

# THE INCIDENCE OF MYCOTOXINS IN THE FODDER MEANT FOR THE ALIMENTATION OF MILCH COWS FROM THE ROMANIAN FAMILY FARMS

Simion Violeta-Elena

*The Veterinary Medicine Faculty, Spiru Haret University, Bucharest, Romania*

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## Introduction

The development of agricultural family farms has started in Romania since there is a favourable economical and legislative environment, but the timing is critical in this period of transition. This study follows the incidence of fungi and mycotoxins in the fodder meant for the alimentation of milch cows, its contamination degree, the assessment of the nutritional value of the fodder and the presence of mycotoxins in the milk produced by the cows that were part of this study.

The researches of mycological and mycotoxicological screening performed in the Romanian family farms have led to the identification of the types of mycetes and mycotoxins that are present in a geographic area in order to establish some prevention measures against contamination in those areas. In the case of farm animals and especially in the case of milch cows, the negative effects of the mycotoxins are implied both in the health, production and reproduction of the animals and also in the health of those people that consume products obtained from the animals previously mentioned.

## Materials and methods

The study was performed in the period May 2003 – May 2004 and it consisted in the investigation by means of mycological and mycotoxicological examination of the fodder used in the alimentation of milch cows bred in family farms, from 5 counties which is an important Romanian agricultural area.

For this study, three family associations were chosen at random from each county. The fodder categories that were investigated and the number of assays were : green fodder – 30 assays, fibrous fodder – 30 and mixtures of concentrated fodder (cereals grains, wheat and corn husk, sunflower groats, pea, soya) – 75 assays.

In order to determine aflatoxin M<sub>1</sub>, there have been analyzed 2 assays of milk coming from each family association from the 5 counties (a total of 30 assays).

The mychological and mychotoxicological determinations were performed by usual laboratory methods. The qualitative mychological analysis of the fodder consisted in the identification of those mycets types that are potentially mycotoxinogenous, by the examination of the cultural characters and by microscopical examination. The quantitative assessment of the mychological charge was performed using the method of serial dilutions (the dilutions  $10^{-3}$  –  $10^{-4}$ ) for the concentrated mixtures and by the direct method for the other categories of fodder (green fodder and fibrous fodder). Sabouraud environment was used in order to grow mycets. The degree of mychological contamination of the analyzed assays was expressed in NTF % and colonies/g fodder and it was divided in quality groups : low, mild and intensive contamination.

The analysis of mycotoxins in fodder and milk was performed using the immunoenzymatic method, ELISA.

## Results

From the total of 135 assays of fodder there have been examined the following : 75 assays of concentrated mixtures, 15 assays of fibrous fodder, 15 assays of fibrous forage, 30 assays of green fodder and 30 assays of milk. Since the green fodder that was analyzed did not develop a significant mychological culture, the results were not included in the following statistics. In the concentrated mixtures the following types of mycets were identified : Aspergillus (69 assays), Fusarium and Mucor (57 assays), leavens (12 assays) and Trichotecium (6 assays).

In fibrous fodder mixtures (Figure 1) there were identified : Aspergillus (15 assays), Penicillium (12 assays), Fusarium (8 assays), leavens (7 assays) and Mucor (6 assays) and in fibrous forage assays (Figure 2) there were identified : Rhizopus (15 assays), Fusarium (14assays), Aspergillus (12 assays) and Penicilium (9 assays)

