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Cadmium in liver and kidneys of domestic Balkan and Alpine dairy goat breeds from Montenegro and Serbia

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Abstract

Concentrations of cadmium (Cd) were determined in the samples of 144 animals around 1 and of 144 animals around 4 years old. Cd was analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), after microwave digestion. Cd concentrations were higher ($p < 0.05$) in kidney than in liver and higher ($p < 0.05$) in older animals than in young ones. In domestic Balkan goat which was raised in a free-ranged system Cd accumulation was lower ($p < 0.05$) than in Alpine goat raised in an intensive production system. Geographic region did influence Cd accumulation only in older animals. Higher Cd levels ($p < 0.05$) were determined in goats from Serbia. The highest obtained Cd concentrations in both tissues were lower than maximum levels set by European and national legislation for ruminants (cattle and sheep).

Keywords: Cadmium, Liver, Kidney, domestic Balkan goat, Alpine goat.

Introduction

The total number of goats in the Republic of Montenegro and in the Republic of Serbia is about 37.5 and 203 thousands, respectively (MONSTAT, 2015; Statistical Office of the Republic of Serbia, 2015). The domestic/indigenous Balkan goat originates from Capra prisca (Adametz), which is reared across south-eastern Europe. Domestic Balkan is a dairy goat breed, very resistant to diseases and undemanding in terms of nutrition and care. In Montenegro, this breed is reared mainly in the least favorable areas characterized by karsts relief and in Serbia in mountainous areas. The domestic Balkan goat population is mostly located in smallholder farming areas and the traditional production system is extensive (Memisi et al., 2004; Marković et al., 2007; Stanišić et al., 2012). The Alpine goat is a variety of domestic goat that is a native to the French Alps. Alpine goats are heavy milkers and are often used for commercial dairy production (Memiši and Žujović, 2012).

Animal by-products (offal) fall into the categories "edible" and "inedible". Edible offal, or variety meat, is also a form of meat which is used as food, but which is not skeletal muscles and in general possesses higher concentrations of some micronutrients, especially minerals and vitamins, than muscular tissue (Lawrie and Ledward, 2006; Tomović et al., 2011). Also, edible offal is often different from skeletal tissue in structure, composition, functional and sensory properties (Ockerman and Basu, 2004). According to Montenegrin and Serbian legislation (Serbian Regulation, 1974) edible organs and glands (edible offal) of a butchered goat that are removed in dressing include: brain, tongue, heart, lungs, thymus, liver, spleen, kidney and testis. Liver is the most widely used edible organ and is used in many types of manufactured meat products. Livers from older animals are better suited to manufactured meats particularly liver sausage and paté because they have strong flavour and may be tough. Kidneys are used as a whole or sliced and generally either grilled, sautéd or braised, but not used to any extent in manufactured meat products (Spooncer, 1988).

Cadmium (Cd) is a heavy metal found as environmental contaminant. In the environment Cd is toxic to plants, animals and micro-organisms. Cd bioaccumulates mainly in kidneys and liver of vertebrates, in aquatic invertebrates and in algae (EFSA, 2009; Rudy, 2009; Adetunji et al., 2014; Canty et al., 2014). Therefore, older or animals with a longer life span have higher Cd concentrations in their tissues. The concentration of Cd in edible offal may be used as an indicator of environmental contamination by Cd (Tomović et al., 2013). In ruminants, Cd exposure may be associated with a number of different activities, including industrial processing, mining and agricultural practices, and is also higher in soils in some geographic regions. Moreover, factors such as type of the tissue, sex, breed and species could also affect Cd bioaccumulation in ruminants (Antoniou and Zantopoulos, 1992, 1995; Khan et al., 1995; Abou-Arab, 2001; Niedziółka et al., 2009; Roggeman et al., 2014; Kar et al., 2015; Lane at al., 2015; Kim et al., 2016).6

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Human exposure to Cd can occur via food, water, air, soil and dust. Food is the main source of Cd exposure for the non-smoking population (EFSA, 2009). The International Agency for Research on Cancer has classified Cd as a human carcinogen (Group 1, IARC, 1993), based on occupational studies. JEFCA (2011) established a provisional tolerable monthly intake (PTMI) for Cd of 25 μg kg^{-1} body weight. The European Commission (2006), as well as the Montenegrin and Serbian authorities (Serbian Regulation 1992a, 1992b, 2002) set maximum limits for Cd levels in cattle, sheep, pig and poultry tissue of 0.05, 0.5 and 1 mg kg^{-1} fresh weight in muscle, liver and kidney, respectively. However, Cd maximum levels have not been set for goat tissue.

