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EVALUATION OF THE HEALTH STATUS OF DAIRY COWS DURING A MYCOTOXIN SCREENING OF FEED IN A FARM FROM NORTH-EAST ROMANIA

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Abstract

The purpose of this study was to analyze the health status of dairy cows from a farm in Moldova. A total of 30 feed samples were analyzed from a mycotoxicological point of view, and 90 blood samples were collected from cattle of the BNR breed, which were biochemically analyzed to investigate the state of health. Adverse effects of mycotoxins are manifested in health, production, and reproduction in ruminants, especially dairy cows, and in the population. During the experimental period, regarding mycotoxin contamination of feed, the levels of mycotoxins investigated (AFL-T, FUM, and DON) did not exceed legal limits. From a biochemical point of view, the parameters that did not fall within the limits of the reference interval and showed a slight increase were represented by ALT, Gamma GT, and urea. All cows involved in the study were apparently healthy at the time of sample collection, but the resulting biochemical analyses suggest possible mild liver disease (by increasing above the normal limits of the previously mentioned parameters).

Key words*: biochemical analyses, bovine serum, dairy cows, mycotoxins.*

INTRODUCTION

Mycotoxins are by-products like antibiotics, only mycotoxins are toxic to animals and humans. Due to their high toxicity, even minute levels can have an impact on an individual's health (Alassane-Kpembi et al., 2016). According to Alonso et al. (2015), nearly all mycotoxins are cytotoxic, capable of rupturing cell membranes and influencing or preventing the creation of DNA, RNA, and proteins. However not every fungal growth produces mycotoxins, and finding fungi does not always mean that mycotoxins are present. Eating a diet tainted with mycotoxins can have both short-term and long-term consequences, such as immune system suppression, teratogenic, carcinogenic, and estrogenic effects (Cheli et al., 2013). Concurrent exposure to numerous mycotoxins is more common in the livestock business, despite the fact that the scientific literature offers a wide range of knowledge regarding the effects of specific mycotoxins on various animal species (Chukwudi et al., 2021). The reported illness signs and/or poor animal performance in commercial operations could be the result of numerous mycotoxins interacting synergistically. Some mycotoxins can enter milk and have negative effects not just on animal health but also on food safety and human health (Gallo et al., 2015; Sirbu et al., 2020). Only aflatoxin has been reported to be transmitted into the milk of nursing cattle at significantly elevated levels of concern out of all the mycotoxins examined (Penagos-Tabares et al., 2021). It is not always the case that fungal contamination is the cause of an animal's illness, as fungi can exist without creating mycotoxins. Because the mycotoxicological, clinical, radiological, microbiological, and histological data alone cannot provide the information required for the correct diagnosis, these data must be corroborated in order to produce an accurate diagnosis. According to Solfrizzo et al. (2014), the effects of mycotoxin ingestion are primarily chronic and involve concealed problems with decreased intake, productivity, and fertility.

Through clinically confusing changes in animal husbandry, decreased food consumption or refusal of feed, altered nutritional absorption and metabolism, effects on the endocrine system, and immune system suppression, these consequences result in significant economic losses (Stoev, 2015). In order to maintain the state of health and obtain production at optimal or even good levels, it is necessary to observe the control regarding the quality of feed, thus the periodic mycotoxicological analysis of the feed is an important way of assessing their quality both for their administration in the nutrition of dairy cows and for the preservation process (Coroian et al., 2017).

The health status of cows can be assessed and depends on the biochemical profile of the blood. If the physiological limits are normal, the biochemical profile reflects a good state of health, being also correlated with milk production (Magdaléna et al., 2020).

MATERIALS AND METHODS

The material is represented by the 30 samples, such as corn grains (15) and mixed fodder ration (15) from a cattle farm whose study the article refers to. Only these feed categories (corn grain and mixed fodder ration) were chosen because they were considered to be the most relevant and important. The structure of the mixed fodder ration for the dairy cow includes corn, triticale, soybean sorghum, rapeseed sorghum, mixed fodder premix, brewer's yeast, etc. The samples were collected on different days and months, namely March-May, respectively June-August 2023. The mycotoxicological analysis of the fodder was carried out by the ELISA method, the method used for the quantitative determination of mycotoxins. The basic principle is the antigen-antibody reaction, and the mycotoxins are detected by means of the color reaction, the intensity of which is measured by means of the ELISA immunoenzymatic line.

