Fumonisin contamination of feeds sampled in forty riding centres in northern Italy

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ABSTRACT
Seventy-two samples of simple and mixed feeds for horses were collected from 40 riding centres located in north-east of Italy in order to evaluate the level of fumonisin (FB1) contamination. Feed samples were stored at –18°C and analyses were performed in duplicate by immuno-enzymatic method (ELISA), using a commercial test kit for B1 fumonisin quantitative detection. As expected, the levels of B1 fumonisin were very low in oat (from <0.01 to 0.84 ppm) and barley samples (from 0 to 0.95 ppm). In mixes of several cereals, containing variable proportions of corn, fumonisin concentration was always lower than 5.00 ppm, i.e. the maximum level recommended by U.S. Food and Drug Administration in corn and corn by-products used for equids. Fumonisin levels of commercial mixed feeds (CMF) were highly variable among feed industries and, within industry, among different commercial products. High levels of fumonisin (>10.00 ppm) were found in CMF formulated for horses with intense activity in one industry, only. This could be due to an occasional and undetected corn contamination. The results of this study indicate the need of a continuous and careful control of the raw materials used in the feed industry in order to reduce the risks of adverse effects of fumonisin in horses.

Key words: fumonisins, FB1, feeds, horses

INTRODUCTION
Ideal diet should contain not only nutritional principles but also healthy raw materials. Ingestion of feeds contaminated by toxins produced by micro-organisms can cause a particular kind of nutritional pathologies called toxicinfections (Adams et al., 1999). Their prevention is one of the most important objectives of a correct animal feeding management (Pietri and Piva, 2000). There are different genera of fungi that can attack plants and produce toxins in normal or particular conditions like drought, presence of parasites, etc.
Mycotoxins are secondary metabolites which can contaminate raw materials in field (primary contamination) or during harvesting, storage, transformation, manipulation or transport of feeds (secondary contamination). They can be responsible for a wide variety of negative effects on health of both humans and animals, because their different chemical structure produces different biological effects. Indeed they can be mutagenic, teratogenic or cancerogenic or they can also cause serious lesions to different organs and apparatuses. More than 350 types of mycotoxins are known but only 20 of them can cause dangerous mycotoxicoses after their ingestion. Those toxins are produced by 5 different genera of fungi: Aspergillus, Fusarium, Penicillium, Alternaria and Claviceps.
Fumonisins (B1, the most frequently isolated, B2 and B3) are important mycotoxins discovered quite recently. They are produced by different species of fungi belonging to Fusarium genus (F. moniliforme, F. proliferatum and F. verticilloides). Cereals like rice, barley, wheat, millet and sorghum and, especially, corn are the most contaminated raw materials by fumonisins. Fumonisins are dangerous not only for people but also for different animal species. They can cause oesophageal cancer in humans and hepatic lesions and pulmonary oedema in cattle and pigs (D'Mello and Macdonald, 1997). In particular, equids are very sensitive to fumonisins because they can develop ELEM, which is a acronym for equine leukoencephalomalacia. It is a mortal neurological disease with liquefactive necrosis of encephalic white matter and oedema under meninges and its symptoms are depression, anorexia, weakness, blindness, head-pressing, ataxia, aimless circling, tremors and paresis (Adams et al., 1999). Indeed, encephalic tissue is rich in sfingolipids and fumonisins interfere with their metabolism because they are chemically similar to sfingosin, the sfingolipid precursor (D'Mello et al., 1999).
Fumonisin levels in animal feeds are not yet regulated by law in European Union but FDA recommends maximum levels of total fumonisins (B1, B2 e B3) of 5 ppm in corn and its by-products and 1 ppm for total mixed ration given to equids (FDA, 2001). EU Commission is trying to fix official maximum levels.

AIMS
The aim of this study was to detect the level of FB1 contamination of horse feeds sampled in some riding centres of North-eastern Italy. In fact there are not law limits but only recommended maximum levels for fumonisins in animal

feeds even if their toxic effect on animals was demonstrated. So the study tried to check the diffusion of fumonisins in horse feeds in Veneto region and to verify if they exceed the recommended maximum levels.

MATERIAL AND METHODS

72 simple and mixed feeds for horses were sampled in 40 riding centres, homogeneously distributed in the different provinces of Veneto region from January to April 2003. The centres housed about 1500 horses of different type or activity as reported in table 1. For each type or activity of horses at the riding centres, data regarding the composition of the diet were collected, in order to estimate a mean intake as hay and concentrate on a DM basis.

Table 1: Type and activity of horses in the riding centres

	Type/Activity					
	Saddle		Western		Pony	
	Race (SR)	Walking (SW)	Race (WR)	Walking (WW)	Race (PR)	Total
Animals (no.)	775	167	148	254	90	1454

Table 2 shows the different feeds collected, 40% of which were commercial mixed feeds (CMF). Among the CMF, more than 50% were produced by the only one industry (Table 3).

Table 2: Type of feed samples collected

Kind of feed	Number of samples
Commercial mixed feed	29
Cereal mixture	13
Flaked barley	11
Whole oat	9
Alfaalfa hay (pellets)	3
Rolled oat	2
Whole corn	1
Rolled barley	1
Soya-bean meal	1
Linseed cake	1
Mixed hay (pellets)	1
Total	72

Table 3: Commercial mixed feeds by feed industry collected

Feed industry	Number of samples
CMF A	16
CMF B	2
CMF C	2
CMF D	2
CMF E	5
CMF F	1
CMF G	1
Total	29

All samples were stored at -18°C until analysis to avoid other fungal proliferations and production of new mycotoxins. A commercial ELISA kit containing materials and procedures to do a quantitative detection of FB1 level was used to verify the content of FB1 in feeds. Each feed sample was analysed in duplicate.