Literature data indicate a large variability in the concentration of Cd in goat offal (Table 1), but data are available only for a few countries. Cd levels in animal products, such as meat, liver and kidney, are monitored throughout national residue monitoring programs, conducted with a view to determine whether there are residues and to prevent the distribution of products that pose a risk to human health. Generally, monitoring of Cd in foods, i.e. red meat and edible offal is needed (Tomović et al., 2013). Nevertheless these available data, in general there is a lack of information about occurrence of Cd in goats. Therefore the aims of this study were: (i) to obtain Cd levels of liver and kidney from 2 dairy goat breeds (domestic Balkan and Alpine goat) reared in Montenegro and Serbia; (ii) to investigate the differences in Cd levels between two goat breeds reared under different production systems; (iii) to compare the obtained values with data reported in available scientific literature; (iv) to compare the obtained values with the maximum levels set by European and national legislation for ruminants (cattle and sheep).

Materials and Methods

During 3 months 576 samples (liver and kidneys) from domestic Balkan and Alpine goats were collected in Montenegro and Serbia. All sampled animals were slaughtered for human consumption. Domestic Balkan goats were reared outdoors (free-range) grazing meadow and pasture and/or browsing trees and shrubs in the central part of Montenegro and in the eastern part of Serbia (the area of "Stara Planina", Old Mountain). In winter the animals were also given locally produced hay and silage. Alpine goats were reared under an intensive system in modern commercial dairy farms in both countries. All animals had remained on their herd of birth until slaughter. Together with each animal, a copy of the passport was received, containing information on the date of birth, sex and type of the animal. Regarding to age, animals were categorized into two groups: one and four years old. The selection was made in the slaughterhouse on the day of slaughter and was based on the information of the animal's passport.

Liver and kidneys were taken from each individual animal. Samples for analysis (approximately 50 g) were taken after homogenization of the whole liver and both kidneys, vacuum packaged in polyethylene bags and stored at – 80 °C until analysis. The content of cadmium (Cd) was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) with an iCAP 6000 series system (Thermo Scientific, Cambridge, UK), after microwave digestion with a MWS-3+ system (Berghof, Eningen, Germany). Cd concentrations in all analyzed samples were above the limit of detection and are all reported as mg kg^{-1} wet weight. Quality control of the analytical measurements was performed using the certified reference material ERM – CE278, mussel tissue (IRMM, Institute for Reference Materials and Measurements, Geel, Belgium). The results of the analytical quality control program are presented in Table 2. The limit of detection (LOD) was calculated as 4.35 times the standard deviation of 6 procedure blanks, multiplied with the dilution factor. The limit of quantification (LOQ) was calculated as mean plus 10 times the standard deviation of 6 procedure blanks, multiplied with the dilution factor. Type A evaluation of measurement uncertainty was applied. Measurement uncertainty was calculated by taking the square root of the average of the squared deviations of the values from their average value divided by n – 1. All data are presented as mean, standard deviation (SD) and range. Independent *t*-test and analysis of variance (one-way ANOVA) were used to test the hypothesis about differences between 2 or more mean values. The software package STATISTICA 12 (StatSoft, Inc., Tuls, OK, USA, 2015) was used for data analysis.

Results and Discussion

Cd concentrations in livers and kidneys of domestic Balkan and Alpine goats from Montenegro and Serbia are presented in Table 3. There were significant differences in Cd concentrations between liver and kidneys in both age groups of both breeds. Concentrations were significantly higher ($p < 0.05$) in kidneys than in the liver, what was in agreement with previous studies in goats (Antoniou and Zantopoulos, 1992, 1995; Khan et al., 1995; Husain et al., 1996; Abou-Arab, 2001; Niedziółka et al., 2009, 2010; Aslam, 2010; Okoye and Ugwu, 2010; Kar et al., 2015; Tomović et al., 1015). In contrast, Akan et al. (2010), Abd El-Salam et al. (2013), Oladipo and Okareh (2015) and Swaileh et al. (2009) determined the opposite trend. The Pearson correlation coefficient (Table 4) between liver and kidney Cd results varied from 0.587 ($p < 0.001$, in Serbia for 1 year old domestic Balkan goat) to 0.937 ($p < 0.001$, in Serbia for 4 years old Alpine goat). Cd is efficiently retained in kidneys and liver with a very long biological half life, ranging from 10 to 30 years (EFSA, 2009; Lane et al., 2015). It is bound to small cysteine-rich peptides, including metallothionein, which are involved in the binding, transport and detoxification of excessive Cd (Nordberg et al., 1994), primarily through high affinity binding, as Cd seemed to be the most important metal for metallothionein induction in kidneys (Roggeman et al., 2014). Furthermore, the Cd concentration in the liver and kidneys was significantly lower (*p*

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< 0.05) in younger groups (1 year old) than in older groups (4 years old). Cd accumulation in liver and kidneys with age was also found in the other studies on ruminants (Rudy, 2009; Adetunji et al., 2014; Canty et al., 2014; Kar et al., 2015; Kim et al., 2016; Tomović et al., 2016).

