ASSESSMENT OF FEED QUALITY AND CONTAMINATION OF FEEDSTUFFS WITH TOTAL AFLATOXIN AND ZEARALENONE IN A DAIRY COW FARM IN BISTRIȚA-NĂSĂUD COUNTY

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Abstract

This study was conducted in a dairy cattle farm in Bistrița-Năsăud County. Assessments were made over the fodder used to feed dairy cows and mycotoxic load. Feed samples were collected two times, first in the fall of 2019 and again in the fall of 2020 from different places from the same batch and they were conditioned shortly after harvest by drying and grinding. A total of 8 samples were collected from the farm and they represent the total of all feed used in the livestock feed in that farm. All of the forages were organoleptically analyzed, we determined the chemical composition; dry matter, crude protein composition, ether extract, crude cellulose, crude ash and nitrogen free extract. Zearalenone and Total Aflatoxins were also determined from each of the samples using RIDASCREEN® test, which are an competitive enzyme-linked immunosorbent assays. Zearalenone was detected in all the samples analyzed with values between 43.83 and 1054.03 µg/kg in 2019 and in 2020 with values between 103.45 and 1818.23 µg/kg. 75% of the samples analyzed in 2019, 50% of the samples analyzed in 2020 exceeding the maximum permissible limit in the European Union (EU). Total Aflatoxins were detected in all the samples analyzed, with values between 0.361 and 2.35 µg/kg, without exceeding the maximum permissible limit in EU.

Key words: bovine, gross chemical composition, zearalenone, aflatoxins

INTRODUCTION

Animal nutrition is a very important aspect in animal husbandry, even if this aspect is not taken seriously by Romanian farmers. Most diseases are caused by poor quality feed, poorly designed rations by empiricists and mold-contaminated feed that leads to mycotoxin poisoning that worsens the health of animals and leads to decreased productivity, especially combined with poor feeding. The quantitative and qualitative control of the feeds is done through a series of laboratory analyzes, intended exactly to detect their non-conformity. An important role that these laboratories play is to test the presence of toxins in feed and to provide the nutritionist with the necessary data on the nutritional value of feed (Tisch, 2005). Among the chemicals involved in the occurrence of biological and economic problems in animals, we mention mycotoxins, produced by certain species of fungi such as: *Aspergillus, Fusarium, Penicillum, Trichobacterium*, etc. Not all mycotoxins are important for human food safety, but aflatoxins, ochratoxins, fumonisins, patulin, ergotoxin, and trichothecine are exceptions (Diaz, 2005). Mycotoxins are toxic substances produced by fungi (molds) that grow on field crops or on stored ones. Of the several thousand species of mold that can grow on feed, only a few produce mycotoxins. Even if over 400 mycotoxins are identified chemically, the biological or veterinary impact is known to only a few (Seglar, 2017). Several studies have identified the action of mycotoxins in ruminants. Dairy cows suffering from mycotoxicosis have been associated with decreased milk production and failure to respond to therapies or dietary changes. Symptoms are usually not specific and may include: reduced food intake, refusal of food, poor body condition and reproductive problems. Field investigations were associated with abomasum displacement, ketosis, placental retention, metritis, mastitis, and fatty liver (Selgar, 2017)

MATERIAL AND METHOD

The study was conducted between 2019 and 2020, on a farm in Bistrița-Năsăud County and in the discipline of Animal Nutrition, within the Faculty of Veterinary Medicine Cluj-Napoca. The farm is located in Orheiu Bistriței, Bistrița-Năsăud County. It consists of 2 halls that house 400 head of cattle, of which 150 dairy cows and the rest cows in

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breastfeeding, pregnant youth, youth for preparation for calving and calves.

