UNIVERSITY OF LIFE SCIENCES
"KING MIHAI I" FROM Timisoara
Multidisciplinary Conference on Sustainable Development
25-26 May 2023

Physico-Chemical and Mycological Evaluation of Fodder from a Cattle Farm

Ioana Porosnicu[1,2], Luminița-Iuliana Ailincăi[1], Andra-Sabina Neculai-Văleanu[2], Mirela-Adina Ariton[2], Mihai Mareș[1]

[1]Iasi University of Life Sciences, 700490, Mihail Sadoveanu Alley, no.3, Romania
[2]Research and Development Station for Cattle Breeding, 707252, Iasi-Ungheni no.9, Dancu, Iasi

Abstract

The purpose of the research was to carry out some analyzes in order to establish the physico-chemical composition and the fungal load of the plant substrates used for raising animals, in a cattle farm in the Moldova area in 2022. There were five categories of samples (75 determinations in total) - alfalfa hay, corn silage, mixed feed ration, concentrates, corn grains and were analyzed randomly from the farm. The results of the physico-chemical composition of the feed by the FT-NIRS technique were in accordance with the regulations in force and no significant differences were found between the samples, it is certain that the humidity had a higher percentage, hence the fact that there were developed species of fungi speaking from the point of view of mycotic contamination. The highest fungal load was recorded in corn grains and corn silage, and the lowest value was recorded in alfalfa hay. The number of colony-forming units per gram of sample was determined by the serial dilution technique in a double agar layer. The results obtained during the analyzed period highlighted the presence of the genus *Penicillium* to the greatest extent (28%), and the lowest percentage was recorded for the genus *Cladosporium* (5.3%).

Introduction

The occurrence of fungi in plant substrates that are intended for animal production is one of the biggest concerns for animal and human health. The identified filamentous fungi belong to a wide range of fungal genera. These fungal genera vary according to many factors - pre-harvest factors (crop location, weather conditions in the field, growing season, agricultural practices), but also post-harvest factors (storage conditions). It is known that the growth of filamentous fungi on plant substrates can cause problems related to spoilage or contamination with mycotoxins. From this point of view, it is important to evaluate both the physico-chemical and mycological aspects of the plant substrates. Crude chemical analysis was the first method used to determine the nutritional value of forages. Thus, it was considered that the feeds that have a higher chemically crude nutrient content also have a higher nutritional value. Feed safety is an important prerequisite for achieving optimal production results, as well as for maintaining animal health, especially in intensive industrial production, therefore constant monitoring of raw materials and compound feed is necessary. Contamination of feed with potentially toxic fungi is a common occurrence worldwide and harmful effects have been observed in all classes of farm animals due to the production of mycotoxins by certain mold species and strains. Potentially toxic fungi are associated with cereals and oilseeds and mainly belong to the genera Fusarium, Aspergillus and Penicillium.

Materials and methods

Figure 1. The sample and the results obtained according to the NIRS technique

In determining the quality of fodder, special emphasis was placed on the NIRS technique for determining the physico-chemical analyzes of fodder. This non-destructive method is a fast, very elegant technique that is in full development. Thus, the sample to be analyzed is poured into a tray and leveled with a line so as not to exceed the walls of the tray. Fix the sample tray in the device (on the black support under the infrared lamp) and start the analysis (figure 1). The second objective of the research aimed at assessing the phenomenon of fungal contamination of concentrates, corn kernels, alfalfa hay, corn silage and mixed fodder rations used in animal feed, taking into account: determining the number of filamentous and yeast-like micromycetes in plant substrates using standardized investigation techniques and the identification of the dominant mycotic flora. The research method included the technique of serial dilutions in a double layer of agar and bacterial inhibitors in order to establish the number of colony-forming units, the focus being thus on the isolation, identification and characterization of micromycete species, to be taxonomically classified. The number of filamentous and yeast-like micromycetes in plant substrates was determined based on the serial dilution method, according to the SR EN ISO 16140-2: 2016 - Food chain microbiology standard. Method validation. Part 2: Protocol for validation of alternative methods. This method was used in order to determine the degree of micromycete contamination of the fodder administered in animal feed.

Figure 2. The working environment regarding the mycological examination of fodder

Results and discussion

For alfalfa hay, moisture content averaged 22.52%, a normal value that highlights the fact that alfalfa hay is often dried and stored in a controlled environment to prevent mold growth and spoilage. During the drying and storage process of alfalfa hay, the fat content may decrease, as the fats are susceptible to oxidation and rancidity. Alfalfa has a high protein content (15.09%) due to its ability to fix atmospheric nitrogen and a chemical composition rich in essential amino acids. The values of the chemical composition of alfalfa hay were in accordance with the specialized literature.

