

Statistical Estimation of the Degree of Contamination with Mycotoxins in Feed for Cattle

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Abstract

The research aimed toxicological monitoring of feed produced in a unit type CFF (combined forage feed) of an intensive type ruminant farm during January-June 2013. To fulfill that purpose were collected and analyzed a total of 10 forage samples represented by CF (combined forage) T2-0-3 steers, soybean grist, corn, CF ruminants, CF muttons, CF 01-0-3 muttons, CF 21-2 for determining mycotoxin load, pursuing the following parameters: concentration of AFB1 (aflatoxin B1), concentration of ZEA (zearalenone), concentration of OTA (ochratoxin). Quantitative determination of residues of aflatoxin B1, ZEA and OTA in cereals and animal food was performed by ELISA immunoassay. Following the research it was established that 80% of the total samples were contaminated with both OTA and AFB1 and ZEA; 3 of the 8 contaminated samples (37.5%) showed high levels of concentration for the three mycotoxins.

Keywords: cattle, CFF, contaminated, mycotoxin

1. Introduction

Mycotoxins are toxic metabolites produced by fungi in the substrates of food and feed. Mycotoxins occur during the growth of parasite fungi on plants which are subsequently used in human and animal nutrition, and they are primary metabolites with a high toxicity both for humans and animals.

Zearalenone (ZEA), also known as F-2 mycotoxin, is a potent estrogenic metabolite produced by some *Fusarium* species [1].

Aflatoxins may be acutely toxic, carcinogenic, mutagenic and teratogenic and they are primarily metabolised in the livers of vertebrates [2]. Ochratoxin is a naturally occurring fungal toxin produced by one species of *Penicillium* and by rare species in the *A. ochraceus* group [3]. The triggering of mycotoxicosis often gains a seasonal character, being influenced by environmental

conditions (temperature and humidity) that favor the formation of mycotoxins.

Generally the acute evolution of mycotoxicosis is found in cases of massive contamination with mycotoxins in feed. Chronic poisoning, the most common form of evolution of the disease is difficult to diagnose. In most cases, the casuistry is complicated by bouts of diarrhea or pneumonia, even in the absence of a clear etiology [4].

In case the food contamination with mycotoxins in moderate the mycotoxicosis can evolve insidious [5].

2. Materials and methods

The studies were carried out on an intensive growth type of farm in the south-western Romania. Also the farm has a unit that produces CFF. The CFF produces the feed destined to be administered in ruminant alimentation.

The research had as a purpose monitoring from a mycotoxicological point of view the feed produced in a unit that makes CFF from an

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intensive growth type of farm of ruminants, during January-June 2013.

In order to fulfill that purpose there were collected and analyzed a total number of 10 feed samples for determining the mycotoxin load, by following the next parameters: the concentration in AFB1, the concentration in ZEA, the concentration in OTA.

The work samples were represented by:

- CF (combined forage) T2-0-3 steers
- Soybean grist; corn; sunflower shrot
- CF (combined forage) ruminants
- CF (combined forage) mutttons
- CF (combined forage) 01-0-03 mutttons
- CF (combined forage) 21-2

The determination of mycotoxins in feed samples was performed with ELISA method according to the following reference documents: users guide kit Ridascreen Aflatoxin B1 30/15-Quantitative determination of aflatoxins B1 residues by immunoenzymatic assay; users guide Kit Ridascreen Zearalenone-Quantitative determination of Zearalenone by immunoenzymatic assay. For the determination of the Zearalenone it was used ELISA Kit containing materials for a total of 96 measurements (including analysis standards).

After preparing the samples, all the reagents are brought to the room temperature, reagents that will be brought back at refrigeration temperature (between 2-8°C) immediately after being used.

The zearalenone conjugate is provided in the test kit, in a concentrated form. It will be diluted, in a ratio of 1:11 (1:10), by means of a micropipette, with conjugate dilution buffer liquid.

For the determination of aflatoxin B1 and ochratoxin has been used the direct method (without purification on immunoaffinity columns). Samples must be kept in cool places, protected from light. For a sample to be representative, it must be well mixed before the extraction procedure [6].

Statistical analysis was done in order to establish relations between the two categories of various events, based on standard deviation and linear correlation with coefficient Pearson (r) ($p < 0.05$; IC 95%) using the program Quattro pro Excel and SPSS Statistics.

