- Pulina, G., A. Nudda, G. Battacone and A. Cannas, 2006. Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. *Anim. Feed Sci. Technol.*, **131**: 255-291.
- Rodriguez, E. M. R., E. D. Uretra and C. D. Romero, 1999. Concentrations of cadmium and lead in different types of milk. *Z. Lebensm.Unters. F. A*, **208**: 162-168.
- Simsek, O., R. Gultekin, O. Oksuz and S. Kurultay, 2000. The effect of environmental pollution on the heavy metal in content of raw milk. *Nahrung*, **44**: 360-363.
- STATISTICA, 2008. Stat Soft, Inc. version 8.0, www.statsoft.com.
- Tajkarimi, M., M. Faghieh, H. Poursoltani, A. Salah Nejad, A.A. Motallebi and H. Mahdavi, 2008. Lead residues levels in raw milk from different regions of Iran. *Food Control*, **19**: 495-498.
- Unnikrishnan, V., M. K. Bhavadasan, B. S. Nath and C. Ram, 2005. Chemical residues and contaminants in milk:A review. *Indian J. Anim. Sci.*, **75**: 592-598.
- Vidovic, M., A. Sadibasic, S. Cupic and M. Lausevic, 2005. Cd and Zn in atmosphere deposit, soil, wheat and milk. *Environ. Res.*, **97**: 26-31.
- Vukadinović, V. and B. Bertić, 1988. Book on agrochemistry and plant nutrition. *Faculty of Agriculture in Osijek, Croatia*, pp. 56.
- Zagorska, J. and I. Ciprova, 2005. The comparison of chemical pollution between organic and conventional milk. *International Scientific Conference Proceedings. Research for Rural Development*. Jelgava, Latvia 19-22 May 2005: pp. 196-198.
- Zagorska, J., I. Ciprova and T. Rakcejeva, 2008. Evaluation of contamination of organic and conventional milk by heavy metals and aflatoxin in M1 in Latvia. *Mai. Chem. Techn.*, **42**: 122-126.