Occurrence of aflatoxins in feedstuff, sheep milk and dairy products in Western Sicily

Carlo Finoli¹, Angela Vecchio²

¹ Dipartimento di Ingegneria e Tecnologie Agro-Forestali. Università di Palermo, Italy
² Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche. Università di Milano, Italy

Corresponding author: Prof. Carlo Finoli. Dipartimento di Ingegneria e Tecnologie Agro-Forestali. Facoltà di Agraria, Università degli Studi di Palermo. Viale delle Scienze 13, 90128 Palermo, Italy - Tel. +39 091 7028134 - Fax: +39 091 484035 - Email: cfinoli@unipa.it

Paper received June 6, 2003; accepted September 11, 2003

ABSTRACT

Samples of feedstuffs (15), milk (40), and cheese (30) coming from sheep and dairy farms (23) or market in Western Sicily were analyzed for their respective content of aflatoxin B₁ (AFB₁) and M₁ (AFM₁) to evidence any possible indirect mycotoxin contamination risk to the consumer. Analyses using HPLC and fluorescence detection were performed after immunoaffinity column sample extraction and cleanup; AFM₁ was detected in 30% of the milk samples at levels ranging from 4 to 23 ng/l and in 13% of the cheeses from 21 to 101 ng/kg; in the feed the AFB₁ ranged from <10 to 769 ng/kg. None of the contaminated samples exceeded the legal limits set down by the European Union for milk (50 ng/l) and feed (5 µg/kg), or that in force in the Netherlands for cheese (200 ng/kg).

Key words: Aflatoxin M₁, Sheep milk, Sheep cheese, Aflatoxin B₁, Feedstuff.

RIASSUNTO

PRESENZA DI AFLATOSSINE IN MANGIMI, LATTE E FORMAGGI DI PECORA PRODOTTI NELLA SICILIA OCCIDENTALE.

E’ stata condotta un’indagine su 15 mangimi e 40 latti di pecora, provenienti da diverse industrie zootecniche localizzate nella zona occidentale della Sicilia, sul contenuto in aflatoossina B₁ (AFB₁) e M₁ (AFM₁), al fine di evidenziare eventuali rischi legati alla presenza di xenobiotici in questa filiera agro-zootecnica. La ricerca dell’AFM₁ è stata effettuata anche su 30 campioni di formaggio reperiti nella rete distributiva o forniti dagli stessi produttori. Le determinazioni sono state condotte mediante HPLC e rivelazione spettrofluorimetrica, previa estrazione e purificazione del campione con colonne ad immunoaffinità. AFM₁ è risultata presente nel 30% dei campioni di latte con concentrazione compresa fra 4 e 23 ng/l e nel 13% dei campioni di formaggio con concentrazione compresa fra 21 e 101 ng/kg. AFB₁ ha oscillato fra <10 e 769 ng/kg nei mangimi. I livelli di micotossine nei campioni positivi di latte e mangimi non hanno superato i limiti stabiliti nell’Unione Europea (50 ng/l e 5 µg/kg) mentre nei formaggi rientrano in quelli fissati dalla legislazione olandese (200 ng/kg).

Parole chiave: Aflatossina M₁, Latte di pecora, Formaggio di pecora, Aflatossina B₁, Mangimi.
Introduction

In order to ensure good animal performance and animal welfare, it is most important to supply feed high in energy quality and of good hygienic quality, also from a toxicological point of view. The deterioration of feed, resulting in loss of nutritional compounds, is caused by the microbial growth, especially moulds, that through the formation of mycotoxins can affect the hygienic quality (Scudamore and Livesey, 1998).

When animals consume toxin contaminated feed the toxins are excreted as is, or after bio-transformation. One of the major xenobiotic contaminants of milk is AFM₁, that comes from AFB₁, and its carry over rate has been established experimentally as being usually 1-2% of the parent toxin (Applebaum et al., 1982; Spahr et al., 1999) but values as high as 5% have been reported (Veldman et al., 1992). Recently the carry over of aflatoxin from feed to ovine milk was studied by Battacone et al. (2002).