Sample	Sample number	Moisture (UM%)	Fat (S.U.%)	Crude protein (PB %)	Raw ash (CB %)	Raw cellulose (CEL B %)
Alfalfa hay	Sample 1	23,30	1,78	14,5	7,9	30,1
	Sample 2	21,30	1,8	15,1	7,7	31,2
	Sample 3	22,20	1,77	16,2	7,3	32,3
	Sample 4	21,70	1,81	15,4	7,8	30,5
	Sample 5	24,10	1,92	14,7	7,55	31,4
	Sample 6	22,70	2,1	14,9	6,99	31,7
	Sample 7	22,50	2	15,2	7,8	30,2
	Sample 8	21,20	2,1	15,8	7,14	32,1
	Sample 9	20,80	1,89	14,1	6,76	30,5
	Sample 10	23,40	1,83	14,5	7,6	30,2
	Sample 11	22,10	1,77	15,22	7,9	32,1
	Sample 12	23,10	1,79	15,71	7,22	32,3
	Sample 13	22,80	1,67	15,2	7,8	30,5
	Sample 14	23,40	1,91	14,89	7,7	30,1
	Sample 15	23,20	1,8	14,9	6,7	31,2
	Average	22,52	1,86	15,09	7,46	31,09

Figure 3. Physico-chemical composition of alfalfa hay

Sample	Sample number	Moisture (UM%)	Fat (S.U.%)	Crude protein (PB %)	Raw ash (CB %)	Raw cellulose (CEL B %)
Corn silage	Sample 1	62,40	2,10	3,50	2,20	18,60
	Sample 2	60,40	2,12	3,44	2,11	18,40
	Sample 3	61,50	2,15	3,12	2,23	18,00
	Sample 4	61,66	2,55	3,17	2,12	17,80
	Sample 5	62,50	1,90	3,55	2,00	17,56
	Sample 6	62,88	1,88	3,80	2,54	17,20
	Sample 7	61,45	2,11	3,89	2,13	18,45
	Sample 8	62,40	2,14	4,10	2,41	18,76
	Sample 9	62,78	1,79	3,22	2,19	18,32
	Sample 10	61,50	2,15	3,44	2,23	17,89
	Sample 11	61,66	2,55	3,12	2,12	18,40
	Sample 12	62,50	1,90	3,17	2,00	17,50
	Sample 13	62,88	1,88	3,55	2,54	17,90
	Sample 14	60,50	2,11	3,10	2,13	18,40
	Sample 15	62,88	2,14	3,66	2,41	18,50
	Average	61,99	2,10	3,46	2,22	18,11

Figure 4. Physico-chemical composition of corn silage

Corn silage is obtained by aerobic or anaerobic fermentation of freshly cut corn. This processing involves retaining moisture to support the fermentation process. Therefore, corn silage has a higher moisture content than alfalfa hay (61.99%). During the fermentation process, some of the protein compounds can degrade and convert into other substances, which can lead to a decrease in the protein content of the final silage (3.46%). Corn has a different chemical composition than alfalfa, being rich in carbohydrates but also containing a significant amount of fiber, including cellulose. During the fermentation process, part of the fibers may be fermented and degraded, causing a reduction in the cellulose content (18.11%).

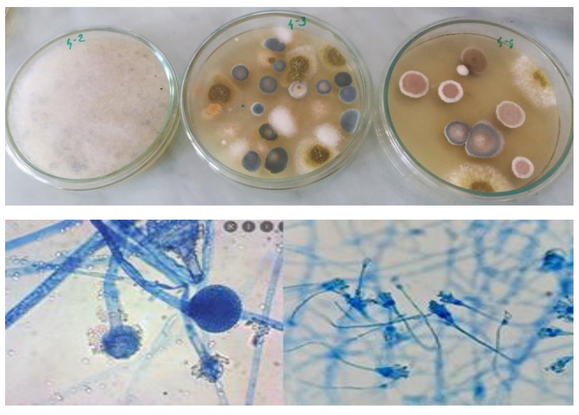

Figure 4. Macroscopic and microscopic appearance of the genera *Mucor* and *Penicillium*

