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

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Abstract

The purpose of the research was to carry out some analyses 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 analysed 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 analysed 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%).

Keywords: contamination, fodder, mycological analyses, physical-chemical analyses.

1. Introduction

The occurrence of fungi in plant substrates that are intended for animal production is one of the greatest concerns for animal and human health [1]. 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) [2]. It is known that the growth of filamentous fungi on plant substrates can cause problems related to damage or contamination with mycotoxins [3]. From this point of view, it is important to evaluate both physico-chemical and mycological aspects of plant substrates [4]. 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 content of chemically crude nutrients also have a higher nutritional value [5]. Feed safety is an important prerequisite for obtaining optimal production results, as well as for maintaining animal health, especially in intensive industrial production, therefore constant monitoring of raw materials and compound feeds is necessary [6]. Compound feed and raw materials can be contaminated with unwanted substances, which can come from the environment or the production process. The presence of pathogens in feed can occur due to the use of contaminated raw materials during transport, in the production facility or on site [7]. Because bacterial contaminants are unevenly distributed in the feed, the bacteria present may be damaged and difficulties may arise during microbial analysis [8]. Contamination of feed with potentially toxic

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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 mould species and strains [9]. Potentially toxic fungi are associated with cereals and oilseeds and mainly belong to the genera Fusarium, Aspergillus and Penicillium. The factors that influence the development of microorganisms are represented by temperature, oxygen, relative humidity, water. activity, pH, nutrients and different types of inhibitors [10].

2. Materials and methods

In determining the quality of fodder, special emphasis was placed on the NIRS technique for determining the physico-chemical analyses of fodder [11,12]. This non-destructive method is a fast, elegant technique that is in full development. Thus, the sample to be analysed is poured into a tray and levelled 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 (the sample must not contain other foreign bodies because it influences the result) The results are displayed on the screen after the analysis is completed (Figure 1).

Figure 1. Prepared corn sample

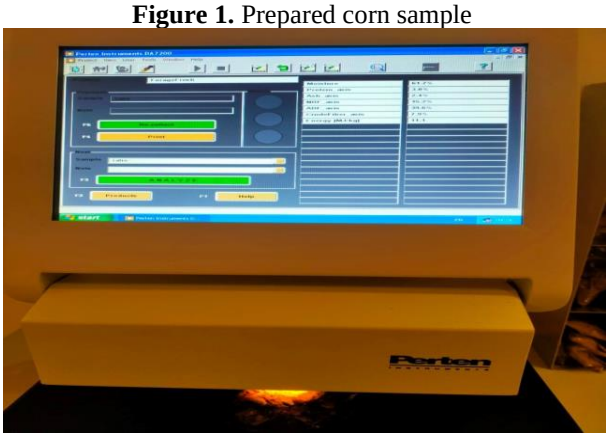

Figure 2. FT NIR-PERTEN analyser

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 from 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 the plant substrates was determined based on the serial dilution method, according to the SR EN ISO 6887-1:2017 standard - Microbiology of the food chain - Preparation of test samples for microbiological examination - General rules the preparation of initial suspension and decimal dilutions [13]. This method was used in order to determine the degree of micromycete contamination of the fodder administered in animal feed (Figure 3).

Figure 3. Mycological examination of feed under the bacteriological hood

3. Results and discussion

The feeds were physico-chemically evaluated and 15 determinations were made per sample by the NIRS technique. The gross chemical composition of the investigated samples was consistent with the values found in the literature. For alfalfa hay, the moisture content averaged 22.52%, a normal value that highlights that alfalfa hay is often dried and stored in a controlled environment to prevent mould growth and spoilage. During the drying and

storage process of alfalfa hay, the fat content may decrease (1.86%), 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 ash in alfalfa hay (7.46%) represents the minerals and other inorganic substances that are present in alfalfa plants and the cellulose in proportion of 31% reflects the fact that there is a high fibre content.