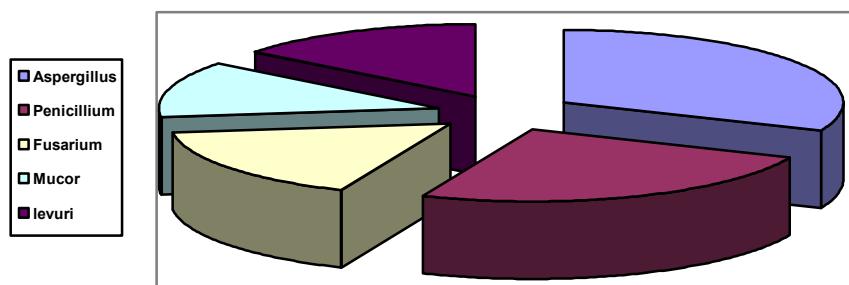


Figura 1 Incidența speciilor de fungi în nutrețurile fibroase

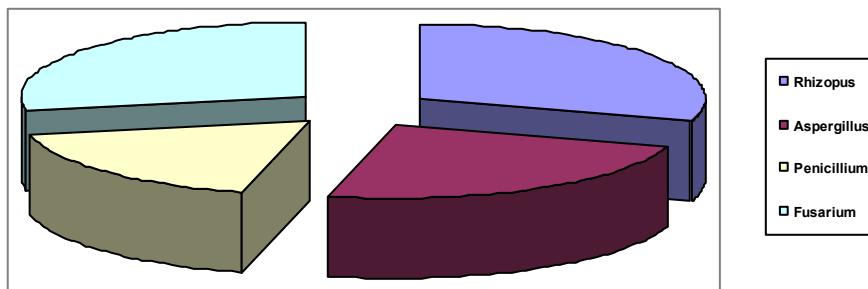


Figura 2 Incidența speciilor de fungi în nutrețurile groșiere

NTF had values between 14 500 and over 200 000 col/g in the concentrated mixtures, between 4-60% in hay assays and 24–90% in fibrous forage assays. At the mychotoxicological examination performed by means of the immunoenzymatic test Elisa for the identification of mycotoxins, values were obtained for : aflatoxin between 0-2,4 ppb, for zearalenone between 55,9 – 537 ppb, for ochratoxin A between 0,37 – 8,6 ppb, for T-2 between 0,18 – 0,33 ppm and for DON between 0 – 0,593 ppm. The reference limit for aflatoxin M<sub>1</sub> (0,14 – 0,24 ppb) was not exceeded in any of the milk assays that were analyzed.

The contamination degree of the assays, on fodder categories was the following : in the concentrated mixtures – low contamination (60 assays), mild contamination (0 assays) and intensive contamination (15 assays), in the hay - low contamination (6 assays), moderate contamination (6 assays) and intensive contamination (3 assays) and in the fibrous forage - low contamination (3 assays), moderate contamination (3 assays) and intensive contamination (9 assays).

## Discussions

The contamination degree of the fodder assays was in proportion of 8% low and 20% intensive at the concentrated mixtures and of 30% low, 30% mild and 40% intensive at the hay and fibrous forage, mostly due to the storage conditions. From the species of mycets, Aspergillus had the highest rate 71,1%, followed by Fusarium 58,5% and Penicillium 15,5%. On fodder categories, in the concentrated mixtures Aspergillus 92% had prevalence, followed by Fusarium and Mucor 71,1%, and in the fibrous fodder Aspergillus 90% was the uppermost followed by Fusarium 73% and Penicillium 70%.

After the mychotoxicological examination performed by means of the immunoenzymatic test Elisa it was observed that the reference limits for these mycotoxins at the milch cows were exceeded for zearalenone (537 ppb and 448 ppb) and ochratoxin (8,6

ppb and 7,1 ppb) only in the hay assays from one county within two family associations. The limit of 20-40 ppb for aflatoxin was not exceeded in any of the analyzed assays. For zearalenone the reference limit is 300 ppb and for ochratoxin 5 ppb, values that were exceeded according to the results that were obtained. For tricotecens, the reference limit for T-2 is 0,1 – 0,3 ppm and for DON it is 2,0 ppm - was not exceeded in the analyzed assays.

The reference limit for the aflatoxin M<sub>1</sub> was not exceeded in any of the milk assays that were analyzed.

### Conclusions

Although the results prove a low contamination of the fodder used in the alimentation of milch cows from the family farms, yet there can be remarked a high incidence of the species Aspergillus (71,1%) as well as of the species Penicillium (15,5), which are the main producers of mycotoxins : aflatoxin și ochratoxin A.

Fusarium (58,5%) also has a high incidence in the analyzed fodder and it is a risk for the health of milch cows by the mycotoxins that it can produce : zearalenone, fumonisin, tricotecens (DAS, T-2, DON). Althogh the presence of mycets was shown in all the fodder assays that were analyzed, the contamination with mycotoxins was under the reference limits, only zearalenone (537 ppb and 448 ppb) and ochratoxin (8,6 ppb and 7,1 ppb) had values exceeding the reference only in the fodder assays from one county, within two family associations.

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