The short working protocol within the ELISA technique involves:

a) adding 200 µL of the conjugated solution to each color-coded dilution well;

b) adding 100 µL of each standard or sample to the dilution wells containing 200 µL of conjugate;

c) mix well, then immediately transfer 100 µl of the contents of each dilution well into a microwell coated with appropriate antibodies and incubate at room temperature for 15 minutes;

d) the contents of the microwells are thrown away and wash the wells with deionized water or buffer solution (5x);

e) dry the wells on an absorbent paper towel;

f) add 100 µl of the substrate solution to each well and incubate for 5-20 minutes;

g) add 100 µl of stop solution to each well;

h) read the absorbance of each well within 10 minutes of the addition of the stop solution at 450 nm (reference wavelength 630 nm) with a microwell reader.

Mycotoxins are extracted from a ground sample (20 g) with 70% methanol, only deoxynivalenol is extracted from a ground sample with distilled water. The extracted sample and enzyme-conjugated mycotoxins are then mixed and added to the antibody-coated microwell. The mycotoxins in the samples are allowed to complement the enzyme-conjugated mycotoxin at the antibody binding sites. After a washing step, the enzyme substrate is added, which results in color development. The intensity of the color is inversely proportional to the mycotoxin concentration in the sample. A stop solution is then added which changes the color from blue to yellow and the absorbance of each well is then measured at 450 nm. The kits provided by Romer Labs were used to analyze the content of total aflatoxins, fumonisins, and deoxynivalenol in the samples, kits that comply with the specifications of EN ISO 9001 and EN ISO 17025 respectively. The working technique refers to the protocol recommended by the manufacturer of the AgraQuant kit.

Figure 1. ELISA immunoenzymatic line (Tecan, Hydro-Flex model) (own source)

The analysis is carried out to identify metabolic disorders in an animal, and lack of nutrients in the body. Ninty blood samples were collected and analyzed from cows of the BNR breed during 6 months (March-August). The cows were divided into three groups, depending on milk production, thus group G1 assumes a milk production of 31 liters, group G2 assumes a milk production of 28 liters and group G3 assumes a milk production of 16 liters/day. After collection, blood samples were centrifuged for 5 minutes at 3000 rpm in a Hettich Zentrifugen Rotofix 32A Centrifuge. The resulting supernatant is called serum. After centrifugation, it is important that the liquid component (serum) is immediately transferred to a graduated, capped Eppendorf tube using a pipette. If the serum is not analyzed immediately, the serum should be stored and transported at -20°C or less. Freeze-thaw cycles are important to avoid, as this is detrimental to many serum components. Blood sera were analyzed for the following biochemical parameters: ALT/GPT (Alanine aminotransferase), ALB (albumin), AST/GOT (Aspartate aminotransferase), TBIL (total bilirubin), CA (calcium), CHOL (cholesterol), CREA (creatinine), gamma-Gt, TP (total protein), TRIG (triglycerides), BUN (urea) and ALKP (alkaline phosphatase). Analyzes were determined using the BioSystems BA200 analyzer, which features a dynamic baseline with SMART LED technology (Figure 2).

Figure 2. BioSystems BA200 analyzer (own source)

Among other things, this analyzer offers the highest degree of flexibility, the dosing is very accurate and it is a compact system with low maintenance. It has a large reagent and sample loading capacity. The values of the biochemical parameters in the serum obtained from the analyses were statistically evaluated by SPSS21. The ANOVA test coupled with the LSD test was performed to highlight the differences in the parameters during the studied period.

RESULTS AND DISCUSSIONS

The most important mycotoxins that contaminate corn and derived products during development and storage are total aflatoxins (AFL-T), fumonisins (FUM), deoxynivalenol (DON), and zearalenone (ZEA) (Table 1).

In the present work, the feed samples were mycotoxicologically analyzed only with regard to the first three mycotoxins previously mentioned.

Aflatoxin is a mycotoxin produced by the species *A. flavus* and *A. parasiticus*. As the most potent natural liver carcinogen known, aflatoxin causes thousands of cases of liver cancer annually worldwide (Jina Wu et al., 2022). Aflatoxin is the only mycotoxin on which the FDA has placed limitations on how much can be found in dairy rations (May et al., 2000). Fumonisins are produced by *F. verticillioides* and *F. proliferatum* plus other species of fungi (Mannaa & Kim, 2007). Fumonisins are often detected in corn silage and can cause important productivity losses but also health problems in both beef and dairy cattle. Even though ruminants are still considered less sensitive to fumonisins, there is evidence that fumonisins, alone or in combination with other *Fusarium* mycotoxins, have a hepatotoxic effect and affect immune functions in ruminants (Chulze, 2010). Deoxynivalenol is also called vomitoxin and is produced by species of the *Fusarium* genus. The mycotoxin deoxynivalenol is among the least toxic of the trichothecenes, and its effects are common. It should be noted that in combination with other mycotoxins (fumonisins, T_2 toxin) it can have much more serious effects (Udovicki et al., 2018). The study regarding the presence of total aflatoxins, fumonisins, and deoxynivalenol was carried out on 15 samples of corn grains and 15 samples of mixed fodder ration, as presented in Table 1. Also, in Tables 2 and 3 the statistical estimators of the main samples are presented of fodder studied (corn grains and mixed fodder ration), respectively the maximum limits allowed.