AFM₁ concentration is not affected by heat treatments, and the association of the mycotoxin with casein leads to an apparent cheese enrichment during cheese-making (Youssef and Marth, 1989; Finoli and Vecchio, 1997).

The International Agency for Research on Cancer (IARC, 2002) has classified both AFM₁ and AFB₁ as carcinogenic agents to humans (Group 1). Consequently, to protect consumers, particularly children, several countries have established, for milk and dairy product contamination, maximum admissible levels of AFB₁ and AFM₁ in feed and in milk respectively.

The monitoring of AFM₁ in cow milk and cow milk products is carried out in many countries (Galvano et al., 1996; 1998; 2001; Blüthgen and Ubben, 2000; Martins and Martins, 2000; Grasso et al., 2001; Palermo et al., 2001; Taveira and Midio, 2001; Virdis et al, 2001) but relatively little is known about the milk and cheese of sheep (Blanco et al., 1988; Martins et al., 1994; Lopez-Diaz et al., 1996; Rossi et al., 1996; Barrios et al., 1996; Minervini et al., 2001; Roussi et al., 2002).

In Italy there are about 6.8 million of sheep, and 10% of these are located in Sicily (ISTAT, 2002). There is only a small national production of sheep milk (650,000 t in the 2001), amounting to around 5% of the overall Italian dairy production (Assolatte, 2003), but there is a very significant variety of sheep cheeses available on the market. In recent years there has been an increasing demand for sheep cheeses in Italy (Anonymous, 2002) and the certain sale, with good profits, of these products is a very good reason to support sheep milk production, especially in marginal areas. It therefore follows, that it is essential that the products obtained be of good quality from nutritional, hygienic and toxicological aspects.

The aim of this work was to investigate AFM₁ contamination in samples of sheep milk and cheese collected in Sicily, and to evaluate, from a toxicological point of view, the quality of some feedstuffs.

Material and methods

Forty samples of sheep milk were collected from 23 sheep and dairy farms, located in Western Sicily, during the November 2001-June 2002 period. Samples were taken from pooled milk and

| Origin     | N. of farms | N. of samples | N. of positive samples | AFM₁ ng/l |
|------------|-------------|---------------|------------------------|-----------|
| Agrigento  | 11 (5)      | 23            | 5                      | 5-10      |
| Caltanissetta | 2 (1)    | 4             | 1                      | 5         |
| Palermo    | 5 (3)       | 7             | 3                      | 4-23      |
| Trapani    | 5 (3)       | 6             | 3                      | 6-22      |
| Total      | 23 (12)     | 40            | 12                     | 4-23      |

() number of farms with contaminated milk
stored at 4 °C until analyzed. Eleven farms were monitored two or three times and samples were collected every three months. The breeding farms varied in sizes and the number of head, belonging to the autochthon Valle del Belice and Comisana breeds, ranged from 20 to 750. Twenty eight sheep cheeses (6 Tuma, 11 Primosal, 9 Pecorino and 2 Vastedda) and 2 salty ricotta, supplied by sheep and dairy farms or obtained commercially, were also analyzed. In 14 cases cheese and milk from which the cheese was made were analyzed. Fifteen feed samples were supplied by some of the farmers for aflatoxin determination.

AFM: extraction from milk and cheese was carried out according to Finoli and Vecchio (1997), and AFB and AFG extraction from the feed was done by the AOAC Official Method (1995). For cleanup, Easi-Extract™ Aflatoxin (Rhône-Diagnostics Technologies Ltd., Glasgow, Scotland) immunoaffinity columns were used. Sample extracts were derivatized with trifluoracetic acid according to Park et al. (1990) and the final volume was 200 µl. HPLC determination was carried out with a Perkin Elmer Series 200 Liquid Chromatograph (Perkin Elmer Corp., Norwalk, CO, USA), equipped with a 20 µl loop, connected to a spectrofluorimetric detector. Perkin Elmer Fluorescence Detector Series 200, set, for AFM: at 365 and 435 nm wavelengths for excitation and emission respectively, and for AFB and G at 360 and 440 nm. The HPLC analyses were performed isocratically at 1 ml/min using water:methanol:acetonitrile (70:18:12) for AFM: and at 1.2 ml/min using water:methanol:acetonitrile (66:17:17) for AFB: on a Supelcosil LC-18 (15 cm x 4.6 mm, 5 µm) column, connected to a Supelguard Cartridge LC-18 (2 cm x 4.6 mm, 5 µm) (Supelco Inc., Bellefonte, PA, USA). The system was linked to a TurboChrom workstation.

