Preliminary study on the presence of aflatoxin B_1 in feeds for lactating cows sampled in the Parmigiano Reggiano production area

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RIASSUNTO – Studio preliminare sulla presenza di aflatossina B_1 in alimenti per bovine da latte provenienti dal comprensorio del Parmigiano Reggiano. *La presenza di aflatossine, in particolare di aflatossina B_1 (AFB_1), negli alimenti destinati alle bovine da latte rappresenta un punto di controllo critico sia per gli animali che li assumono, che per l'uomo consumatore di latte e derivati. E' stato quindi condotto un monitoraggio sugli alimenti zootecnici prodotti in 180 aziende nel corso dell'anno 2003 all'interno del comprensorio del Parmigiano Reggiano, nelle provincie di Bologna, Mantova, Modena, Parma e Reggio Emilia, per un totale di 774 campioni (738 di fieno e 36 di granella). I risultati relativi al contenuto di AFB_1 determinato con metodica ELISA (Veratox® HS), dimostrano una buona qualità degli alimenti considerati. Per quanto riguarda i fieni la percentuale di positività risulta pari al 35,9% considerando positivi quelli che superano il limite di rilevabilità della metodica (0,5 µg/kg), con solo 4 campioni caratterizzati da livelli di AFB_1, di poco superiori al limite di legge, pari a 0,005 mg/kg. Relativamente alle granelle, solo i campioni di mais evidenziano una certa presenza di aflatossina (88,9% sul totale), il 31,25% dei quali risulta caratterizzato da livelli di AFB_1 superiori ai limiti di legge (0,020 mg/kg).*

Key words: aflatoxin B_1, feedstuff, lactating cows.

INTRODUCTION – Toxigenic fungi can affect the most common and relevant crops during the growth, harvest, or storage steps and produce toxic substances known as mycotoxins. Among the reasons for which these substances contaminate approximately 25% of the world's food crops (Lawlor and Lynch, 2001) are prominently inappropriate agricultural and storage practices, intercontinental shipping, as well as environmental and climatic conditions (relative humidity and temperature) (Moss, 1991). Aflatoxin B_1 (AFB_1) is the most potent of all aflatoxins and is of great concern because of its detrimental effects on the health of humans and domestic animals, including carcinogenic, mutagenic, teratogenic and immunosuppressive effects (Eaton and Callagher, 1994). Aflatoxin M_1 (AFM_1) is a hydroxilated metabolite of AFB_1 characterized by a rapid elimination through the milk and the urine, and by a lower toxicity than its parent compound. In the lactating cow the levels of AFM_1 excreted *via* the milk ranges between 1 and 3% of the ingested quantities. Considering EU and Italian regulations, the maximum levels for AFB_1 in feeds are: 0.020 mg/kg in corn and derivates, 0.05 mg/kg in other simple feeds and 0.005 mg/kg in mixed feed for lactating cow. In addition, the maximum level for AFM_1 in milk is 0.05 µg/kg. To check the safety of feed for lactating cow, the Authors verified the presence of AFB_1 in hay and grain samples from the Parmigiano Reggiano production area.

MATERIAL AND METHODS – The sampling process was managed between September 2003 and February 2004. Samples of hay and grain produced during 2003 inside the farms located in different provinces within the production area of Parmigiano Reggiano cheese (Bologna, Mantova, Modena, Parma, and Reggio Emilia) were collected (774 samples; 738 hay samples: 592 lucerne; 93 permanent meadows, and 53 samples of other forage crops from different harvest times and 36 grain samples: barley, wheat, soybean and maize). To carry out the sampling it was used a manual core drill "Quas" specially built for sampling bales because it allows to sample its whole section. The sampling was done according the ASPA methods. Taking elementary samples from three bales doing two holes in each one, the first 20 cm under the edge and the other at ¾ high. Elementary samples were joined to obtain a global sample. Each one was milled up to 3 mm. For grain samples, ASPA methods were used, taking a variable number of elementary samples according to the weight of the lots. Each elementary sample, of about 100 g was jointed to form a global sample which weight was variable from 1 to 10 kg, milled up to 3 mm. The levels of AFB_1 were determined in all the different matrices by a competitive enzyme immunoassay for aflatoxin Veratox® HS "Quantitative Aflatoxin High Sensitivity Test" (Neogen Corporation, 620 Lesher Place, Lansing, MI 48912). According to the manufacturer, the range of quantification was 1-8 µg/kg; the detection limit was 0.5 µg/kg and the limit of quantification was 2 µg/kg. The percentages of cross-reactivity were: AFB_1 100%, AFB_2 18%, AFG_1 13%, and AFG_2 1%. The procedure used to extract AFB_1 from samples was that given in the kit instructions and was the same for all the different matrices. A photometer was used to read the plates with a 650 nm filter (Tecam Spectra Classic). All samples were analysed in duplicate and when a false positive or negative was suspected, the samples were re-assayed on a different plate. The samples with an aflatoxin concentration over the upper limit of the range were repeated after proper dilution.
The study was performed according to ISO 9001:2000 requirements.