Table 1. The results of the mycotoxicological examination of the analyzed feed samples

The analysis period	Sample	AFL-T Total aflatoxins (µg/kg)	FUM - Fumonisins (µg/kg)	DON- Deoxynivalenol (µg/kg)
March-May	Corn grains	4.66	255.16	819.35
March-May	Corn grains	3.15	317.11	982.71
March-May	Corn grains	4.25	114.8	1108.5
March-May	Corn grains	6.84	195.3	986
March-May	Corn grains	2.11	266.2	1117.36
March-May	Corn grains	4.30	178.16	892.4
March-May	Corn grains	3.68	418.22	788.31
June-August	Corn grains	6.30	542	1144.5
June-August	Corn grains	4.77	314.2	799
June-August	Corn grains	3.14	351	412.33
June-August	Corn grains	6.72	216.5	652.81
June-August	Corn grains	6.47	180.11	839.99
June-August	Corn grains	5.22	237.66	1087
June-August	Corn grains	4.16	167.2	960.5
June-August	Corn grains	3.95	206.8	1116.1
March-May	Mixed fodder ration	0.11	115	1002.4
March-May	Mixed fodder ration	0.05	198.4	716.55
March-May	Mixed fodder ration	0.01	94.2	608.1
March-May	Mixed fodder ration	0.63	131.4	422.83
March-May	Mixed fodder ration	0.01	188.9	315.22
March-May	Mixed fodder ration	1.20	156.28	414
March-May	Mixed fodder ration	0.83	60.9	262.95
June-August	Mixed fodder ration	1.45	124.1	212
June-August	Mixed fodder ration	0.05	210.88	320.4
June-August	Mixed fodder ration	0.96	131	412.33
June-August	Mixed fodder ration	0.08	96.3	247.01
June-August	Mixed fodder ration	0.55	168.66	188.6
June-August	Mixed fodder ration	1.76	214.7	308.6
June-August	Mixed fodder ration	1.82	140.62	254
June-August	Mixed fodder ration	1.09	110.83	372.98

Table 2. Statistical estimators - corn grains

Mycotoxicological parameters	Standard limit *(REGULATION (EC) NO. 1881/2006)*	Statistical estimators - corn grains				
		$\bar{X}$	s	CV, %	Min.	Max.
Total aflatoxins (µg/kg)	10	4.65	1.42	0.31	2.11	6.84
Fumonisins (µg/kg)	4000	264.03	264.03	0.42	114.8	542
Deoxynivalenol (µg/kg)	1750	913.79	203.36	0.22	412.33	1144.5

Table 3. Statistical estimators - mixed fodder ration

Mycotoxicological parameters	Standard limit *(REGULATION (EC) NO. 1881/2006)*	Statistical estimators - mixed fodder ration				
		$\bar{X}$	s	CV, %	Min.	Max.
Total aflatoxins (µg/kg)	4	0.71	0.65	0.93	0.01	1.82
Fumonisins (µg/kg)	4000	142.81	45.92	0.32	60.9	214.7
Deoxynivalenol (µg/kg)	1750	403.86	219.28	0.54	188.6	1002.4

Analyzing the results presented in Tables 1, 2, and 3, we can note that the determined values do not exceed the limits established by Regulation (EC) no. 1881/2006, thus for corn grains, the maximum allowed limit regarding total aflatoxins is a maximum of 10 µg/kg, while for the mixed fodder ration the maximum allowed limit is 4 µg/kg. The average value obtained for corn grains, in the case of total aflatoxins, was 4.65 µg/kg, while for the mixed fodder ration, it was 0.71 µg/kg, values according to the standard.

The level of fumonisins allowed in feeds represented by corn and derived from corn, according to Regulation (EC) no. 1881/2006, is 4000 µg/kg. From the samples analyzed in this study, it emerged that the average obtained fell within the limit allowed by the standard: corn grains - 264.93 µg/kg, respectively mixed fodder ration - 142.81 µg/kg.

For the deoxynivalenol parameter, the specialized literature shows that the determined values of samples from Romania, compared to other studies from other parts of the world, are low. In the present case, the determined values of the analyzed feed samples did not exceed the maximum established limit of 1750 µg/kg according to the results obtained: corn grains - 913.79 µg/kg, respectively mixed fodder ration - 403.86 µg/kg (Tables 1, 2 and 3).