The analyses were carried out in triplicate and the quantitation by external standard method.

The quantitation limits were 4 ng/l in milk and 15 ng/kg in cheese for AFM: and 10 ng/kg for AFB and AFG in feedstuffs. The recovery of AFM: after spiking milk at 25 ng/l and cheese at 150 ng/kg, was 92 ± 3% (relative standard deviation 3%) and 85 ± 4% (relative standard deviation 5%), respectively. The feeds were spiked with AFB: and AFG: at 1.7 µg/kg, and with AFB: and AFG: at 1.0 µg/kg. The recoveries were 97 ±

Table 2. Aflatoxin M₁ occurrence in the sheep cheeses and ricotta

| Origin  | Producer | Cheese type | N. of samples | N. of positive samples | AFM₁ ng/kg |
|---------|----------|-------------|---------------|------------------------|------------|
| Farms   |          |             |               |                        |            |
| Agrigento | A        | Pecorino 2 | 0             | < 15                   |            |
|          |          | Vastedda 2 | 0             | < 15                   |            |
| Caltanissetta | B   | Tuma 1     | 0             | < 15                   |            |
|          |          | Primosale 1| 0             | < 15                   |            |
|          |          | Pecorino 3 | 0             | < 15                   |            |
|          |          | Salty ricotta 1 | 0             | < 15                   |            |
|          |          | Primosale 2 | 2             | 27 - 101               |            |
|          |          | Salty ricotta 1 | 1             | 44                     |            |
| Palermo | D        | Tuma 2     | 0             | < 15                   |            |
|          | E        | Tuma 1     | 0             | < 15                   |            |
| Trapani | F        | Primosale 2 | 0             | < 15                   |            |
|          |          | Pecorino 2 | 0             | < 15                   |            |
| Market  |          | Tuma 2     | 0             | < 15                   |            |
|          |          | Primosale 6 | 0             | < 15                   |            |
|          |          | Pecorino 2 | 1             | 21                     |            |
| Total   |          |             | 30            | 4                      | 21 - 101   |
8% (relative standard deviation 8%) for AFB₁, 95 ± 12% (relative standard deviation 13%) for AFB₂, 102 ± 8% (relative standard deviation 8%) for AFG₁, and 104 ± 14% (relative standard deviation 13%) for AFG₂.

Results and discussion

The results, not corrected for recovery, are shown in Tables 1-3. Among the 40 milk samples, 12 exhibited AFM₁ levels between 4 and 23 ng/l, similar to the literature (Rossi et al., 1996; Roussi et al., 2002), and always lower than the tolerable level set down by the European Community (50 ng/l) (REG, 2001); in 70% of the samples the AFM₁ levels were lower than the quantitation limit of the analytical method (Figure 1). Of the 23 dairy farms 12 delivered contaminated samples; the milk of 4 out of 11 farms, monitored more times, was always toxin free.

The AFM₁ concentrations in the four positive cheeses were higher than 50 ng/kg in only one sample, but always below the 200 ng/kg limit recommended by the Netherlands legislation (Weidenbörner, 2001). Out of the 20 cheeses supplied by the dairy farmers only 3 samples, all from the same producer, were found to have AFM₁ at levels ranging from 27 to 101 ng/kg; of the 10 commercial cheeses one showed AFM₁ at 21 ng/kg. Our results seem a little better than that obtained by Minervini et al. (2001) who, on analyzing 94 samples of sheep cheese in Southern Italy, found 17% of the samples positive in the 50 to 210 ng/kg range. In 13 out of 14 cases in which both milk and cheese have been analyzed they were free from toxin; in the only case in which it was possible to calculate the apparent enrichment factor it was 5.4. Assuming this factor also for the most contaminated cheese, of the same type and supplied from the same producer, the AFM₁ concentration of the milk would be approximately 20 ng/l.

