BENI C.[1], MARCONI S.[1], PENNELLI B.[1], RONCHI B.[2]

Xenobiotics concentration and mobility in bovine milk from Italian farms

PROGRESS IN NUTRITION
VOL. 9, N. 1, 39-45, 2007

TITOLO
Concentrazione e mobilità di xenobiotici nel latte bovino in Italia

KEY WORDS
Xenobiotic, metal, law limits, bovine milk

PAROLE CHIAVE
Xenobiotici, metalli, limiti di legge, latte bovino

Summary
In sixty Italian dairy farms, with both conventional and biological bovine milk production, representative of the national situation, samples of different matrixes were analyzed in order to evaluate the presence of potential contaminant elements. In bovine milk chain (soils, irrigation and clear water, forages, integrators, unifeed and milk), quantitative analysis were performed with regard to heavy metals (Cd, Hg, Ni, Pb, Cu, Zn), metalloides (As, B) and non-metals (Se). The results obtained show that in some cases Cd, Pb and Zn concentrations in unifeed are over the legal limits; concerning milk, we have a decrease of boron and copper and an increase of selenium. As a conclusion the results obtained suggest that no toxic risk can be expected on milk production and consequently on human health. However, screening of matrix and contamination source are necessary to fix appropriate reference law limits for xenobiotics concentration in milk.

Riassunto
In 60 aziende italiane produttrici di latte bovino, sia con tecniche convenzionali che biologiche, rappresentative della situazione nazionale, sono state analizzate differenti matrici per valutare la presenza di potenziali elementi contaminanti. Le analisi quantitative, condotte sulla filiera di produzione (suolo, acque di irrigazione e di abbeveraggio, foraggi, integratori, unifeed e latte), sono state effettuate su metalli pesanti (Cd, Hg, Ni, Pb, Cu, Zn), metalloidi (As, B) e non-metalli (Se). I risultati mostrano che, in alcuni casi, le concentrazioni di Cd, Pb e Zn sono state superiori ai limiti di legge per l'unifeed; nel latte, si evidenzia un decremento di boro e rame e un aumento di selenio rispetto ai valori riscontrati in letteratura. In conclusione, i risultati del lavoro indicano che non sussiste il rischio di contaminazione della produzione di latte bovino e, conseguentemente, di tossicità sulla salute umana. Si è evidenziata, infine, la necessità di continuare il monitoraggio delle matrici e delle fonti di contaminazione per poter fissare appropriati limiti di legge per gli xenobiotici nel latte.

[1] CRA - Istituto Sperimentale per la Nutrizione delle Piante, Roma
[2] Department of Animal Production, Università della Tuscia, Viterbo

Indirizzo per la corrispondenza:
Dr. Claudio Beni
Istituto Sperimentale per la Nutrizione delle Piante
Via della Navicella 2-4
00184 Roma
Tel: 06-7008260
Fax 06-7005711
E-mail: claudio.beni@entecra.it

Introduction

Within the programme "Prevention of contamination in conventional and biological agricultural system" supported by Italian Ministry of Agriculture and Forest, the Experimental Institute of Plant Nutrition has studied the concentration and potential mobility in bovine milk of nine elements: As, B, Cd, Hg, Ni, Pb, Cu, Se and Zn. Samples of soil, forage crops, unifeed and other food, beverage and irrigation water from Italian farms have been analyzed.

For what regards the elements analyzed, copper, selenium and zinc are essential microelements (toxic at high doses); arsenic, cadmium, mercury, nichel and lead have a toxic effect on humans and animals when they entry the food chain (1-3).

In previous works (4, 5) the presence of metallic elements in animal diet and the eventual risk in human diet have been studied.