Even if the results obtained were in accordance with the specialized literature and fell within the maximum limits allowed by the standard, from a percentage point of view, deoxynivalenol had a higher level of contamination in the 30 feed samples (77.30% - corn grains, respectively 73.78% - mixed fodder ration) (Table 4, Figure 3).

Table 4. Obtaining the percentage of mycotoxins in the analyzed samples

Mycotoxins	Corn grains		Mixed fodder ration	
	Average (n=15)	Mycotoxin percentage (%)	Average (n=15)	Mycotoxin percentage (%)
AFLA-T (µg/kg)	4.32	**0.37**	0.71	**0.13**
FUM (µg/kg)	264.03	**22.33**	142.81	**26.09**
DON (µg/kg)	913.79	**77.30**	403.86	**73.78**

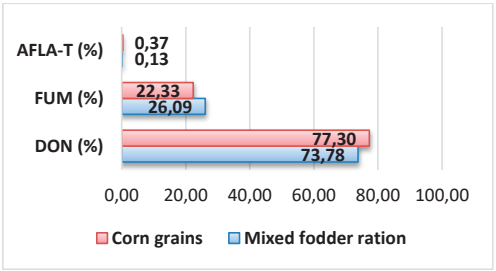

Figure 3. The percentage of mycotoxins in the analyzed samples

In second place was the class of fumonisins, in which only 22.33% were present in corn grains and 26.09% in mixed fodder ration (Table 4, Figure 3). In third place were the total aflatoxins, which represented the lowest level of contamination, such as 0.37% (corn grains) and 0.13% (mixed fodder ration) (Table 4, Figure 3).

Between the two categories of fodder, corn kernels presented a higher concentration of mycotoxins in contrast to the mixed fodder ration, with the mention that they were within legal limits. According to the literature, the negative impact of fumonisins in cattle is observed only when the animals are exposed to extremely high levels of mycotoxin, such as 90 mg/kg, levels that are much higher than the maximum recommended levels. The presence of fumonisins does not cause damage to ruminants (Antonio, 2022).

The degradation of deoxynivalenol depends a lot on the functions of the rumen, therefore chronic exposure should not be neglected. Thus, it is known that this mycotoxin - deoxynivalenol can produce inflammatory processes, even if its presence in the diet is below the maximum recommended levels (Gallo et al., 2020). The adverse effects of mycotoxins are manifested both in health, production, and reproduction in ruminants, especially dairy cows, and in the human milk-consuming population.

A biochemical profile is recommended whenever necessary because biochemical parameters change their values, which is characteristic of certain pathological conditions. The serum samples were analyzed from a biochemical point of view, and the average of the parameters, respectively the non-conforming average values obtained are in Table 5.

Table 5. The average of the biochemical parameters for each month

Biochemical Parameters	Reference interval *(The Merck Veterinary manual)*	The average of the parameters ($\bar{X}$) / Month					
		March	**April**	**May**	**June**	**July**	**August**
Albumin (g/L)	29-39	34.87	33.87	29.60	34.27	33.47	34.13
ALP-DEA (U/L)	27-127	87.33	93.33	87.60	74.67	120.47	122.87
ALT-GPT(U/L)	5-18	24.80	24.67	23.07	24.13	27.27	25.93
AST-GOT(U/L)	60-125	102.27	104.93	95.60	109.80	84.73	78.13
Total bilirubin(mg/dL)	0-1.6	0.08	0.09	0.05	0.08	0.23	0.08
Calcium (mg/dL)	8-11.4	8.46	8.46	7.48	8.39	8.54	8.34
Cholesterol(mg/dL)	163-397	201.73	216.53	180.87	192.27	196.33	175.13
Creatinine(mg/dL)	0.5-2.2	0.71	0.68	0.69	0.75	0.56	0.57
Gamma-GT(U/L)	6-17.4	34.20	37.80	25.00	37.87	25.20	26.67
Total protein(g/L)	59-81	71.67	74.80	67.80	82.93	75.33	79.27
Triglycerides (mg/dL)	10-19	6.94	5.14	2.80	14.47	18.27	16.67
Urea(mg/dL)	10-25	35.80	34.00	27.53	22.53	8.87	12.40

