International Food Research Journal 23(3): 1012-1016 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Heavy metals in small ruminant's milk from Algerian area steppe

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Article history

Received: 10 January 2015
Received in revised form: 9 September 2015
Accepted: 11 September 2015

Keywords

Algeria
Heavy metals
Ewe's milk
Goat's milk
Health
Contamination

Abstract

The total contents and distributions between soluble and colloidal phases of heavy metals (Pb, Cd and Cr) in milk of two ewe's breed (Ouled-Djellal and Rumbi) (n = 20 each) and one local goat (n = 10) reared in Algerian area steppe, were evaluated. Ewes and goats were conducted in the same herd with similar housing and feeding. All animals were pastured in non-polluted region of Djelfa. Individual milk samples were collected three times during spring season and analyzed for their heavy metal contents. Ewe's milk exhibited higher ($P \leq 0.001$) levels of Pb and Cd than goat's milk but lower ($P \leq 0.001$) value of Cr. The average Pb and Cd contents were 0.181 ± 0.062 ppm and 0.061 ± 0.021 ppm for ewe's milk and 0.070 ± 0.023 and 0.012 ± 0.004 for goat's milk respectively, which was considered as tolerable levels for human consumption. The concentrations of Cr were 0.054 ± 0.023 ppm and 0.131 ± 0.058 ppm for ewe and goat milks, respectively. There was no significant difference between the two breeds of Algerian ewe's milk. Theses toxic elements were not in dangerous concentrations.

Introduction

Human activities and geological background are the two important origins of trace elements in the environment (Baize and Sterckeman, 2001; Imperato *et al*., 2003). Industrialization and increasing number of cars explain the presence of these harmful elements in the nature. Among twenty three metals, lead (Pb) and cadmium (Cd) are considered the most toxic (Dana, 2014) while chromium (Cr) is known as essential and important in health and metabolism at low concentration (Bowen, 1979) but toxic at high concentration (Llobet *et al*., 2003). They can be absorbed by soil and then transferred in plants that constitute feeding stuff. In final, these elements can be present in animal products like milk (Trancoso *et al*., 2009). According to Nwude *et al.* (2010), blood being a major medium of transfer of heavy metals into milk. Thus, milk can be considered as bioindicator of industrial pollution (Kashamov *et al.*, 2005). The chemical form in which a macro mineral and trace element is found in milk is important, because it will influence the degree of intestinal absorption and utilization, transport, cellular assimilation, and conversion into biologically active forms, and thus bioavailability (Cashman, 2006). Consumption of contaminated milk may be dangerous for human health, especially for infants.

In Algeria, sheep and goat milks are mainly produced in steppe for sheep and in mountain region for goat. They are directly consumed by family or transformed at artisanal level in "smen" (traditional butter), "l'ben" (fermented milk) or "j'ben" (fresh cheese). The mineral composition of Algerian ewe's milk was studied (Yabrir *et al*., 2014) but not those of goat's milk. Moreover, there is no information on the trace and toxic elements in these milks, produced in non-polluted Algerian area steppe. The objective of this study was to determine the total concentrations of lead, cadmium and chromium and their distributions between the soluble and colloidal phases in raw sheep and goats' milks. The effect of type of breed was also discussed.

Materials and Methods

Animals and sampling

This study was carried out with two breeds of ewes (Ouled-Djellal "OD" and Rumbi "RB") (n = 20 each) and one local breed of goats (n = 10)

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located in Algerian area steppe. Ewes and goats were conducted in the same herd with similar conditions of housing and feeding. They were pastured in non-polluted region of Djelfa. Individual milk samples were collected three times during spring season. After hand milking, milks were cooled 24 h and then analyzed for their mineral composition.

Analytical procedure

Mineral concentrations were determined on whole milk for total contents and on diffusible fraction for soluble contents. The soluble phase was obtained after coagulation of milk by rennet (Texel-Poulenc France) followed by recovery of whey as described by De La Fuente *et al.* (1996). Each individual milk was dried overnight at 103±2°C and then ashed in a muffle furnace at 550°C for 8h (Miles *et al.*, 2001). The ash obtained was dissolved in 50%, 10% HCl solutions and deionized water. After each addition, solutions were dried to appropriate volume on a hot plate according to Miles *et al.* (2001). Then, they were filtered and diluted to appropriate detection level as suggested by aforementioned authors. Trace elements analysis (Cr, Pb and Cd) were performed using atomic absorption spectrometry (AAnalyst 400, Perkin Elmer). Three standard solutions of 0.01, 0.1 and 0.5 ppm were prepared for calibration curve by using stock standard solution ISO (1.000 ppm for each element) immediately before analysis. Deionized water with 18 MΩcm resistance was used in the preparation of all sample and standard solutions. Each sample were analyzed in duplicate.