The analyzes were performed on a number of 8 samples of fodder taken from the farm located in Bistrița-Năsăud county from Orheiu Bistriței, 4 fodder samples from 2019 (sample 1- maize silage, sample 2 – maize and grass silage, sample 3 - combined feed for cows, sample 4 – natural hay) and 4 samples from 2020 of the same feedstuffs . Partial samples were taken from several lots, 0.1 kg each. After homogenization, average laboratory samples (maximum 1 kg mass) were extracted by square method. The organoleptic examination of the fodder on the farm was performed; the analysis of the raw chemical composition of the 8 fodder samples was determined by the Weende method and the mycotoxicological examination, determining the level of Zearalenone and total Aflatoxins in the examined samples. To perform the mycotoxicological examination we used the RIDASCREEN® Zearalenone test, which is based on competitive enzyme-linked immunosorbent assays to determine the quantitative determination of zearalenone in cereals, feed, beer, serum and urine. For the quantitative analysis of total aflatoxins, 4 feed samples were used. Laboratory analyzes were performed with the RIDASCREEN®FAST Aflatoxin ELISA competitive immune-enzymatic ELISA test for the quantitative determination of aflatoxins in feeds. The interpretation of the results was performed according to the EC Regulations No. 1881/2006 and No. 1126/2007 regarding the limits of mycotoxins in fodder and food. The statistical analysis was performed using Microsoft Excel.

RESULTS AND DISCUSSIONS

The results of the organoleptic examination of fodder used in cow feed in 2019 ranged from good to very good quality. The sample of maize and grass silage showed a greenish-yellow color, with a pleasant smell, it did not show impurities, so the assessment was as having a good quality. The hay sample was appreciated as a good quality, presenting a green color, a pleasant smell and without impurities. The corn silage did not show any impurities, had a pleasant smell, a yellow color and was considered of good quality. Combined pelleted fodder (complementary feed) is the only one that was considered of a very good quality, with a pleasant smell, it did not present impurities and the color was the specific one, yellow-brown. Regarding the results of the chemical composition of the 2019 feed samples, there is a good quality of natural hay, with dry matter values of over 87%, the protein having slightly higher than average values of about 8%, lower values are observed only in the case of crude cellulose. Regarding the values of complementary compound feed, it has a good value in terms of energy and nitrogenous substances, with a value of crude protein of over 24%. The value of the crude protein from the corn silage, compared to the dry matter, has lower values than its average in these varieties of fodder, namely 5.17% compared to an average value of approximately 8%. For the determination of Zearalenone, samples were collected from each batch of fodder from 2019. Zearalenone was detected in all samples examined, 3 of the 4 exceeding the maximum values allowed in the feed of dairy cows. Zearalenone values ranged from 41.83 µg / kg to 1054.03 µg / kg. The contamination is of moderate to high intensity, the feed with the highest concentration of Zearalenone being the corn silage with 1054.03 µg / kg, and the lowest concentration is in the complementary feed with 41.83 µg / kg. Of the 4 samples analyzed, 3 exceed the maximum allowed value of 500 µg / kg in cow feed. The results show that there are problems in the processing and storage of feed and this is a risk factor in terms of animal health, most often affecting the reproductive system. Given the fact that we have high concentrations of Zearalenone and after discussions with the farmer, it was concluded that there are problems caused by this mycotoxin. The farmer reported that he had problems specific to zearalenone intoxication: decreased milk production, infertility, abortions and embryonic resorption. A study on mycotoxin poisoning in Africa shows that due to the lack of awareness of the danger of feed containing mycotoxins, animals are still fed with this feed.