RESULTS AND DISCUSSION

FB1 level in commercial mixed feed samples was highly variable among different feed industries and among different kinds of feed produced by each industry (Figure 1). This was mainly linked to the level of corn included in the mixture. FB1 contamination of feeds produced by the most important industry of the region was on average 8.32±7.16 ppm, i.e. higher than the threshold level suggested by the FDA.

FB1 concentration in cereal mixes with different percentages of corn inclusion was lower than 5 ppm (Figure 1). Therefore, the corn used to prepare the cereal mixes showed a generally lower contamination in FB1 than that used to produce commercial mixtures.

Single cereals showed a generally low FB1 contamination (Figure 1).

As expected, FB1 level in oat and barley samples was very low (from <0.01 to 0.84 and from 0 to 0.95 ppm respectively; Figure 1).

Moreover, pelleted alfalfa and mixed hay showed a detectable level of FB1 contamination, although in the latter case only one sample was collected. However, it seems that during the formulation of commercial feeds the selection of raw materials (especially corn) by some feed industries is not sufficiently effective. Nevertheless, looking at the composition of the DM intake estimated for horses of different type and/or activity (Figure 2), only

animals with intense activity, and therefore with more than 20% of DM from CMF, seem to run the risk of an FB1 intake over the threshold established by FDA. However, no symptoms of fumonisin toxicity were detected during the visits at the riding centres. On the other hand, it has to be pointed out that analysis method for FB1 quantitative detection by ELISA kit can give some problems of interferences when matrix is complex as CMF. Indeed, more reliable results are obtained when matrix is homogeneous like corn and other single cereals.

CONCLUSIONS

The study improved the knowledge about the fumonisin contamination of feeds for horses. Results suggest to pay more attention to the choice of the raw materials, especially corn, and to the formulation of commercial feeds. However, research has to be extended to a higher number of samples and on other more effective detection methods (i.e., HPLC), particularly when matrix is complex as in CMF. Results suggest the need of a continuous monitoring of corn and cereal mixes or commercial mixed feeds with high percentages of corn in riding centres in order to avoid negative effects on the horse health.

Figure 1: FB1 contamination level of horse feeds (ppm)

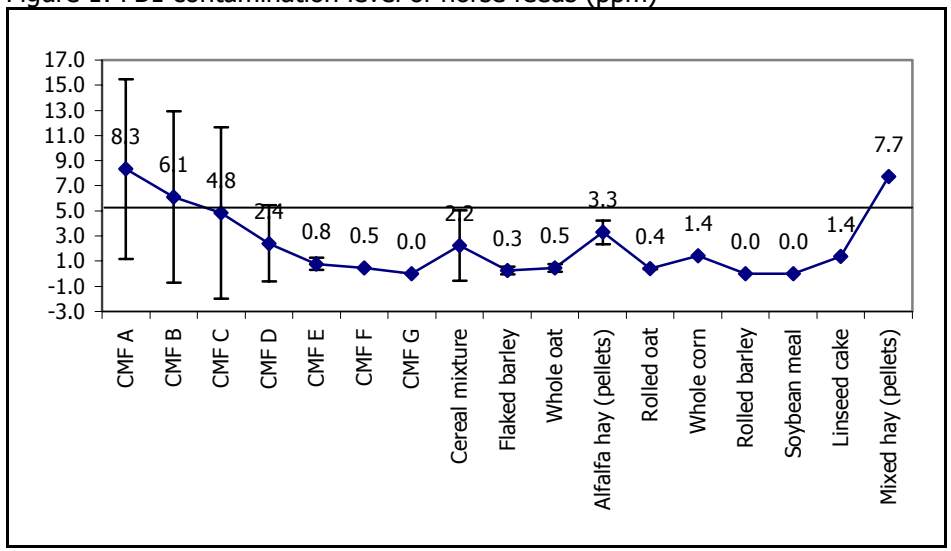
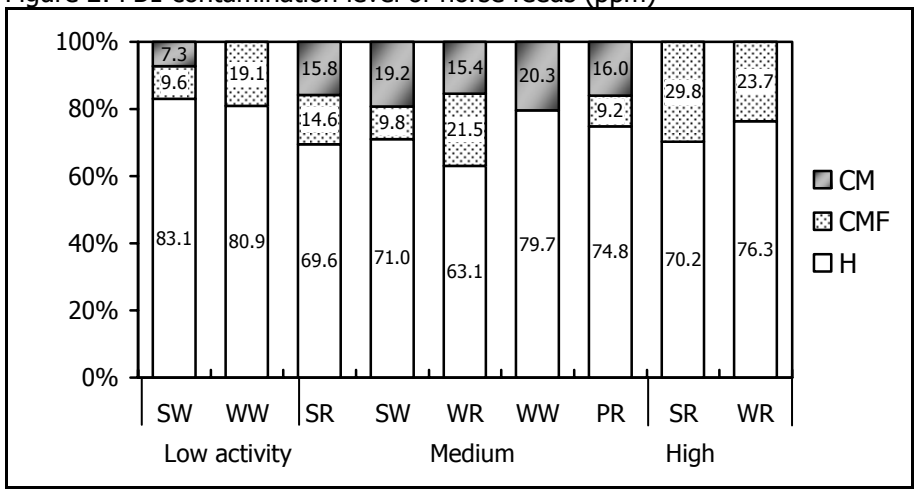


Figure 2: FB1 contamination level of horse feeds (ppm)



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