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 4. Physico-chemical composition of alfalfa hay

Corn silage is obtained by aerobic or anaerobic fermentation of freshly cut corn. This processing involves retaining moisture to support the fermentation process. Therefore, according to figure 5, corn silage has a higher moisture content than alfalfa hay (61.99%). Corn itself has a low-fat content and during the fermentation process to obtain corn silage, the amount of fat can remain relatively constant (2.10%). Also, during the fermentation process, some of the protein compounds can degrade and transform 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. During the fermentation process, part of the fibres may be fermented and degraded, causing a reduction in the cellulose content (18.11%) (Figure 5).

Mixed ration is a combination of several feed sources, including hay, corn silage, concentrates and other ingredients. The fat, protein, ash, cellulose content of the mixed ration may vary depending on the proportions and sources used in the mixture. For fat, concentrates or other added ingredients can contribute to its increase, for ash the individual content of minerals and inorganic substances matters and for cellulose the high level of fibre matters, such as a higher proportion of alfalfa hay. The average values obtained, such as moisture (68.11%), fat 3.68%), protein (5.38%), ash (3.18%) and cellulose (35.39%) they fell within the limits provided by the specialized literature (Figure 6).

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 5. Physico-chemical composition of corn silage

Sample	Sample number	Moisture (UM%)	Fat (S.U.%)	Crude protein (PB %)	Raw ash (CB %)	Raw cellulose (CEL B %)
Mixed feed ration	Sample 1	68,12	3,70	5,33	3,10	33,00
	Sample 2	67,12	3,60	5,45	3,20	40,00
	Sample 3	66,23	3,55	5,14	3,11	33,67
	Sample 4	69,56	3,89	5,89	3,17	33,56
	Sample 5	68,17	3,50	5,66	3,50	33,12
	Sample 6	68,15	4,10	4,90	2,90	34,50
	Sample 7	69,12	3,68	5,35	3,14	38,22
	Sample 8	67,66	3,89	5,45	3,18	38,20
	Sample 9	68,00	3,12	5,14	3,11	34,12
	Sample 10	67,89	3,87	5,89	3,17	36,40
	Sample 11	68,12	3,89	5,66	3,50	40,00
	Sample 12	67,80	3,67	4,90	3,14	33,67
	Sample 13	69,11	3,50	5,35	3,18	33,56
	Sample 14	68,65	3,68	5,45	3,11	33,12
	Sample 15	68,00	3,54	5,14	3,17	35,70
	Average	68,11	3,68	5,38	3,18	35,39

Figure 6. Physico-chemical composition of mixed feed ration

The moisture content of the concentrates can vary depending on the processing and packaging, as well as the natural water content present in the concentrated components, in the present case, the average value being 22.85% (Figure 7).

Sample	Sample number	Moisture (UM%)	Fat (S.U.%)	Crude protein (PB %)	Raw ash (CB %)	Raw cellulose (CEL B %)
Concentrates	Sample 1	18,68	4,30	9,60	1,00	5,30
	Sample 2	17,00	4,14	9,56	1,14	5,30
	Sample 3	16,80	4,67	8,90	1,55	4,20
	Sample 4	16,70	4,89	8,77	2,10	4,77
	Sample 5	25,70	4,77	9,45	1,89	4,89
	Sample 6	26,40	4,76	9,67	1,57	4,80
	Sample 7	25,50	3,90	9,78	1,13	5,23
	Sample 8	24,77	4,67	9,47	1,20	5,67
	Sample 9	16,70	4,67	9,60	1,14	5,13
	Sample 10	25,70	4,89	9,56	1,55	4,20
	Sample 11	26,40	4,77	8,90	2,10	4,77
	Sample 12	25,50	4,76	8,77	1,89	4,89
	Sample 13	24,77	3,90	9,45	1,57	4,80
	Sample 14	25,70	4,11	9,67	1,13	5,67
	Sample 15	26,40	4,19	9,78	1,20	5,13
	Average	22,85	4,49	9,40	1,48	4,98