3. Results and discussion

Of the total number of samples (10 samples) there were gathered 5 samples of the feed in order to determine AFB1 content, representing a rate of 35.71% (Table 1).

From toxicological analysis of samples of feed, and the interpretation of laboratory data, there were revealed the following:

a. 60% of the samples showed higher levels than the limit for the concentration of AFB1 on the type of feed as follows (Figure 1).

Table 1. Determining the level of contamination of samples with AFB1

No. sample	Type forage	Origin forage	Normal values (mg/Kg)	Results (mg/Kg)
1.	NC21-2	CFF	LOD=0.004 LOQ=0.004	0.0016+0.003
2.	Sunflowers shrot	CFF	LOD=0.00042 LOQ=0.00084	0.0015+0.0003
3.	Soybean grist	CFF	LOD=0.00042 LOQ=0.00084	22686-1.1 outside the limit of detection
4.	CF ruminants	CFF	LOD=0.0004 LOQ=0.0008	0.0162+0.0029
5.	CF mutttons	CFF	LOD=0.00042 LOQ=0.00084	0.003+0.0005

Sunflower shrot had a concentration of 0.0015 mg/kg, compared to the amount allowed by the LOD=0.00042 mg/kg;

CF-ruminants with the presence of mycotoxins in the amount of 0.016 mg/kg compared with LOD=0.00042 mg/kg;

CF-mutttons had a concentration of 0.003 mg/kg to a maximum permissible value of LOD=

0.00042 mg/kg;

b. in a single sample, represented soybean grist, the quantity of AFB1 was below the detection limit for reference value LOD=0.00042 mg/kg;

c. in the fodder NC-21-2 it was recorded an amount of 0.001 mg/kg, negligible less comparing to the reference value of LOD=0.004 mg/kg, considered with negative results for evaluation;

d. the highest AFB1 concentration was recorded in the sample n=4 (CF-ruminants) (0.016 mg/kg).

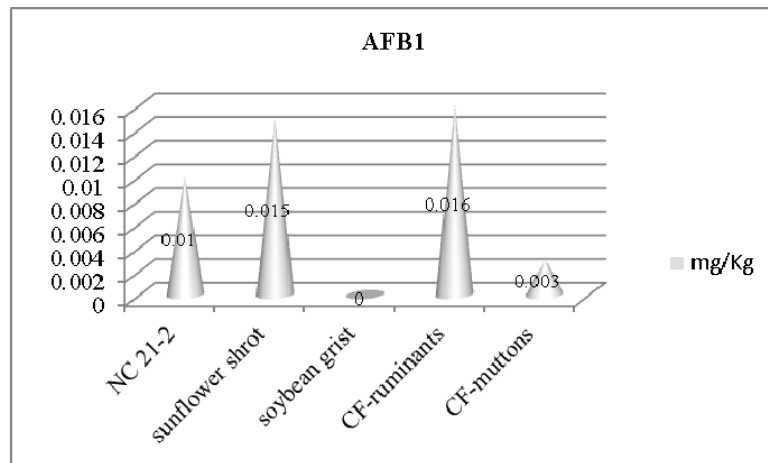


Figure 1. AFB1 concentration in feed samples

From statistical analysis of the laboratory data based on the index value $r^2=1$, at a threshold of significance $p>0.05$; CI-95% for all three contaminated samples significant differences were established between the values obtained from

analysis and the value of the concentration maximum allowed for sunflowers shrot, CF-ruminants and CF-muttons (LOD=0.00042 mg/Kg) (Figure 2, Figure 3, Figure 4).

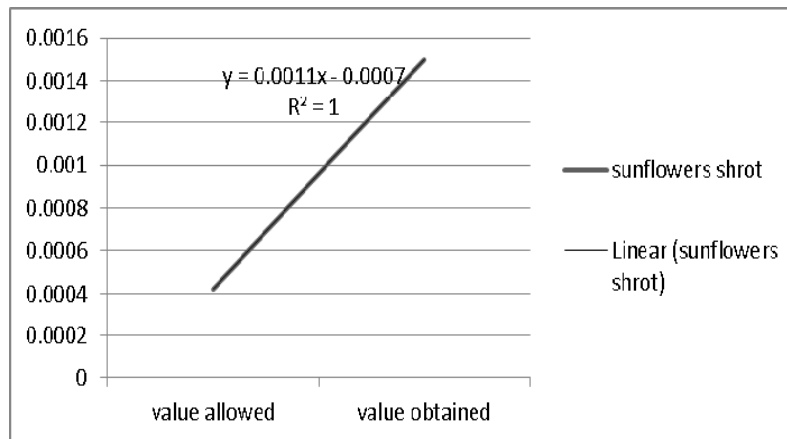


Figure 2. Statistical estimation of AFB1 concentration in sunflowers shrot based index r^2