Statistical analysis

Statistical analysis was carried out using Statistica program. The significant differences between means were calculated by one-way Analysis of Variance (ANOVA) using Turkey range test. Breed and species were the factors studied and the probability level was 95 or 99%.

Results and Discussion

Concentration of heavy metals in ewe and goat milks

Pb and Cd elements - Results are reported in Tables 1 and 2 for ewe and goat milks, respectively. On the one hand, there is no statistical difference related to the ewe's breed for all trace elements. On the other hand, ewe milk exhibited higher ($P \leq 0.001$) level of Pb and Cd than goat milk and lower ($P \leq 0.001$) value of Cr. Generally and according to Park *et al*. (2007) mineral contents of sheep milk are higher than those of goat milk.

The average contents of Pb and Cd in ewe milk (Table 1) were in accordance with the previous results of the same species (Anastasio *et al*., 2006) but lower than that reported by other authors (Antunovic *et al*., 2005; Borys *et al*., 2006; Ivanova *et al*., 2011). Our values were intermediate between the values obtained by Caggiano *et al.* (2005) in summer (0.05 and 0.17 µg/g for Pb and Cd, respectively) and winter (0.07 and 0.22 µg/g, respectively). This difference can be attributed to changes in ovine feeding during the year. For goat milk, similar values of Pb and Cd were determined than concentrations reported by Güler (2007). For Cd, we determined lower value than Güler (2007). The presence of Pb in goat milk corresponded to the limit of quantification considered by Trancoso *et al*. (2009) while Cd concentration was higher. Rahimi (2013) found lower values of Pb and Cd in sheep and goat milks than the concentrations determined in our study. The comparison of the results of Antunovic *et al.* (2012) for goat milk from organic breeding with our results indicated that these elements were at higher concentrations. The presence of Pb in milk is attributed to many factors such as transhumance along roads and/or motorways, fodder and water contaminations and climatic factors (Birghila *et al*., 2008). Cd in milk might have natural or anthropogenic origins - fertilizers and atmospheric deposition in soil - (Maas *et al*., 2011) and is considered as an industrial risk (Massanyi *et al*., 1995). On the other hand, the presence of these elements at higher amount than the permissible norms in sheep milk is an indicator of environmental contamination (Licata *et al*., 2004; Ivanova *et al*., 2011). Cd and Pb are note known to serve any essential biological function (Liu, 2003) but are well known for the toxic effects (Tona *et al.,* 2013; Khan *et al.,* 2014) because their biological activity is perceived to be largely confined to toxic reactions (McDowell, 2003) and can be cumulative (Ogabiela *et al*., 2011). In human body, Cd accumulates in liver and kidney and Pb in bone (Garcia-Esquinas *et al*., 2011). Accumulation of excessive levels of Cd and Pb causes renal damage and dysfunction (Salah *et al*., 2013). Pb is poorly absorbed by mammals and their concentrations in milk are generally low and milk is not considered as an important source of exposure (Casey *et al*., 1995). A provisional tolerable weekly intake (PTWI) have been established by FAO/WHO expert committee for Cd, 7 µg/kg and for Pb, 25 µg/kg bodyweight (FAO/WHO, 1993). From these remarks, it is necessary to control their concentrations in food (Birghila *et al*., 2008). In our cases, the concentrations of these elements determined in milk can be considered as not excessive.