Figure 7. Physico-chemical composition of concentrates

Concentrates may contain concentrated fat sources (vegetable oils or oilseeds), providing additional energy and essential fatty acids in the animal ration, which may result in a higher fat content (4.49%). The protein content is 9.40% due to the fact that there is an increased amount of nutrients. Concentrates are often formulated to be higher in carbohydrates and protein than fibre because they provide a concentrated source of energy. As the cellulose content is lower in the concentrates (4.98%), this may contribute to the reduction of the total cellulose content in the ration (Figure 7). According to figure 8, the average values obtained for corn kernels were: 14.18% moisture, 3.68% fat, 7.08% protein, 1.62% ash and 2.34% cellulose. Corn kernels can be harvested and dried to a certain moisture level to ensure proper storage and preservation. Low humidity can help prevent mould and grain damage. A maximum level of 14% humidity is recommended. Corn kernels themselves are not naturally high in fat, being relatively low (3.68%). It also contains a modest amount of protein (7.08%) and the amount of ash is relatively small, since they are mainly a source of carbohydrates and fats, and their mineral content is generally low (1.62%), by also the cellulose content is lower (2.34%).

Sample	Sample number	Moisture (UM%)	Fat (S.U.%)	Crude protein (PB %)	Raw ash (CB %)	Raw cellulose (CEL B %)
Corn grains	Sample 1	13,50	3,80	7,00	1,50	2,20
	Sample 2	14,30	3,88	6,89	1,44	2,13
	Sample 3	14,70	3,65	6,77	1,70	2,30
	Sample 4	13,89	3,57	7,50	1,89	2,61
	Sample 5	13,90	3,56	7,40	1,75	2,11
	Sample 6	14,34	3,89	7,32	1,45	2,22
	Sample 7	14,76	3,64	6,78	1,68	2,61
	Sample 8	13,80	3,88	6,80	1,50	2,20
	Sample 9	13,50	3,65	7,11	1,44	2,13
	Sample 10	14,30	3,57	7,50	1,70	2,30
	Sample 11	14,70	3,56	7,40	1,89	2,61
	Sample 12	13,89	3,89	7,32	1,75	2,22
	Sample 13	13,90	3,65	6,78	1,45	2,61
	Sample 14	14,90	3,57	6,89	1,68	2,20
	Sample 15	14,30	3,50	6,77	1,50	2,60
	Average	14,18	3,68	7,08	1,62	2,34

Figure 8. Physico-chemical composition of corn grains

Regarding the mycological examination, a screening of the fodder used in animal feeding was carried out. Table 1 shows the genera of fungi identified in the 75 determinations on the 5 sample categories, respectively their percentage expression.

Table 1. The qualitative mycological exam of samples

Sample	Number of samples analysed	*Aspergillus*		*Penicillium*		*Fusarium*		*Cladosporium*		*Alternaria*		*Mucoraceae*		*Yeasts*	
		P.s	%	P.s	%	P.s	%	P.s	%	P.s	%	P.s	%	P.s	%
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***		***Yeasts***	

P.s. - positive sample

Following the mycological examination, the highest degree of mycotic contamination was corn silage and corn kernels (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 the *Mucoraceae* family (Figures 9 and 10).