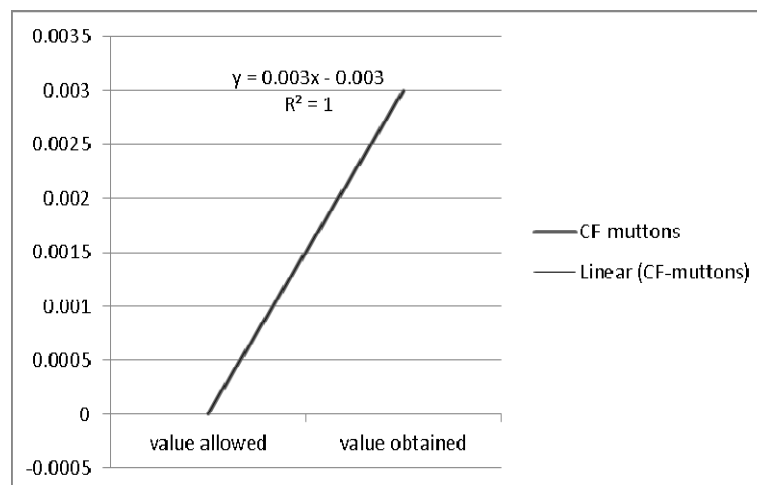


Figure 3. Statistical estimation of AFB1 concentration in CF- muttons meal based index r^2

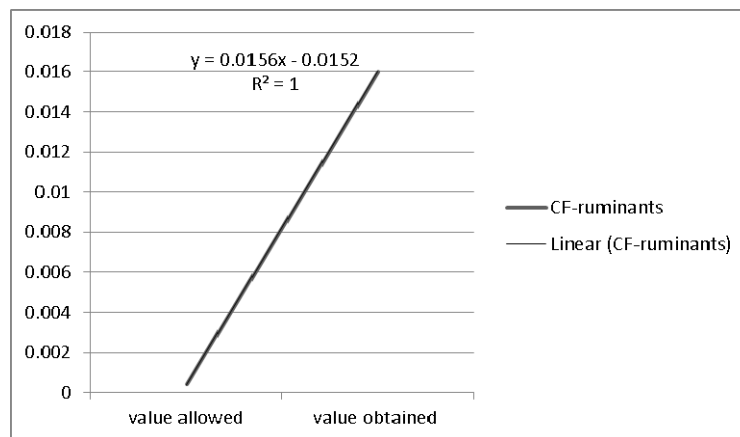


Figure 4. Statistical estimation of AFB1 concentration in CF- ruminants based index r^2

For the determination of zearalenone after performing mycotoxicological tests on samples of corn and NC01-0-3, the following values were obtained (Table 2). For the determination of in the ZEA, both corn sample and sample CF-muttons

did not record concentrations above the maximum admitted level-LOD=0.0018, 0.019 mg/Kg. In corn, ZEA concentration values were 0.0002 mg/kg and in the sample CF-01-0-3 muttons-LOD =0.0004 mg/kg (Figure 5).

Table 2. Determining the level of ZEA

No. sample	Type forage	Origin forage	Normal values (mg/Kg)	Results (mg/Kg)
1.	Corn	CFF	LOD=0.0018 LOQ=0.0024	0.0002 corresponding to provisions
2.	CF 1-0-3 muttons	CFF	LOD=0.019 LOQ0.0024	0.0004 corresponding to provisions