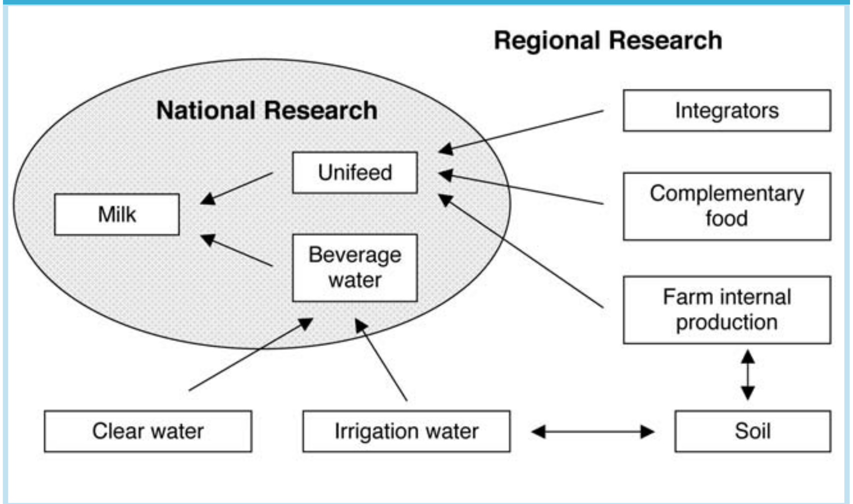
Food contamination is an unavoidable effect due to environmental and anthropic activities. Industrial, agricultural and urban activities are the main sources of pollution (6- 8).

The concentration of B, Se, Zn and Cu in milk mainly depends on nutritional factors as, being essential microelements for vegetables, they are a part of daily ration; as for Zn and Cu also the contamination from pipes into drinking water could be important. For what concerns undesired elements as As, Cd, Hg, Ni, Pb, only very small concentrations are allowed. Possible contamination of milk from ingestion of metals is circumscribed by physiological detoxification mechanisms (1-3, 9-14).

Present law limits on concentration of metals in the animal diet (Tab. 3) have been improved with respect to the one mentioned in two our previous publications (4, 5). However, in our opinion some gap in regulation persists. Indeed, only the presence of As, Cd, Hg, Pb, Cu and Zn in unifeed is regulated in Italian Legislation (16-20). For what concerns milk, only the limit of lead is fixed (21). For beverage water no legal limits exist. These limits must be necessary because daily ration of water can reach 150 L head^{-1} per day at the top of milking. Therefore one cannot exclude negative effects when the concentration of one of the mentioned metals is over the threshold value. Apart from the case of milk productions from farms, regulated by D. Lgs 31/2001 (22), that imposes the use of net water for beverage of cows, there are still possible gaps in checking levels of metals in beverage water.

The present monitoring considered the potential contamination of the above mentioned elements in bovine milk produced both in conventional and biological systems. Two different levels of investigation have been adopted in the study: national and regional (Fig. 1). The national research examined 60

Figure 1 - Sampling design of the investigation



farms distributed in North, Centre and South Italy; the second one 10 farms in Lazio, three of them being biological.

Materials and methods

National research regarded nine elements (As, B, Cd, Hg, Ni, Pb, Cu, Se e Zn) by means of the following methods of sampling (Tab. 1); Regional research is about the same elements but in a higher number of matrixes (Table 2).

All matrixes were collected with 3 repetitions by means of the Italian official reference methods for water (23), unifeed (24) and milk (25).

The determination of As, B, Cd, Hg, Ni, Pb, Cu, Se and Zn, has been respectively performed by:

Soil: Official methods of analysis (26).

Water: EPA standard methods 3120 and 3125 (27, 28).

Milk: acid digestion with microwave, mass/plasma spectrometer, certified standard CRM63R.

Unifeed and other vegetal samples: acid digestion with microwave, mass/plasma spectrometer.

Table 1 - Matrixes and methods of sampling for national research

Matrix	Sample
beverage water	2 x 125 ml from tap
milk	5 x 125 ml from refrigerator after mixing
unifeed	8 manger samples

Table 2 - Matrixes and methods of sampling for regional research

Matrix	Sample
farm soils	5 bulked samples (3 sub-samples 0-30 cm) on standard polygon
irrigation water	2 x 125 ml from irrigation
beverage water	2 x 125 ml from tap milk 5 x 125 ml from refrigerator after mixing
unifeed	8 manger samples
forage, complementary food and integretors	8 sampled

The data derived from the national research have been analysed with a statistical pattern: multiple comparison regression (multivariate) blocks analysis (ANOVA): as dependent variable we chose elements content in milk and the elements concentration in water and unifeed as independent variable.

In regional research the elements content in unifeed has been considered as depending on xenobiotics amount in soil and every kind of food.

Results

When a comparison between our results and the law limit has been not possible, for solving the "legal empty", national literature reports for contamination in water, milk and food have been considered. In regard to beverage water we took into account the legal limits in force for drinking water.