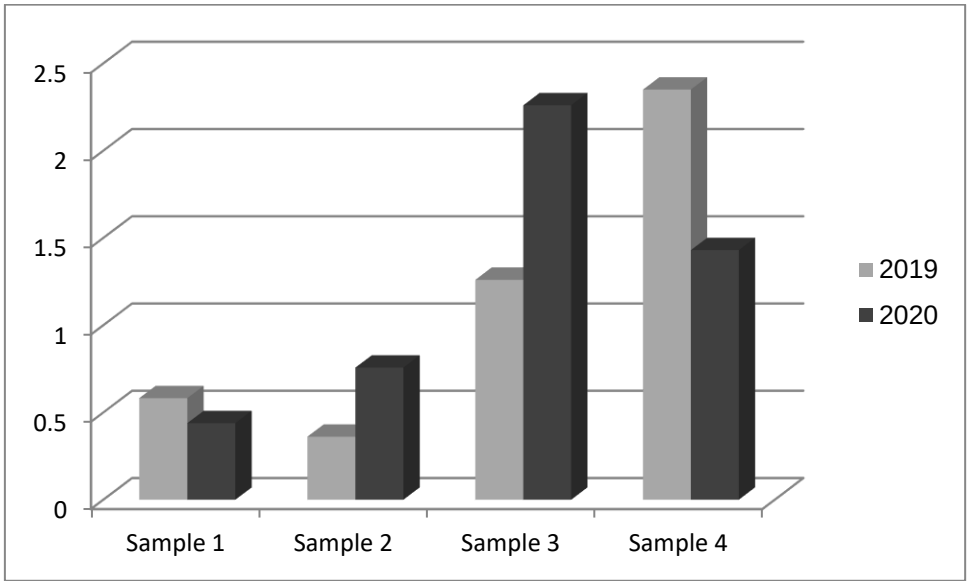

Figure 1 Total aflatoxin in the investigated samples in the years 2019 and 2020

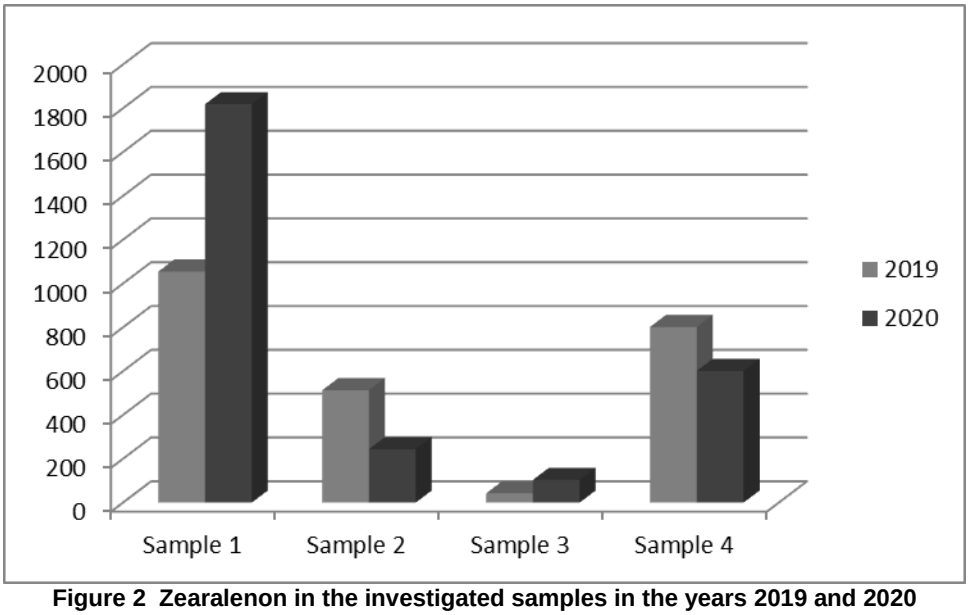

Figure 2 Zearalenon in the investigated samples in the years 2019 and 2020

Table 1

Contamination with Total aflatoxin and zearalenon in the investigated samples analysis

	Total aflatoxin – 2019	Total aflatoxin - 2020	Zearalenone - 2019	Zearalenone -2020
Mean value	1.13825	1.2215	602.4025	691.575
Median value	0.921	1.0935	656.875	422.31
Maximum	2.35	2.26	1054.03	1818.23
Minimum	0.361	0.439	41.83	103.45
Stdev	0.893	0.806	434.353	779.77