In March, the biochemical parameters that did not fall within the limits and showed a significant increase were: ALT-GPT, Gamma-Gt, and urea. Only the triglyceride content was lower than the maximum provided by the specialized literature. In April, the results of biochemical parameters are similar to March. Slight-moderate increases in ALT-GPT, Gamma-Gt, and urea can indicate diseases with liver location and not only. The reduced content of triglycerides demonstrated the fact that the animals were in a negative energy balance. Regarding the month of May, all the parameters fell within the stipulated limits, with the exception of 5 of them, thus ALT, Gamma, and urea showed a higher increase than normal values, while the content in calcium and triglycerides was more reduced. For the month of June, the average values of the analyzed blood biochemical parameters that did not fall within the limits provided by the specialized literature and that showed a significant increase were: ALT-GPT -24.13 U/L, Gamma-GT - 37.87 U/L, and total proteins - 82.93 g/L. Unlike the previously mentioned months, in July, in addition to the increase in the biochemical parameters represented by ALT and Gamma, in the blood of the animals sampled in the study, a reduced content of urea is added, symbolizing the fact that in July, the protein intake was not provided in the feed. For the month of August, following the determinations made on the serum samples, all the parameters were in the graph with the exception of two, thus a relatively higher content of alanine aminotransferase ALT-GPT was observed, the average value being 25.93 U/l, and for gamma -GT showed an average value of 26.67 U/L. Elevated alanine aminotransferase is an indicator of liver disease. Mild to moderate elevations of ALT (up to four to five times normal) may occur in nonhepatic conditions such as inflammatory gastrointestinal disease, heart failure, and hemolytic anemia (Stoev, 2015). Increased gamma-glutamyltransferase - indicates diseases with liver localization. GGT is an enzyme that is found throughout the body but is mostly found in the liver. When the liver is damaged, GGT can leak into the blood. High levels of GGT in the blood can be a sign of liver disease or damage to the bile ducts. The increase in urea indicates an alteration of liver function, but also other changes, such as dehydration, heart disease, shock, and urethral obstructions (Streit et al., 2012). In several studies, diets contaminated with FUM led to an increase in liver enzymes, such as gamma-glutamyl transpeptidase (GGT). Blood GGT levels can be used to detect chronic, subacute, or acute liver disease and an increase in these two liver enzymes can be associated with fatty liver syndrome in ruminants. Increases in these liver enzymes were detected in dairy cows receiving a combination of 994 ppb FUM and 733 ppb DON (Gallo et al., 2020), as well as beef cattle receiving 3.5 ppm FUM and 1.7 ppm DON in combination (Duringer et al., 2020). Elevated levels of these liver enzymes are a sign of liver damage caused by ingestion of FUM. Regarding the biochemical analyses, Table 6 presents the statistical interpretation of the values of biochemical parameters of the blood in the serum.

Table 6. Statistical interpretation of the values of blood biochemical parameters in the serum