National research:

- For what regards milk, the results of the statistical analysis are shown in Table 3. Ni, Se and Zi concentration increased significantly.
- The mean content of metals observed in food is within the legal limits. Only for copper, selenium and zinc there is an increase that in our opinion depends on the unsuitable use of integrators.
- Zn level in beverage water is

Table 3 - Results of national research on bovine milk chain

Element	Limits	Milk ($\mu g\ L^{-1}$) Certified concentration (min-max)	Mean concentration[4]
Arsenic[2]	20-60	5-10.4	5.29
Boron[2]	500-1000	108-574	272.58
Cadmium[2]	0.05-3	0.08-0.32	0.11
Mercury[2]	0.05-2	1.00	1.00
Nichel[2]	0.4-6	2.4-59.6	4.58
Lead[1]	20.00	0.16-3.2	1.06
Lead[2]	20.00		
Copper[2]	90.00	22.20-58.5	39.84
Seleium[2]	10.00	16.66-86.20	36.64
Zinc[2]	3900.00	2379-7560	4073.10

Element	Limits	Food ($mg\ kg^{-1}$ s.s.) Certified concentration (min-max)		Mean concentration[4]	
		Ensilate	Unifeed	Ensilate	Unifeed
Arsenic[1]	2.00	0.50	0.50-0.58	0.500	0.501
Boron	-	2.64-26.12	5.06-65.90	6.99	14.09
Cadmium[1]	1.00	0.008-0.407	0.015-0.106	0.028	0.046
Mercury[1]	0.10	0.0007-0.0840	0.0006-0.0150	0.0045	0.0045
Nichel	-	0.070-7.710	0.220-7.040	0.84	2.48
Lead[1]	5.00	0.060-16.080	0.060-1.840	0.24	0.39
Copper[1]	35.00	2.54-13.72	2.98-42.21	4.37	8.59
Copper[2]	11.00				
Selenium[2]	0.30	0.018-0.50	0.030-0.71	0.09	0.22
Zinc[1]	150.00	5.80-64.30	8.50-235.00	16.42	45.93
Zinc[2]	48.00				

Element	Limits [3]	Water ($\mu g\ L^{-1}$) Certified concentration (min-max)	Mean concentration[4]
Arsenic	10.00	1.00-43.70	1.16
Boron	1000.00	10-10003	29.42
Cadmìum	5.00	0.20-1.31	0.22
Mercuriy	1.00	0.20-1.50	0.22
Nichel	20.00	1.00-76.50	1.65
Lead	10.00	0.20-12.20	0.56
Copper	1000.00	1.00-64.00	3.39
Selenium	10.00	1.00-8.50	1.48
Zinc	-	2.30-7420	93,25

[1] Legislative limits in force; [2] Mean value indicated in "Encyclopedia of Dairy Science"; [3] Legislative limits for drinking water; [4] Data are expressed as geometric mean of total value

above the one prescribed for standard limits.

Regional research:

- An analysis of the regional research results, besides a confirmation of the national research data, helped us out to observe how metals enter animals diet (Tab. 4, data not reported).
- The concentration of metals in unifeed, except for Hg and Se, is related to farms production. Cd and Cu levels have secondary origin from industrial product (complementary food and integrators).
- With regard of mercury concentration there is a direct contribution from soil, while Se compound comes from industrial matrix.

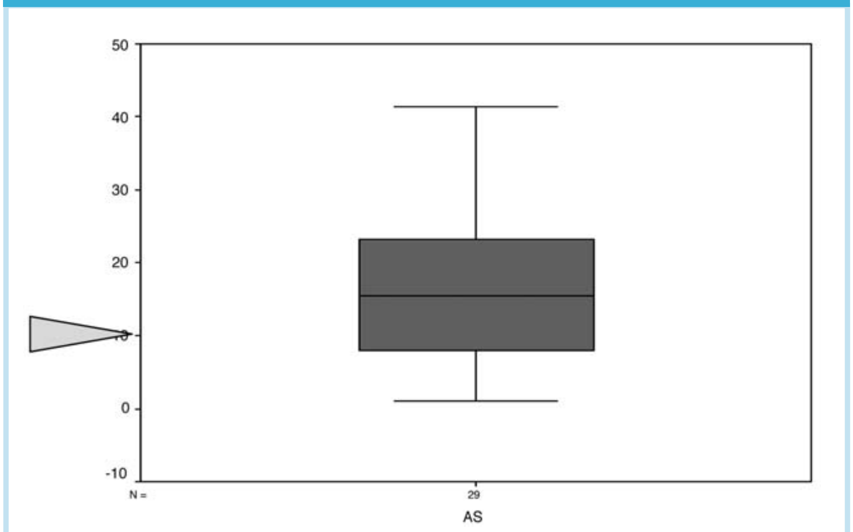
The present work underlines also the risk of high amounts of arsenic in net and bearing stratum water, due to the volcanic origin of the soils of Lazio Region. Figure 2 shows legal threshold limit (triangle indicator) in force for drinking water (10 mg L^{-1}); 75 % of samples from Lazio are above the standard for drinking water.