This negatively influences this branch of milk processing by decreasing milk production and the impact that mycotoxins have on animal health (Kemboi D. et al., 2020) Several cases have correlated zearalenone with the estrogenic response and severe fertility problems in cows,

even abortions. Symptoms include vaginitis, vaginal discharge, poor reproductive performance and enlargement of the mammary gland in virgin calves. (Smith G., 2019) The results of the analyzes of the gross chemical composition of the fodder samples from 2020, show higher values of the dry matter of the hay compared to 2019, with values of crude protein of almost 8.5%. There is an improved value of the crude protein value in corn silage compared to the dry matter of over 6%, but still below the average of 8%. Regarding the supplemented pelleted compound fodder, its nutritional value is higher, finding values of crude protein of almost 26.5%. We can appreciate that the nutritional value of fodder in 2020 is higher than in the fodder samples in 2019. For the determination of Zearalenone, samples were taken from each batch of fodder in 2020 and was found in all samples examined, 2 of the 4 exceeding the maximum permitted values in the feeding of dairy cows. Zearalenone values ranged from 103.45 µg / kg to 1818.23 µg / kg. The contamination is lower than the analysis from 2019 in 3 of the samples, the feed with the highest concentration of Zearalenone being the corn silage, exactly as in the 2019 sample, with a concentration of 1818.23 µg / kg, and the lowest concentration is in complementary feed, as in the 2019 analysis with 103.45 µg / kg. The measurements carried out in 2019 and 2020 show that the processing and storage of fodder is inadequate animal health and milk production being also affected. The farmer was advised to add mycotoxin inhibitors to feed. The addition of mycotoxin inhibitors in contaminated feed is considered the best method to reduce the effects of mycotoxins (Galvano et al., 2001). Activated carbon is considered a substance that binds very well to zearalenone and deoxynivalenol (Whitlow, 2014). The chemical structure of zearalenone is similar to that of estrogen. ZEA intoxications lead to reproductive problems, including symptoms of estrus in calves before puberty, irregular heat, silent heat, emission resorption, abortions, placental retention, metritis and mastitis (Obremski et al., 2012). European Community directives on the presence of deoxynivelenol, zearalenone, ochratoxin A, T2, HT2 and fumonizine; the zearalenone content must not exceed 250 µg / kg in the case of pig feed and a maximum value for cows and calves of 500 µg / kg. For young animals the values are much lower, 5 µg / kg for calves and lambs (EC, 2006). The productivity of dairy cows can be severely impaired by the presence of zearalenone in feed. Diagnosing and isolating zearalenone can sometimes be difficult and even stressful. The diagnosis of zearalenone poisoning and the induction of estrogenic effects are based on clinical signs and the detection of zearalenone in feed. Treatment is based on removing contaminated feed from animal feed and replacing it with high-quality feed. Although the toxicity of zearalenone varies quite a bit, farmers and veterinarians should take into account the estrogenic effects of this substance and the repercussions on the reproductive health of cows (Witte, 2003). In determining the aflatoxin, each feed sample from 2019 was analyzed separately. Aflatoxin was detected in all samples examined, but none exceeded the maximum values allowed in the feeding of dairy cows by European legislation. The determined values were between 0.361 µg / kg and 2,35 µg / kg in 2019 and between 0.439 µg / kg and 2.26 µg / kg in 2020. Contamination with total aflatoxin was in the investigated years constant, the average varying in a small margin. The maximum values of total Aflatoxins in the samples analyzed by us reveal values much lower than in other countries, such as Pakistan where values of up to 15 µg / kg for wheat and 13 µg / kg for maize were recorded (Lutfullah, 2012). Aflatoxins are very toxic to animals and humans. Even in non-lethal amounts, aflatoxins can endanger animal health and productivity. For dairy cows it should not exceed 20 µg per ration (Jordan, 2012). In a study to test the theories by which mycotoxins can bind to other compounds, activated carbon was added in high doses and shown to reduce aflatoxicosis in goats (Hatch et al., 1982).

CONCLUSIONS

The organoleptically analyzed feeds ranged from good to very good quality. The raw chemical composition of the fodder showed values quite close to those cited in the literature, except for the low values of crude cellulose in hay and a low value of crude protein in corn silage. Correlating the values of the gross chemical composition for the two years, we can appreciate that the nutritional value of the feeds in 2020 is higher than in 2019. Total aflatoxin and zearalenone were identified in all analyzed samples. Only zearalenone exceeded the maximum admitted limit in European Union in the majority of samples.

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