Parameter		Month						ANOVA	LSD
		March (3)	April (4)	May (5)	June (6)	July (7)	August (8)		
Milk production (L)	X±sD	31.02±2.93	31.22±2.34	28.11±1.49	28.03±1.06	16.13±3.90	16.09±3.40	$p = 0.000$ ($p < 0.001$ ***)	3:5,3:6,3:7,3:8 4:5,4:6,4:7,4:8 5:7,5:8,6:7,6:8
	Min.	26.33	27.88	25.72	26.54	10.88	10.01		
	Max	35.50	35.98	30.63	29.99	22.16	20.98		
Albumin (g/L)	X±sD	34.87±3.20	33.87±3.23	29.60±5.90	34.27±5.54	33.47±3.66	34.13±5.38	$p = 0.035$ ($p < 0.05$ *)	3:5,4:5, 5:6,5:7, 5:8
	Min.	26.00	28.00	15.00	27.00	27.00	18.00		
	Max	40.00	41.00	40.00	48.00	40.00	38.00		
ALP-DEA (U/L)	X±sD	87.33±17.51	93.33±19.76	87.60±20.22	74.67±42.22	120.47±18.15	122.87±49.58	$p = 0.000$ ($p < 0.001$ ***)	3:7,3:8,4:7,4:8 5:7,5:8,6:7,6:8
	Min.	62.00	59.00	46.00	30.00	104.00	68.00		
	Max	111.00	134.00	127.00	176.00	167.00	255.00		
ALT-GPT (U/L)	X±sD	24.80±5.60	24.67±4.37	23.07±6.62	24.13±5.33	27.27±4.35	25.93±6.95	$p = 0.417$ ($p > 0.05$ ns)	5:7
	Min.	16.00	15.00	6.00	18.00	15.00	12.00		
	Max	37.00	33.00	35.00	36.00	35.00	36.00		
AST-GPT (U/L)	X±sD	102.27± 35.20	104.93± 34.01	95.60± 22.25	109.80± 45.29	84.73± 27.87	78.13± 15.67	$p = 0.054$ ($p > 0.05$ ns)	3:8,4:8,6:7,6:8
	Min.	58.00	64.00	48.00	50.00	47.00	54.00		
	Max	190.00	171.00	142.00	200.00	136.00	116.00		
Total bilirubin (mg/dL)	X±sD	0.08±0.08	0.09±0.09	0.05±0.06	0.08±0.04	0.23±0.45	0.08±0.14	$p = 0.199$ ($p > 0.05$ ns)	5:7,6:7,7:8
	Min.	0.01	0.01	0.01	0.01	0.01	0.01		
	Max	0.26	0.33	0.20	0.19	1.64	0.54		
Calcium (mg/dL)	X±sD	8.46±0.60	8.46±0.63	7.48±0.94	8.39 ±1.04	8.54±0.47	8.34±0.55	$p = 0.001$ ($p < 0.001$ ***)	3:5,4:5,5:6,5:7 5:8
	Min.	7.40	6.69	5.46	7.15	7.41	6.61		
	Max	9.68	9.41	9.00	11.75	9.26	8.88		
Cholesterol (mg/dL)	X±sD	201.73±55.77	216.53±60.97	180.87±59.29	192.27±41.43	196.33±33.65	175.13±37.86	$p = 0.248$ ($p > 0.05$ ns)	4:8
	Min.	99.00	89.00	41.00	110.00	124.00	129.00		
	Max	292.00	351.00	302.00	273.00	242.00	242.00		
Creatinine (mg/dL)	X±sD	0.71±0.11	0.68±0.12	0.69±0.15	0.75 ±0.15	0.56±0.15	0.57±0.11	$p = 0.000$ ($p < 0.001$ ***)	3:7,3:8,4:7,4:8 5:7,5:8,6:7,6:8
	Min.	0.51	0.53	0.50	0.56	0.17	0.38		
	Max	0.90	0.94	1.04	1.12	0.83	0.74		
Gamma-GT (U/L)	X±sD	34.20±18.66	37.80±24.23	25.00±11.28	37.87±15.28	25.20±10.22	26.67±7.14	$p = 0.045$ ($p < 0.05$ *)	4:5,4:7,5:6,6:7
	Min.	13.00	6.00	6.00	15.00	7.00	17.00		
	Max	83.00	93.00	53.00	73.00	41.00	39.00		
Total protein (mg/dL)	X±sD	71.67±6.79	74.80±6.16	67.80±8.17	82.93±9.49	75.33±6.78	79.27±3.69	$p = 0.000$ ($p < 0.001$ ***)	3:6,3:8,4:5,4:6 , 5:6,5:7,5:8,6:7
	Min.	60.00	60.00	49.00	75.00	65.00	73.00		
	Max	82.00	86.00	83.00	107.00	92.00	85.00		
Triglycerides (mg/dL)	X±sD	6.94±3.40	5.14±3.81	2.80±2.88	14.47 ±3.72	18.27±4.32	16.67±6.31	$p = 0.000$ ($p < 0.001$ ***)	3:5,3:6,3:73:8, 4:6,4:74:8,5:6, 5:7,5:8,6:7
	Min.	0.10	0.01	0.00	10.00	12.00	5.00		
	Max	12.00	11.00	8.00	24.00	28.00	30.00		
Ureea (mg/dL)	X±sD	35.80±6.61	34.00±5.36	27.53±5.00	22.53±8.95	8.87±5.53	12.40±6.23	$p = 0.000$ ($p < 0.001$ ***)	3:5,3:6,3:7,3:8 ,4:5,4:6,4:7,4: 8,5:6,5:7,5:8,6 :7,6:8
	Min.	21.00	21.00	14.00	11.00	1.00	0.00		
	Max	48.00	42.00	35.00	49.00	19.00	22.00		

p> 0.05 = insignificant / ns, p< 0.05 = significant / *, p< 0.01 = distinctly significant / **, p< 0.001 = highly significant / ***
ANOVA - Analysis of variance, LSD - Least Significant Difference

Milk production was significantly higher in the months of March, and April compared to the other months (p<0.001), while the average values of milk production were significantly lower in the months of July and August. Through the ANOVA test, it is found that there are very significant differences in milk production over the six months, and through the LSD test we note what these are, thus the months of March, and April show very significant differences (p<0.001) with the months of May, June, July, August, while the

months of May, June show very significant differences ($p<0.001$) with the months of July and August. As the temperature is higher in the summer months, and as the body temperature also increases, cattle reduce their feed intake to alleviate heat stress, thus leading to a gradual decrease in milk production and a change in milk fat content milk (Jiangjing et al., 2019).

The albumin content was significantly higher in March, June, and August compared to the other months ($p<0.05$), while the mean values were significantly lower in April, May, and July. The lowest value recorded was 15.00 g/L, while the highest value was 48.00 g/L (Table 6). Between the albumin content during the six months, it is found that there are significant differences ($p<0.05$), according to the ANOVA and LSD statistical test.