All the feed samples contained aflatoxins at a levels ranging from 17 to 1715 ng/kg, expressed as the total. The AFB₁ maximum was observed in a field bean sample (Vicia narbonensis var. velox) with a value of about 770 ng/kg, however the AFB₁ levels in the feeds were always below 5 µg/kg, the maximum tolerable concentration in feed for lactating animals (DM, 2003; DIR, 2002). Therefore, for what concerns aflatoxin presence, it can be considered that the feeds were of good quality. Furthermore the contamination levels appear lower than those reported in studies carried out in Northern and Southern Italy (Pietri et al., 2001; Esposito et al., 2001).

Figure 1. Distribution of sheep milk samples according to AFM₁ concentration.
Conclusions

AFM1 is an undesirable residue that occurs in milk, and, consequently, in the cheese made from this milk, when animals are fed AFB1 contaminated feed. Although the deductions made in our study on ovine milk are from a survey with limited sampling, it can be seen that the AFM1 levels detected in the sheep milk samples are reassuring since they were always well below the European tolerance level; furthermore only 13% of the cheese samples were found contaminated. However it is important to remember that AFM1 is cancerogenic and its presence must be avoided in foods as emphasized in the Reg. (CE) 466/2001 (REG, 2001). Nevertheless, climatic conditions in particular periods can lead to the occurrence of AFB1 in feedstuffs, and therefore also in feed of lactating animals, so the monitoring of sheep milk for AFM1 contamination should never be neglected.

It is quite obvious that health precaution strategies can reduce mycotoxin uptake upstream from milk production. One such strategy is to improve the quality of feeds and hence minimize the risk of mycotoxicoses in animals (Guerre et al., 2000).

The authors wish to thank Dr. S. Bruno for his valuable contribution to the study.

This research was supported by a grant of the Ministero dell’Istruzione, dell’Università e della Ricerca (60%).

REFERENCES

ANONYMOUS, 2002. Mercato favorevole ai formaggi ovicaprini. Informatore Agrario. 58(18): 120-121.
APPLEBAUM, R.S., BRACKETT, R.E., WIESEMAN, D.W., MARTI, E.H., 1982. Aflatoxin: toxicity to dairy cattle and occurrence in milk and milk products. A review. J. Food Prot. 45: 752-777.
ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1995. Official Methods of Analysis of AOAC International, 16th Ed., Gaithersburg, USA.
ASSOLATTE, 2003. Home page address: http://www.assolatte.it.
BARRION, M.J., GUALDA, M.J., CARANAS, J.M., MEDINA, J.M., JORDANO, R., 1996. Occurrence of aflatoxin M1 in cheeses from the South of Spain. J. Food Prot. 59: 898-900.
BLANCO, J.L., DOMINGUES, L., GOMEZ-LUCIA, J.F., GARAYZARAL, F.F., GOYACHE, J., SUAREZ, G., 1988. Behavior of aflatoxin during the manufacture, ripening and storage of Manchego-type cheese. J. Food Sci. 53: 1373-1376.
BATTACONE, G., NIBBA, A., PALOMBA, M., PULINA, G., 2002. Trasferimento di aflatossina dalla razione al latte ovino e alla cagliata. Sci. Tecn. Latt.-Cas. 53: 283-293.
BLUITHGEN, A., UBBEN, E.H., 2000. Zur Kontamination von Futtermitteln und Tankwagensammelmilch mit den Aflatoxinen B und M in Schleswig-Holstein - ein aktueller Überblick. Kieler Milchwirtschaftliche Forschungsberichte. 52: 333-354.
DIR, 2002. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Off. J. EC, L 140, May 30, 2002.
DM, 2003. Decreto Ministeriale 23 dicembre 2002, n. 317. Regolamento interministeriale recante norme di attuazione della direttiva 1999/29/CE, relativa alle sostanze ed ai prodotti indesiderabili nell’alimentazione degli animali. G.U.R.I. n. 155 del 7 luglio 2003.
ESPOSITO, M., ARACE, O., GRIMALDI, M., SOPRANO, V., SERPE, L., 2001. Indagine sulla contaminazione da...
La presenza di aflatossina M1 nel latte e nei suoi derivati provenienti dalle regioni Campania e Calabria. Riv. Sci. Alim. 30: 29-34.