Cr element - In our study, the average

Table 1. Content of trace elements (in ppm) in ewe milk

Element	Breed	min	Mean ± sd	max	P*	Overall mean
Pb	OD	0.092	0.181 ± 0.076	0.318	ns	0.181 ± 0.062
	RB	0.122	0.181 ± 0.045	0.259		
Cd	OD	0.031	0.056 ± 0.021	0.093	ns	0.061 ± 0.021
	RB	0.049	0.068 ± 0.019	0.099		
Cr	OD	0.020	0.053 ± 0.019	0.082	ns	0.054 ± 0.023
	RB	0.029	0.055 ± 0.029	0.099		

*: Analysis of variance (ns: not significant at 95%)

concentration of Cr in ewe milk was lower than those determined in goat milk (Tables 1 and 2). Our results obtained with the milk concentration of Cr for the two breeds of ewes were not significantly different (Table 1) and similar to that obtained by authors (Anastasio *et al.,* 2006; Ivanova, 2011). For goat's milk, Trancoso *et al.* (2009) reported 0.002 and 0.018 ppm as the minimum and maximum contents during the lactation period. In the opposite, Güler (2007) reported higher concentration (0.77 ± 0.08 ppm). A Cr concentration lower than of 0.003 mg /100 ml for goat milk was found in the study carried out by Lopez *et al.* (1985). While Pb and Cd are known as toxic elements, Cr is considered as essential trace element (Bowen, 1979) but it can be a poison at higher level (Qin *et al*., 2009). Cr compounds are mutagenic and carcinogenic in variety of test systems (Zodape *et al*., 2012). However, it is essential to maintain the metabolic systems of human body (Qin *et al*., 2009) and plays a role in sugar metabolism as a cofactor with insulin (Hoekstra *et al*., 1970). According to Mertz (1993), Cr deficiency results in insulin resistance which can be improved by Cr supplementation. This latest resulted in reduction of total cholesterol, reduction of LDL cholesterol and increasing of HDL cholesterol (Press *et al*., 1990). The essential roles of Cr in human nutrition and health are highlighted in the review of Krejpcio (2001) and Pechova and Pavlata (2007); and the recommended dietary intake of Cr during the first half of infancy is 0.01-0.04 mg/day (Casey *et al*., 1995).

Distribution of Cd, Pb and Cr between the soluble and colloidal phases of milk

Cd and Cr - Globally, Cd was more retained in the colloidal phase of ewe milk than those of goat milk (Table 3). For ewe milk, our study showed that the Cd concentration in the micellar fraction was in agreement with the percent reported by Milhaud *et al.* (1998) (77 vs 75%). This was not the case for goat milk where 75% was determined in our study against 59% by Milhaud *et al.* (2000). These authors have also determined that the rennet curd retained more Cd than lactic curd for ewe or goat milks. On the other hand, Anastasio *et al*. (2006) reported that Cd was more present in the soluble fraction and probably equally distributed between casein micelles and components of low molecular mass in the aqueous phase. The same authors observed, during cheese manufacture, an increase of Cr in cheese compared to raw milk. This increase suggested that Cr was preferentially bound to casein molecules in milk and remained attached to these compounds during the coagulation step because Cr was also determined in the curd.

Table 2. Content of trace elements (in ppm) in goat milk

Element	min	Mean ± sd	max
Pb	0.043	0.070 ± 0.023	0.118
Cd	0.008	0.012 ± 0.004	0.021
Cr	0.048	0.131 ± 0.058	0.278

Pb and Cd – These elements were also more retained in the colloidal phase of ewe milk than those of goat milk (Table 3). Anastasio *et al.* (2006) and Tona *et al.* (2013) observed high residual Pb concentration in cheese. Coni *et al*. (1996) also stated that Cd and Pb were mainly present in the curd of cow milk compared to raw milk, which is in agreement with the hypothesis that Pb and Cd are essentially linked to the casein fraction (Mata *et al*., 1996). It is noteworthy that after heat treatment, the distribution of Cd in milk change due to the formation of complexes between the whey proteins and the metal or to the dissociation of Cd initially bound to casein micelles (Salah *et al*., 2013). In the opposite, the distribution of Pb did not change significantly either

Table 3. Distribution of metals between soluble and colloidal phases

		Pb	Cd	Cr
Soluble (ppm)	Sheep	0.00	0.0138	0.0169
	Goat	0.004	0.0029	0.075
% of soluble	Sheep	0	23	19
	Goat	6.13	24.82	31.25
Colloïdal (ppm)	Sheep	0.178	0.0462	0.0371
	Goat	0.066	0.0091	0.056
% of colloïdal	Sheep	100	77	81
	Goat	93.87	75.18	68.75

in cow's milk or milk products (Mata *et al.*, 1996).

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