Alkaline phosphatase content (ALP-DEA) was highly significant in July and August and less so in the other months ($p<0.001$). The lowest value recorded for all months was 30.00 U/L, while the highest value was 255.00 U/L (Table 6). The differences are highly significant ($p<0.001$), according to the ANOVA statistical test coupled with the LSD test.

The content in alanine aminotransferase (ALT-GPT) was significantly higher in July compared to the other months, but we are talking about a statistically insignificant correlation ($p>0.05$). Between the content of alanine aminotransferase during the six months, it is found that there is only one insignificant difference ($p>0.05$) and this is between May and July, according to the ANOVA statistical test coupled with the LSD test (Table 6).

It was found that the content in aspartate aminotransferase (AST-GOT) was found insignificant during the six months ($p<0.05$), according to the ANOVA test. The lowest value recorded for all months was 47.00 U/L, while the highest value was 200.00 U/L (Table 6). The LSD test highlights that there were insignificant differences ($p>0.05$) (Table 6).

Regarding the dynamics of total bilirubin during the experimental period March-August, the correlations are insignificant (*$p>0.05$*) according to the ANOVA statistical test coupled with the LSD test, there being differences between the months of May and July, June and July, and July and August. The lowest value recorded for total bilirubin was 0.01 mg/dL, while the highest value was 1.64 mg/dL for all months (Table 6).

Regarding the calcium content, very significant differences ($p<0.001$) are found during the six months, according to the ANOVA statistical test. The LSD test highlights what these differences are, thus we note that between March and May, April and May, May and June, July and August there are very significant differences ($p<0.001$).

Correlations of cholesterol content are insignificant ($p>0.05$) according to the ANOVA statistical test. According to the LSD test, there were differences only between April and August. The lowest value recorded for cholesterol content was 41.00 mg/dL, while the highest value was 351.00 mg/dL for all months (Table 6). Regarding the creatinine content, the correlations are highly significant ($p<0.001$) according to the ANOVA statistical test coupled with the LSD test, there are differences between March and July and August, April, and July and August, May, and July and August and June with July and August (Table 6).

Glutamyl transpeptidase (Gamma-GT) was significantly higher in April, and June compared to the other months ($p<0.05$), while the lowest values significantly recorded were in May, July, and August. During the six months, it was found that the differences were significant ($p<0.05$), according to the ANOVA and LSD statistical test.

The total protein content was significantly higher in June compared to the other months ($p<0.001$). The correlations are highly significant ($p<0.001$) according to the ANOVA statistical test coupled with the LSD test, there are differences between March and June and August, April and May and June, May and June, July and August, but also between June and July (Table 6).

Triglyceride content was highly significant in July and August and less so in the other months ($p<0.001$), with differences between March and May, June, July, August, April, and May with June, July, August and between June and July ($p<0.001$) (Table 6).

According to the ANOVA test, coupled with the LSD test, urea had a higher content in March and April compared to the other months, the differences being highly significant ($p<0.001$).

To highlight the differences in the parameters during the studied period, it is observed that milk production, alkaline phosphatase, calcium content, creatinine, total proteins, triglycerides, and urea showed very significant differences during the 6 months, where $p<0.001$. Significant differences, where $p<0.05$ were found only in 2 of the 13 parameters, such as albumin and glutamyl transpeptidase, and non-significant differences were for ALT, AST, bilirubin, and cholesterol. The overview of the correlations of the analyzed biochemical parameters on bovine serum is presented in Table 7.

Table 7. Pearson correlation on the analyzed biochemical parameters of bovine serum