Nevertheless distribution curve of As (Fig. 3) in soil samples satisfied normal curve. This event may suggests that high level of arsenic have geological origin. However no toxic As levels has been observed in milking production. This is probably due to the accumulation of the element in other animal organs, such as liver, loin, intestine and spleen (29).

Table 4 - Results of regional research

Elements	Primary origin	Secondary origin
As	Internal farm production	
B	Internal farm production	
Cd	Internal farm production	Commercial products
Hg	Soil	
Ni	Internal farm production	
Pb	Internal farm production	
Cu	Internal farm production	Commercial products
Se	Commercial products	
Zn	Internal farm production	

Figure 2 - Arsenic in drinking water ($\mu g\ L^{-1}$) resulted in regional research



Comparison of conventional and biological farming proves that different production managements have no effect whatsoever on metal contamination. Moreover is not possible to carry out statistical analysis because there are a few replications (samples from three biological farm in Lazio) but we may suggest a different trend in both type of farming: biological and conventional.

Table 5 displays the main characteristics of soils in the different farming models: biological soil had a higher organic matter and nutrients content than conventional one.

In conclusion, these results show how important is the knowledge of interaction between these elements and soil (xenobiotic content available for cultivated forages) in order to evaluate the risk of contamination.

Conclusions

The investigation of nine xenobiotics in 60 farms was sufficient to describe milking production at a national level.

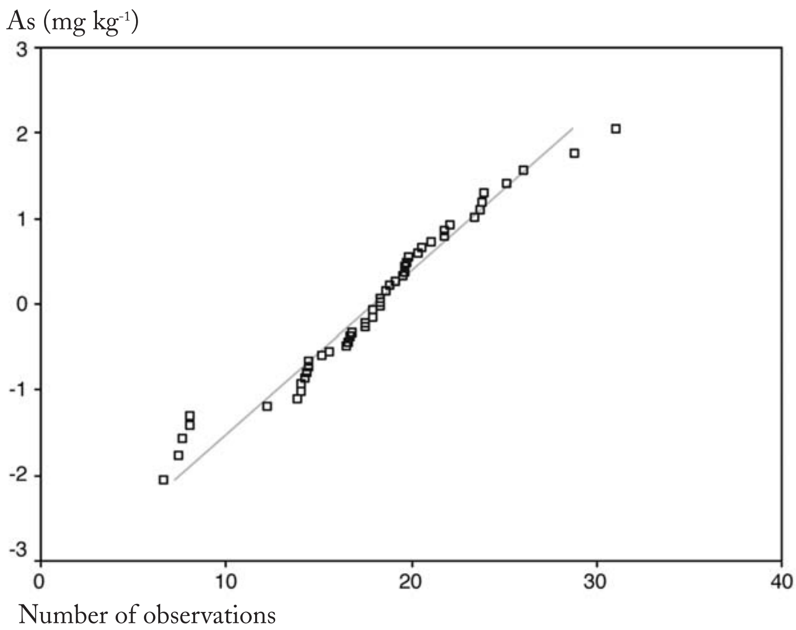
Milk is a important and basic food in the human diet, so it is necessary to fix legal thresholds for all toxic elements.

In brief: Cd, Pb and Zn levels are frequently above the standard limits in unifeed; Arsenic concentration is higher in soil and water of Lazio. With regard to milk quality in Italy, as found out in other studies, a low content in B and on the contrary high Se level have been confirmed.