Production system (free-range and intensive) and/or goat breed (domestic Balkan and Alpine) significantly influenced Cd accumulation in liver and kidneys in all groups. Cd in liver and kidneys from domestic Balkan goat was significantly lower ($p < 0.05$) than in liver and kidneys from Alpine goat for both ages. This is probably due to a different life style and diet, because domestic Balkan goat was raised under a free-range and Alpine goat under an intensive system. In ruminants, Cd levels in tissue are largely dependent on the Cd content of the diet (Smith et al., 1991; Rogowska et al., 2008; Lane et al., 2015). In general, geographic region significantly influenced Cd accumulation in liver and kidneys in older groups (4 years old), but had no effect in younger groups (1 year old, $p > 0.05$). Cd in liver and kidneys from 4 years old domestic Balkan and Alpine goat in Serbia was significantly higher ($p < 0.05$) comparing with corresponding values in goats from Montenegro. These results suggest a lower environmental pollution level in Montenegro.

The highest goat liver and kidney Cd concentrations have been reported in Nigeria, with mean values of 5.51 and 4.77 mg kg^{-1} wet weight, respectively (Oladipo and Okareh, 2015) for animals from 18 to 48 months old. In Poland Niedziółka et al. (2009) determined the lowest Cd concentrations in liver and kidneys from 150 days old goats (mean 0.021 and 0.038 mg kg^{-1} wet weight, respectively), which was in agreement with results in this survey. Also, results obtained in this study for mean Cd levels in liver and kidneys in goats were similar to those determined in India, where goats older than 1 year from a reference site had Cd levels in liver and kidneys of 0.27 and 0.54 mg kg^{-1} wet weight, respectively (Kar et al., 2015). Table 1 gives an overview of relevant literature data. However, in some studies precise age of slaughtered animals were not reported. Age-related effects on Cd concentrations in liver and kidneys in goats are scarce. Antoniou and Zantopoulos (1992) found that Cd concentrations in liver and kidneys increased with age, while Kar et al. (2015) found an increase with age only in kidneys. Antoniou and Zantopoulos (1992, 1995) also found influence of area on Cd concentrations in liver and kidneys. In contrast, Khan et al. (1995) did not find significant differences in liver and kidney Cd levels between different age and sex groups of goats.

Conclusions

Cd content of all analyzed liver and kidneys ranged from 0.018 to 0.459 mg kg^{-1} and from 0.034 to 0.753 mg kg^{-1} of fresh tissue weight, respectively. No sample exceeded the European and National maximum limits of 0.5 mg kg^{-1} wet weight for liver and 1 mg kg^{-1} wet weight for kidney for cattle and sheep, i.e. ruminants (Serbian Regulation 1992a, 1992b, 2002; Commission Regulation (EC) No 1881/2006). Even 87.5% of liver samples and 91.7% of kidney samples had Cd levels below half of the maximum level. The results of the present study show that the content of Cd obtained in liver and kidney of goats was influenced by all analyzed risk factors: type of the tissues, age, production system (and/or breed) and geographic region.

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Table 1. Cd (mg kg^{-1} wet weight) in liver and kidney of goats, published in literature [mean ± standard deviation (range)]