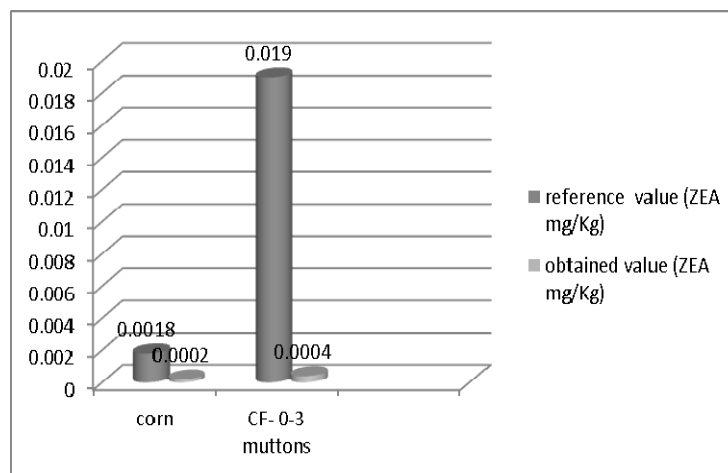


Figure 5. ZEA concentration in the samples examined

For the determination of ochratoxin in feed samples there were analyzed sunflower shrot, CF T2-0-2, CF muttons (Table 3).

The results of analyzes have pointed that:

a. all three samples were contaminated with OTA mycotoxin, which could be a problem for cattle in case of long-term consumption of sunflower shrot, and nephrotoxicity phenomena may occur on a background of chronic evolving mycotoxicoses [7].

b. bearing in mind that ruminants because of rumen degradation of OTA are more resilient, we can estimate that the risk of developing ochratoxicosis is limited [8].

c. the highest recorded value was of a sample of FC muttons (0.0022 0.00035 mg/kg) compared with the reference value of 0.0007 mg/kg;

d. for sunflower shrot the permissible limits were exceeded, but the result meets the requirements of OTA for this type of feed;

e. CF T2 0-2 feed -presented values within the limits of the reference value (0.0007 mg/kg) (Figure 6).

Table 3 Determining the level of OTA

No. sample	Type forage	Origin forage	Normal values (mg/Kg)	Results (mg/Kg)
1.	CF -muttons	CFF	LOD=0.0007 LOQ=0.0014	0.0022+0.00035
2.	CF T 2-02	CFF	LOD=0.0007 LOQ0.0014	0.005+0.0008
3	Sunflowers shrot	CFF	LOD=0.0007 LOQ=0.0014	0.0016+0.0003 corresponding to provisions

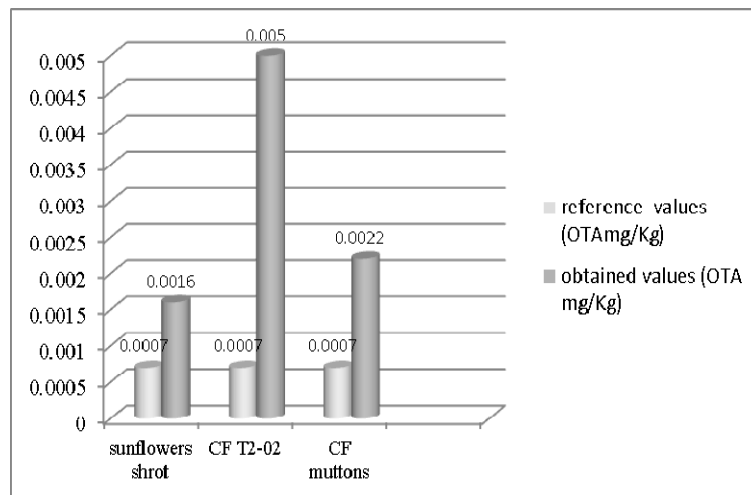


Figure 6. OTA concentration in the samples examined

4. Conclusions

As a result the analyses performed the following conclusions could be established:

- Out of the 10 feed samples examined a total of 8 samples representing 80% of the feed samples were contaminated with both OTA and AFB1 and ZEA;
- 3 samples of the 8 contaminated (37.5%) showed high levels of concentration for the three mycotoxins;
- Highest concentration was established for AFB1, which is a warning sign, knowing that aflatoxicosis has an increased incidence in cattle;
- Of the 8 kinds of feed examined, soy grits is only fodder that has not been contaminated by any of the three types of mycotoxins, for AFB1 laboratory results indicating undetectable levels;
- In the fodder CF 21-2 has been identified only one mycotoxin (AFB1; 0.001 mg/Kg).

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