These findings suggest that milking production in Italy is safe from risk of the mentioned elements contamination.

The relation between milk quality and animal production, vegetal production and soils characteristics exists. This can outfit a control on

Figure 3 - Curve of arsenic distribution in soils from Lazio region (mg kg^{-1})



how pollutants enter milking production.

Different control measures for integrators and complementary food coming from foreign countries (cotton, soybean and vegetal meal) seem to be obligatory.

In order to implement a prevention for metals pollution, further studies are in progress, aiming to investigate toxic levels of contamination. In most part of cases, these elements are fixed in soil by kind and amount of clay, organic matter compound, iron oxides etc. and transmitted to organism at a higher trophic levels. This kind of study is very complex because of the interaction of several factors: species-specific aspects, contact and interaction time with other pollutants. The foreseeing of how these substances will end up and their interaction with the environment are important in order to evaluate the assessment risk and to fix tolerable concentration.

Table 5 - Main physical-chemical features of soil from farm in Lazio

System	pH	CEC meq/100 g %	Sand %	Silt %	clay USDA	Textural %	OM %	N mg/kg	P_2O_5 mg/kg	K_2O
Biological	6.7	31.79	43.4	40.6	16	Loamy	1.65	0,02	46.17	419.17
	6.2	11.6	18.5	63.5	18	Silty loam	1.71	0,12	28.85	584.01
	6.6	16.54	40.8	45.2	14	Loamy	2.01	0,09	26.38	645.24
Traditional	7.5	9.84	68.7	18.3	13	Sandy loam	1.64	0,13	134.38	461.56
	6.8	31.46	33	50	17	Silty loam	3.22	0,2	125.31	2981.29
	7.3	19.57	47.9	28.1	24	Loamy	1.61	0,15	30.5	169.55
	6.7	23.08	35.1	40.9	24	Loamy	2.69	0,22	61.01	767.69
	6.8	26.92	31.9	40.1	28	Clavey loam	2.64	0,17	78.32	880.73
	7.8	15.81	17.2	58.8	24	Silty loam	2.08	0,18	32.15	282.59
	7.2	33.45	56.3	37.7	6	Sandy loam	1.57	0,13	131.9	4271.77