RESULTS AND CONCLUSIONS – The ELISA kit assay for AFB_1 proved to be rapid and easy to perform. In relation to hay, the percentage of positiveness to AFB_1 was 35.9%. Within the 265 hay positive samples to AFB_1, only 4 were characterised by mycotoxin levels higher than the legal limit of 0.005 mg/kg for complete feedstuff for dairy animals (G.U.C.E. L285, 1/11/2003 - Directive 2003/100/CE; G.U. 139, 16/06/2004 - Dlg 149, 10/05/2004), these levels ranged from 5.3 ppb to a *maximum* of 6 ppb. On the other hand, grain, barley, soybean, and wheat samples were all negative for aflatoxin, while the 16 samples of maize showed a high percentage of positiveness (87.5%) as well as the flours (100%). Despite this high level, only 5 were above the legal limit (0.020 mg/kg). The results are shown in Table 1.

Table 1. Concentration and frequency of occurrence of aflatoxin B_1.

Sample	Total number	Positiveness		Over the EU maximum level		Concentration range (mg/kg)
		Number	%	Number	%	
Hays	738	265	35.9	4	0.5	2-6
Barley	15	0	-	-		-
Wheat	2	0	-	-	-	-
Soybean	1	0	-	-	-	-
Maize	16	14	87.5	5	31.25	1.10-47.20
Other	2	2	100	0	-	1.10-4.30

Other: mixed flour and maize+barley.

Even if the number of grain samples was low (because most farms do not produce grains, instead of they buy them), this situation represents the universe of farms which produce grain as a raw material for feedstuff. In general our data agree with the concentrations observed in different studies to evaluate the AFB_1 contamination of animal feedstuffs in Northern Italy (Cinti *et al.*, 2004; Pietri *et al.,* 2004; Piccaglia *et al.*, 2004). It should be observed that the AFB_1 levels we found in maize destined for lactating cows consumption are not dangerous for this species. In cattle, clinical signs occurred after exposure to concentrations of 1.5-2.23 mg/kg feed; decreasing milk production of exposed animals, and a photosensitising effect can precede gross clinical signs of intoxication (Miller and Wilson, 1994). The low susceptibility to aflatoxins can be explained by pre-systemic elimination by the rumen flora of cattle. Nevertheless it is very important to keep in mind that Frobish *et al.* (1986) suggest that AFB_1 in feedstuff produces levels of AFM_1 in the milk according to the *ratio* 55:1; moreover Veldman *et al.* (1992), and Battacone *et al.* (2003) remark that cows and ewes fed rations contaminated with AFB_1 below the EC tolerance level (0.05 mg/kg) may produce milk containing AFM_1 concentrations above EC limit (0.05 µg/kg). It is also important to keep in mind that year 2003 was very dry and hot, but also with periods of time with high levels of relative humidity. These climatic conditions were propitious for the development of fungi producing AFB_1 in feedstuffs, where maize and its derivates represent the highest risk. The results of the present study underline the good and the safe quality of feedstuff analyzed for lactating cows. Anyway, it should be considered that this is a part of a three years study so these are partial results.

ACKNOWLEDGEMENTS – This study was supported by a grant from Regione Emilia-Romagna (Year 2003).

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