GUERRI, P., BAILLY, J.D., BENARD, G., BURGAT, V., 2000. Excrétion lactée des mycotoxines: quels risques pour le consommateur? Revue Med. Vet. 151: 7-22.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, 2002. Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs Vol. 82, Lyon, France.

ISTITUTO NAZIONALE DI STATISTICA, 2002. Home page address: http://www.istat.it.

LOPEZ-DIAZ, T.M., ROMAN-BLANCO, C., GARCIA-ARIAS, M.T., GARCIA-FERNANDEZ, M.C., GARCIA-LOPEZ, M.L., 1996. Mycotoxin in two Spanish cheese varieties. Int. J. Food Microbiol. 30: 391-395.

MARTINS, M.L., MARTINS, H.M., 2000. Aflatoxin M1 in raw and ultra high temperature–treated milk commercialized in Portugal. Food Addit. Contam. 17: 871-874.

MARTINS, H.M., MARTINS, M.L., CRUZ, M.B., 1994. Metodos E.L.I.S.A e T.L.C. aplicados a pesquisa de aflatoxina M em leites de origem ovina e caprina. Vet. Tecn. 4(6): 20-25.

MINERVINI, F., VISCONTI, A., BOTTLACCO, A., MONTAGNA, M.T., 2001. Presenza di aflatossina M in formaggi dell’Italia Meridionale. Ind. Alim. 40: 513-516.

PALERMO, D., PALERMO, C., ROTUNNO, T., 2001. Survey of aflatoxin M level in cow milk from Puglia Italy. Ital. J. Food Sci. 13: 435-442.

PARK, D.I., NESHEIM, S., TRUCKSESS, M.W., STACK M.E., NEWELL, R.F., 1990. Liquid chromatographic method for determination of aflatoxin B1, B2, G1 and G2 in corn and peanut products: collaborative study. J. Assoc. Off. Anal. Chem. 73: 260-266.

BIETTI, A., BERTUZZI, T., GUALLA, A., GADOLINI, F., 2001. Aflatoxin M occurrence in milk samples produced in farms of the Po Valley. pp 243-245. in: Recent Progress in Animal Production Science. 2. Proc. 14th Nat. Congr. ASPA, Firenze, Italy.

REG, 2001. Commission Regulation (EC) N. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Off. J. EC, L 077, March 16, 2001.

ROSSI, J., CATALUSSI, L., GORDETTI, M., 1996. La presenza di aflatossina M nel latte vaccino ed ovino di alcune aziende dell’Umbria. Industria latte. 32(1-2): 37-40.

ROUSSI, V., GOVARIS, A., VARAGOLLI, A., BOTSOGLIO, N.A., 2002. Occurrence of aflatoxin M in raw and market milk commercialized in Greece. Food Addit. Contam. 19: 863-868.

SCUDAMORE, K.A., LIVESEY, C.T., 1998. Occurrence and significance of mycotoxins in forage crops and silage: a review. J. Sci. Food Agric. 77: 1-17.

SPARR, U., WALTHER, B., SIEBER, R., 1999. Vorkommen von Mycotoxinen in Futtermitteln und Carry-over in die Milch: Eine Übersicht. Mitt. Lebensm. Hyg. 90: 575-609.

TAVEIRA, J.A., MINDO, A.F., 2001. Incidence of aflatoxin M1 in milk marketed in São Paulo, Brazil. Ital. J. Food Sci. 13: 443-447.

WEIDENBÖRNER, N., 2001. Encyclopedia of food mycotoxins. Springer-Verlag, Berlin, Germany.

YOUSSEF, A.E., MARTI, E.H. 1989. Stability and degradation of AFM1. In: H.P. Van Egmond (ed.) Mycotoxins in dairy products. Elsevier, London, UK, pp 127-161.