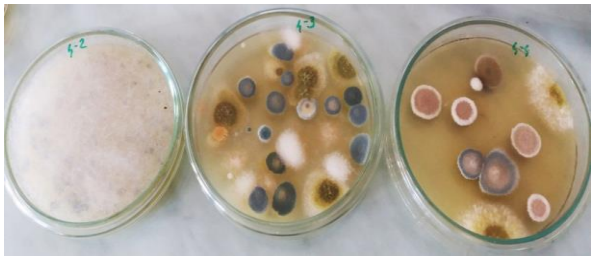

Figure 9. Macroscopic aspect of the genera *Mucor* and *Penicillium*

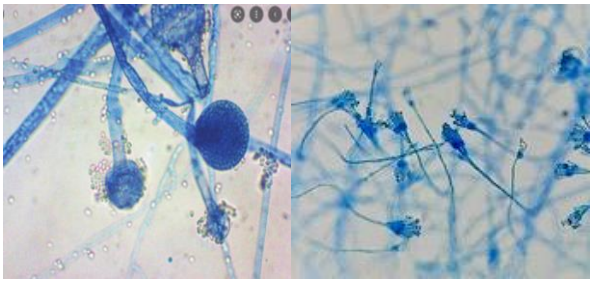

Figure 10. Microscopic aspect of the genera *Mucor* and *Penicillium* (col. LFAA x 400)

On the third place were the concentrates, in which species belonging to the *Mucoraceae* family as well as yeast colonies were identified in a percentage of 26.6%. In the samples of alfalfa hay, species of the genus *Penicillium* and yeasts predominated (20%), followed by species of the genera *Aspergillus*, *Cladosporium* and the *Mucoraceae* family with the same percentage of 13%, and in the corn silage, fungi from the genera *Fusarium* and the *Mucoraceae* family (13%) were isolated by incidence. *Alternaria* and *Cladosporium* genera species were not present in the mixed forage ration and concentrates.

The characters observed during the microscopic examination of fodder samples grown in PDA (Potato Dextrose Agar) medium revealed that the fungi with the highest incidence belong to the *Penicillium* genus that dominates the entire mycotic mosaic that characterizes the fodder analysed, being present in a proportion of 28% (Figure 11).

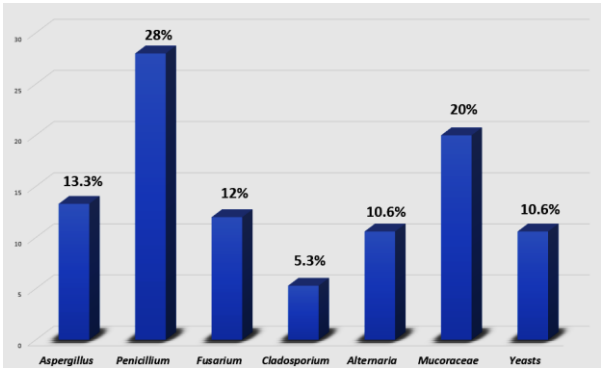

Figure 11. Percentage graphic representation of the genera of fungi

Species from the *Mucoraceae* family had a participation rate of 20%, ranking second. It is interesting to emphasize that the very high incidence of *Mucoraceae* implies a higher degree of humidity, which could be a signal of a potential alteration process. The species of the genus *Aspergillus* presented a contamination level of 13.3% in the analysed samples (place III). It is worth noting that the species of the genus *Fusarium* are in fourth place, occupying 12% percent in this hierarchy.

4. Conclusions

The five categories of samples (alfalfa semi-hay, corn silage, mixed fodder ration, concentrates, corn kernels) were analysed in order to establish the physico-chemical composition and mycotic load. The results of the physico-chemical composition of the feeds were in accordance with the regulations in force and no significant differences were found between the samples. It is important to mention that the specific values may vary according to different sources and local conditions, and the results of laboratory analyses by standardized techniques are the most accurate in determining the nutrient content of animal feed. The value of humidity is the most important aspect regarding the mycological aspect of the evaluation of the quality and storage capacity of the fodder, and the investigated samples presented values considered normal, specifying that the humidity had a percentage close to the maximum limits allowed, this explains the development of the species of fungi from the point of view of mycotic contamination, especially those from the *Mucoraceae* family in the mixed fodder and concentrate ration (33% and 26.6%, respectively). Also, the dominant mycotic flora that characterizes the fodder used in a cow farm, belonged to the *Penicillium* genera (28%), fam. *Mucoraceae* (20%), *Aspergillus* (13.3%) and *Fusarium* (12%) which obtained the highest contamination levels.

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