Pearson Corelation	a)	b)	c)	d)	e)	f)	g)	h)	i)	j)	k)	l)	m)
Milk production[a)] (L)	1	.002	-.419**	-.178	.290**	-.224*	-.091	.194	.389**	.214*	-.214*	-.665**	.740**
Albumin[b)] (g/L)	0.002	1	.179	.631**	.278**	-.043	.760**	.391**	.412**	.079	.252*	.254*	.293**
ALP-DEA[c)] (U/L)	-0.419**	0.179	1	.220*	-.065	.190	.306**	-.019	-.103	.006	-.008	.266*	-.298**
ALT-GPT[d)] (U/L)	-0.178	0.631**	0.22*	1	.368**	-.201	.437**	.570**	.080	.047	.133	.253*	.200
AST-GOT[e)] (U/L)	0.290**	0.278**	-0.065	0.368**	1	-.106	.228*	.313**	.301**	.627**	.134	-.235*	.377**
Total bilirubin[f)] (mg/dL)	-0.224*	-0.043	0.190	-0.201	-0.106	1	.087	-.167	.034	.062	.109	.182	-.194
Calcium[g)] (mg/dL)	-0.091	0.760**	0.306**	0.437**	0.228*	0.087	1	.292**	.401**	.109	.521**	.338**	.148
Cholesterol[h)] (mg/dL)	0.194	0.391**	-0.019	0.570**	0.313**	-0.167	0.292**	1	-.034	.166	.110	-.070	.338**
Creatinine[i)] (mg/dL)	0.389**	0.412**	-0.103	0.080	0.301*	0.034	0.401**	-0.034	1	.121	.184	-.142	.440**
Gamma-GT[j)] (U/L)	0.214*	0.079	0.006	0.047	0.627**	0.062	0.109	0.166	0.121	1	.195	-.114	.202
Total protein[k)] (g/L)	-0.214*	0.252*	-0.008	0.133	0.134	0.109	0.521**	0.110	0.184	0.195	1	.390**	-.143
Triglycerides[l)] (mg/dL)	-0.665**	0.254*	0.266*	0.253*	-0.235*	0.182	0.338**	-0.070	-0.142	-0.114	0.390**	1	-.553**
Urea[m)] (mg/dL)	0.740**	0.293**	-0.298**	0.200	0.377**	-0.194	0.148	0.338**	0.440	0.202	-0.143	-0.553**	1

* - statistically significant correlation ($p<0.05$); **- distinctly significant correlation ($p<0.01$).

The Pearson correlation test was used to evaluate the relationships between biochemical parameters during the 6 months, thus it is observed that milk production was significantly positively correlated with AST, creatinine, and urea and negatively correlated with ALP and triglycerides ($p<0.01$). Albumin was significantly positively correlated with ALT, AST, calcium, cholesterol, creatinine, and urea. ALP was significantly positively correlated with calcium and negatively with milk and urea production. ALT was significantly positively correlated with albumin, AST, calcium, and cholesterol, and AST was significantly positively correlated with milk production, albumin, ALT, cholesterol, creatinine, gamma, and urea. Calcium was significantly positively correlated with albumin, ALP, ALT, cholesterol, creatinine, total proteins, and triglycerides. Cholesterol was significantly positively correlated with albumin, ALT, AST, calcium, and urea. Creatinine was significantly

positively correlated with milk production, albumin, calcium, and urea. Gamma GT was significantly positively correlated only with AST. Total proteins were significantly positively correlated only with calcium and triglycerides. Triglycerides were significantly positively correlated only with calcium and total protein and negatively correlated with milk production and urea. Also, urea was significantly positively correlated with milk production, albumin, AST, and cholesterol and significantly negatively correlated with ALP and triglycerides. The other correlations are insignificant. We observe that positive correlations are distinguished for most blood biochemical parameters and only a few negative ones such as milk production, ALP alkaline phosphatase, triglyceride, and urea content. The evaluation of urea, triglycerides, cholesterol, creatinine, and other parameters provides an opportunity to expect a healthy production in animals, this evaluation bearing the name of blood profile test. Unlike other authors, where ALT usually presents a low level, in the present article, from the results of biochemical analyses on bovine serum, it emerged that the concentrations of ALT, gamma-Gt, and urea were higher than the rest of the parameters. Blood concentrations of enzymes including AST, GGT, and ALT are considered indicators of liver function and are also correlated with metabolic diseases such as ketosis (Li et al., 2016). As in Joo S.'s article, triglycerides showed a lower value. The reduction of triglycerides can lead to the accumulation of triglycerides from the blood in the liver, and this phenomenon can induce metabolic diseases (Joo, 2021).

CONCLUSIONS

During the experimental period, from a mycotoxicological point of view, the results of the feed samples were in accordance with the specialized literature and fell within the maximum limits allowed by the standard (Regulation (EC) no. 1881/2006).

The only biochemical parameters that did not fall within the limits of the reference range and that showed a relative increase were represented by ALT-alanine aminotransferase and Gamma Gt-glutamyl aminotransferase. An above-normal content was also observed in the case of urea. Parameters that had a value lower than the accepted limit were represented by triglycerides. All cows involved in the study were apparently healthy at the time of sample collection, but the resulting biochemical analyses may suggest mild liver disorders, according to the literature. Regarding the increase above the normal limit of ALT, Gamma-Gt, and urea parameters, it should be mentioned that the differences were quite small. Likewise in the case of triglycerides. Reduced content of triglycerides showed that the studied animals were in a negative energy balance at that time.

The content of mycotoxins present in the feed, even if it was relatively low and within the limits stipulated by the standard, had no negative effects on the health of the animals studied, except in a very small percentage, the biochemical analyses show.

Consequently, certain scientific evidence regarding the adverse effects of mycotoxin ingestion on cattle health and performance is scarce and remains to be proven.

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