References

1. Coni E, Caroli S, Ianni D, Bocca A. A methodological approach to the assessment of trace elements in milk and dairy products. Food Chemistry 1994; 50: 203-10.
2. Coni E, Bocca A, Ianni D, Caroli S. Preliminary evaluation of the factor influencing the trace element content of milk and dairy products. Food Chemistry 1995; 52: 123-30.
3. Coni E, Bocca A, Coppolelli P, Caroli S, Cavallucci C, Trabalza Marinucci M. Minor and trace element content in sheep and goat milk and dairy products. Food Chemistry 1996; 57: 253-60.
4. Pennelli B, Sequi P. Contenuto in metalli pesanti nella dieta animale. L'informatore Agrario 2001; 41: 28-29.
5. Pennelli B, Sequi P. Microelementi negli integratori dietetici umani. L'informatore Agrario 2001; 50: 15-6.
6. Beretta C. Tossicologia veterinaria. Milano: Grasso, 1984.
7. Cerruti G. Residui di addittivi e contaminanti negli alimenti. Milano: Tecniche Nuove, 1999.
8. Casarett LJ, Doull J. I fondamenti dell'azione delle sostanze tossiche. Roma: EMSI, 2000.
9. Maggi E, Campanini G, Bracchi PG, Bagnoli R. Determinazione dell'arsenico in alcuni prodotti di origine animale. Atti Società Italiana Scienze Veterinarie 1975; 34: 577-80.
10. Gregorio P, Siracusano C. Sulla contaminazione da piombo nel latte vaccino. Ig Mod 1976. 69: 296-305.
11. Bertolani G, Mazzati P, Barbieri G, Fogliari G, Conti E. Determinazioni e considerazioni relative al dosaggio di piombo nel latte. Industrie Alimentari 1977; 16 (39): 102-4.
12. Feller E. Indagine preliminare sul contenuto in piombo del latte conferito alla provincia di Trento. Latte, 1983; VIII (6): 446-8.
13. Baldini M, Coni E, Stacchini A, Stacchini P. Presence and assessment of xenobiotic substances in milk and dairy products. Annals of Istituto Superiore Sanità 1990; 26 (2): 167-6.
14. Pittia P, Mazzilis D, Dalla Rosa M, Corradini C. Indagine sul contenuto in piombo in latte della provincia di Udine. Scienza e Tecnologia Lattiero-Casearia 1992; 43 (3): 201-8.
15. Imparato E, Esposito M, De Grado G, Grasso L, Oliviero G, Maddaluno F. Livelli di piombo e cadmio, quali indicatori ambientali, nel latte in aziende zootecniche dell'Irpinia. Latte 1999; 24 (5): 106-9.
16. Direttiva CE (32). Sostanze e prodotti indesiderabili nell'alimentazione degli animali. In Direttiva 2002/32/CE del 07/05/2002. Gazzetta Ufficiale dell'Unione Europea del 30/05/2002, L 140.
17. Decreto Ministeriale (317). Regolamento interministeriale recante norme di attuazione della direttiva 1999/29/CE, relativa alle sostanze ed ai prodotti indesiderabili nell'alimentazione degli animali. In Decreto del Ministero della Salute n. 317 del 23 dicembre 2002. Gazzetta Ufficiale della Repubblica Italiana n. 155 del 07/07/2003.
18. Regolamento CE (1334). Modifica delle condizioni per l'autorizzazione all'impiego di additivi appartenenti al gruppo degli oligoelementi nell'alimentazione animale. In Regolamento CE 1334/2003 del 25/07/03. Gazzetta Ufficiale dell'Unione Europea del 26/07/2003, L 187.
19. Decreto del Presidente della Repubblica (433). Disciplina di preparazione, commercio, distribuzione e impiego di additivi delle premiscele e dei mangimi che li contengono. In DPR 433/2001 del 02/11/2001. Gazzetta Ufficiale della Repubblica Italiana n. 291 del 15/12/2001, Supplemento Ordinario n. 269.
20. Direttiva CE (100). Limiti massimi consentiti di sostanze indesiderate nei mangimi. In Direttiva 2003/100/CE del 31/10/2003. Gazzetta Ufficiale dell'Unione Europea del 01/11/2003, L 285.
21. Regolamento CE (466). Definizione dei tenori massimi di alcuni contaminanti presenti nelle derrate alimentari. In Regolamento CE 466/2001 del 08/03/2001. Gazzetta Ufficiale dell'Unione Europea del 16/03/2001, L 77.
22. Decreto Legislativo (31). Attuazione della direttiva 98/83/CE relativa alla qualità delle acque destinate al consumo umano. In D. Lgs. 31/2001. Gazzetta Ufficiale della Repubblica Italiana n. 52 del 03/03/2001, Supplemento Ordinario n. 41.
23. Decreto Ministeriale (37) Approvazione dei "Metodi ufficiali di analisi delle acque per uso agricolo e zootecnico". In Decreto del Ministero delle politiche agricole e forestali del 23/03/2000. Gazzetta Ufficiale della Repubblica Italiana n. 87 del 13/04/2000, Supplemento Ordinario n. 60.
24. Decreto Ministeriale (52). Modalità di campionamento degli alimenti per uso zootecnico. In Decreto del Ministero della sanità del 20/04/1978. Gazzetta Ufficiale della Repubblica Italiana n. 165 del 15/06/1978.
25. Decreto Ministeriale (31). Attuazione della decisione n. 91/180/CEE concernente la fissazione di metodi di analisi e prova relativi al latte crudo e al latte trattato termicamente. In Decreto del Ministero della sanità del 26/03/1992. Gazzetta Ufficiale della Repubblica Italiana n. 90 del 16/04/1992, Supplemento Ordinario n. 67.
26. MiPAF. Metodi di analisi chimica del suolo. Ministero per le Politiche Agricole e Forestali, Osservatorio Nazionale Pedologico e per la Qualità del suolo. Milano: Franco Angeli, 2000.
27. American Water Works Association. Method n. 3120. In Analytical methods for ground water and drinking water. Standard methods, 1998. 20th Edition.
28. American Water Works Association. Method n. 3125. In Analytical methods for ground water and drinking water. Standard methods, 1998. 20th Edition.
29. National Academy of Science. Arsenic medical and biological effects of environmental pollutions. Washington, 1977: National Research Council, Division of Medical Sciences, Assembly of Life Sciences.