Country	Sex	Age	N	Liver	Kidney	Reference
Egypt (rural areas)	NM	NM	6	0.091±0.05	0.980±0.35	Abou-Arab (2001)
Egypt (industrial areas)	NM	NM	6	0.260±0.14	0.911±0.46	Abou-Arab (2001)
Greece (total)	NM	3–8 years	36	0.29	2.52	Antoniou and Zantopoulos (1992)
Greece (Arnea area)	NM	3–4 years	10	0.17	1.05	Antoniou and Zantopoulos (1992)
Greece (Arnea area)	NM	5–8 years	8	0.23	2.14	Antoniou and Zantopoulos (1992)
Greece (Olymplas area)	NM	4–7 years	34	0.53±0.05	3.51±0.40	Antoniou and Zantopoulos (1995)
Greece (Arnea area)	NM	NM	18	0.19±0.01	1.30±0.16	Antoniou and Zantopoulos (1995)
India (reference site)	NM	<1 year	10	0.14 (0.081–0.16)	0.25 (0.11–0.54)	Kar et al. (2015)
India (reference site)	NM	>1 year	10	0.27 (0.11–0.49)	0.54 (0.26–0.86)	Kar et al. (2015)
India (polluted site)	NM	<1 year	10	1.49 (1.07–2.19)	1.98 (1.42–2.93)	Kar et al. (2015)
India (polluted site)	NM	>1 year	10	2.12 (1.52–3.62)	3.92 (2.58–5.11)	Kar et al. (2015)
Kuwait	NM	NM	11	0.047 (0.022–0.110)	0.442 (0.073–1.250)	Husain et al. (1996)
Nigeria	NM	NM	NM	0.44±0.03	0.39±0.07	Akan et al. (2010)
Nigeria	NM	NM	13	0.35±0.36 (ND–1.15)	0.83±0.73 (0.07–3.08)	Okoye and Ugwu (2010)
Nigeria	Male and female	18–48 months	20	5.51±2.92 (ND–11.67)	4.77±1.93 (ND–8.89)	Oladipo and Okareh (2015)
Palestinian Authority	NM	NM	7	0.53±0.02*	0.46±0.01*	Swaileh et al. (2009)
Pakistan	NM	NM	NM	1.588±0.002	1.075±0.001	Abd El-Salam et al. (2013)
Pakistan (winter season)	NM	NM	48	0.052±0.002	0.092±0.003	Aslam (2010)
Pakistan (summer season)	NM	NM	48	0.097±0.003	0.118±0.002	Aslam (2010)
Poland	Male	150 days	10	0.021±0.01	0.031±0.01	Niedziółka et al. (2009)
Poland	Female	150 days	10	0.020±0.01	0.038±0.01	Niedziółka et al. (2009)
Poland	Castrated male	up to 35 kg	7	0.03±0.006		Niedziółka et al. (2010)
Serbia	Male	18.7 to 24.3 kg	15	0.064±0.005	0.114±0.013	Tomović et al. (2015)
USA	NM	<1.5–7	20	0.32	0.51	Khan et al. (1995)
USA	Male	NM	13	0.23	0.33	Khan et al. (1995)
USA	Female	NM	7	0.10	0.80	Khan et al. (1995)
USA	NM	<1.5 year	11	0.14	0.19	Khan et al. (1995)
USA	NM	2–7 years	9	0.23	0.88	Khan et al. (1995)

NM – not mentioned; ND – not detected; *mg kg^{-1} dry weight.

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Table 2. Comparison of certified and measured Cd values (n = 5) in CRM (ERM – CE278, Mussel tissue, IRMM, Geel, Belgium) and other relevant validation data.

Certified (mg kg^{-1})	Found (mg kg^{-1})	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Recovery (%)	RSDr (%)	Measurement uncertainty (%)	Accreditation
0.348	0.349	0.011	0.028	100.4	2.53	2.84	Yes

Table 3. Cd levels (mg kg^{-1} wet weight) in liver and kidneys from domestic Balkan and Alpine goats in Montenegro and Serbia

Goat breed	Country	Montenegro				Serbia			
	Age (year)	1		4		1		4	
	Organ	Liver (n=36)	Kidney (n=36)	Liver (n=36)	Kidney (n=36)	Liver (n=36)	Kidney (n=36)	Liver (n=36)	Kidney (n=36)
Domestic Balkan (n=288)	Mean± SD	0.037±0.012^{gB}	0.066±0.019^{efB}	0.093±0.032^{dB}	0.169±0.092^{bB}	0.049±0.016^{fgB}	0.089±0.027^{deB}	0.127±0.053^{cB}	0.201±0.091^{aB}
	Range	0.020–0.067	0.039–0.099	0.028–0.145	0.038–0.327	0.019–0.081	0.034–0.136	0.018–0.211	0.041–0.369
Alpine (n=288)	Mean± SD	0.078±0.028^{fA}	0.153±0.071^{eA}	0.212±0.103^{dA}	0.364±0.152^{bA}	0.088±0.017^{fA}	0.187±0.055^{deA}	0.266±0.130^{cA}	0.439±0.189^{aA}
	Range	0.034–0.133	0.042–0.291	0.055–0.371	0.080–0.661	0.049–0.121	0.066–0.272	0.065–0.459	0.098–0.753

^{abcdefg} Means with different letters in the same row indicate significant differences at $p < 0.05$; ^{AB} Means with different letters in the same column indicate significant differences at $p < 0.05$.

Table 4. Pearson correlation coefficient (PCC) between cadmium concentrations in liver and kidney

Goat breed	Domestic Balkan				Alpine			
Country	Montenegro		Serbia		Montenegro		Serbia	
Age (year)	1	4	1	4	1	4	1	4
PCC	0.620*	0.880*	0.587*	0.905*	0.836*	0.875*	0.665*	0.937*

*$p < 0.001$.

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