Table 1. *The qualitative mycological exam of samples*

Sample	Number of samples analyzed	*Aspergillus*		*Penicillium*		*Fusarium*		*Cladosporium*		*Alternaria*		*Mucoraceae*		*Trichosporon*	
		Pr. poz.	%	Pr. poz.	%	Pr. poz.	%	Pr. poz.	%	Pr. poz.	%	Pr. poz.	%	Pr. poz.	%
Alfalfa hay	15	2	13	3	20	1	6.6	2	13	2	13	2	13	3	20
Corn silage	15	1	6.6	6	40	2	13	1	6.6	3	20	2	13	-	-
Mixed feed ration	15	3	20	3	20	3	20	-	-	-	-	5	33	1	6.6
Concentrates	15	3	20	3	20	1	6.6	-	-	-	-	4	26.6	4	26.6
Corn grains	15	1	6.6	6	40	2	13	1	6.6	3	20	2	13	-	-
TOTAL	75	10	13.3	21	28	9	12	4	5,3	8	10,6	15	20	8	10,6
		Aspergillus		*Penicillium*		*Fusarium*		*Cladosporium*		*Alternaria*		*Mucoraceae*		*Trichosporon*	

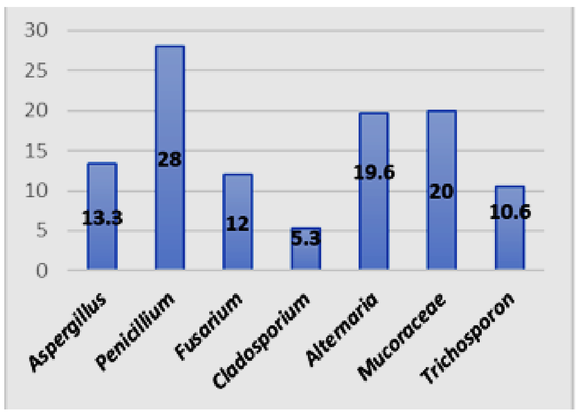

Figure 5. Graphic representation of micromycete genera isolated from feed

After the mycological examination, it was found that the corn silage and corn kernels presented the highest mycotic load (40%) with species belonging to the *Penicillium* genus, and in second place was the mixed fodder ration with a percentage of 33% with species from family *Mucoraceae*. In third place are the concentrates, which offer optimal conditions for the multiplication of micromycetes that also belong to the *Mucoraceae* family and the *Trichosporon* genus (26.6%). In the samples of alfalfa semi-hay, the dominant genera were *Penicillium* and *Trichosporon* (20%), followed by the genera *Aspergillus, Cladosporium* and the *Mucoraceae* family with the same percentage of 13%, and in corn silage fungi from the genera *Fusarium* were isolated by incidence and the *Mucoraceae* family (13%). In the mixed forage ration, the genus *Fusarium* had a higher percentage of contamination (20%) in the 3 analyzed samples, in contrast to the other samples, but it is noteworthy that it includes numerous adapted strains, which seem to prefer geographical areas of Moldavian Plateau. The genera *Alternaria, Cladosporium* were not present in the mixed fodder and concentrate and the genus *Trichosporon* was absent in the corn silage and corn kernels. The characters observed during the microscopic examination of the samples grown in PDA medium revealed that the fungi with the highest incidence belong to the genus *Penicillium* which dominates the entire mycotic mosaic that characterizes the plant substrates examined, being present in 28% of the analyzed samples and the species of the *Mucoraceae* family with a rate participation of 20% and the genus *Aspergillus* (13.3%). It is worth noting that the species of the genus *Fusarium* are in fourth place, occupying 12% percent in this hierarchy. It is interesting to underline the fact that the very high incidence of *Mucoraceae* occupying 20 percent, second place in this hierarchy (in plant substrates) implies a higher degree of humidity, which could be a signal of a potential alteration process.

Conclusions

Five categories of samples (plant substrates) were analyzed - alfalfa hay, corn silage, mixed fodder ration, concentrates, corn kernels randomly from the farm and 75 determinations were made in total (15 determinations per sample) , in order to establish the chemical composition and the fungal load. The results of the chemical composition of the feed were in accordance with the regulations in force and no significant differences were found between the samples, it is certain that the humidity had a higher percentage, this also explains the development of fungal species from the point of view of mycotic contamination . The dominant mycotic flora that characterizes the plant substrates in the geographical area of the Moldavian Plateau belongs to the genera Penicillium (28%), fam. Mucoraceae (20%), Aspergillus (13.3%) and Fusarium (12%) which obtained the highest contamination levels. The climatic conditions in this geographical area are favorable for these species of micromycetes because in specialized laboratories, on environments specific to micromycetes, they develop a vigorous vegetative apparatus with a phenotypic expression that is hard to confuse and easy to categorize taxonomically, if the respective data is also corroborated with the morphological and structural peculiarities of the fruiting bodies.