Alteration of some Biochemical and Haematological Parameters in the Dairy Cows Due to the Intake of Mycotoxin Contaminated Feeds

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Abstract
The hepatogenic role of mycotoxins is well-known, but their action on some biochemical and haematological parameters in the dairy cows is yet to be completely clarified. The investigation monitored the modification of blood test values and of some biochemical parameters from the serum and urine of dairy cows reared in small family farms and which consumed feeds contaminated with mycotoxins.

Ten blood samples and 10 urine samples were collected and subsequently analysed. The samples were collected from 5 family farms, one in each of the following counties: Dolj, Giurgiu, Ilfov, Călărași and Galați.

The blood and urine samples were analysed for their biochemical parameters. The results of the biochemical analyses of the blood and urine samples were correlated with the results of the mycotoxicological analysis of 105 samples of forages collected from the surveyed family dairy farms.

The results showed that some biochemical parameters exceeded the normal physiological level, which may be the result of a defence reaction of the organism to the aggressive action of the mycotoxins identified in the forages given to the dairy cows.

Keywords: biochemical parameters, dairy cows, feed, mycotoxins.

1. Introduction
The adverse effects of mycotoxins manifest both on the health status, production and reproduction in ruminants, dairy cows specifically, and on the human population which consumes milk from dairy cows.

The biochemical parameters change their values, which is characteristic for certain pathological affections, so that a biochemical panel is recommended each time the suspicion of mycosis and mycotoxicoses in animals appears.

The hepatogenic role of mycotoxins is well established, but their significance in natural episodes of hepatic failure in cattle, except for aflatoxicosis, is not yet fully elucidated. Aflatoxin, like other mycotoxins, induces a severe dysfunction confirmed by biochemical tests as reported by many studies [1-7]. Cattle seem to have natural protection against the action of ochratoxin, but in case of high dietary levels or if the organism’s detoxification capacity is altered, ochratoxin symptoms can be observed in dairy cows. The target organism for ochratoxin is the kidney, as accounted for by the selective action of ochratoxin on the proximal tubules of the renal cortex.

The investigation aimed to monitor the health state of the dairy cows by determining the blood test values and blood and urine biochemical panel parameters in cows fed on forages contaminated with mycotoxins.

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2. Materials and methods

For the paraclinical exploration of the dairy cows we collected and analysed 10 blood samples and 10 urine samples from cows reared in 5 family farms, one farm from each of the following counties: Dolj, Giurgiu, Ilfov, Călărași and Galați. The blood and urine samples were subjected to a biochemical panel.

The following biochemical parameters were determined on the 10 blood samples: glucoses, total protein, albumin, urea, GOT and FA activities, mineral components (total Ca, P, Mg); the biochemical parameters determined on the 10 urine samples were: pH, density, glycosuria, proteinuria, urobilinogen, bilirubin, ketone bodies, nitrites, leukocytes and red cells.

The results of blood and urine biochemical panel have been correlated with the results of the mycotoxicological analysis of 105 samples of forages collected from the surveyed family dairy farms.

3. Results and discussion

A) The monthly clinical examination of the dairy cows didn’t reveal the presence of any symptom characteristic to mycoses or mycotoxicoses in the investigated animals.

B) The forage samples analysed by ELISA, AF for mycotoxin contamination produced values between 0.64 – 2.40 ppb, in proportion of 100% (105 samples) being below the admitted limit; OTA ranged between 0.37 – 8.60 ppb, in proportion of 0.95% (1 sample) exceeding the admitted level; ZEA ranged between 55.9 – 537.1 ppb, in proportion of 47.6% (50 samples) exceeding the admitted level; DON ranged between 0.017 – 0.889 ppm, in proportion of 100% (105 samples) being below the admitted limit; T-2 ranged between 113.7 – 324.3 ppb, in proportion of 100% (105 samples) exceeding the admitted level.

C) Table 1 shows the results of the biochemical panel of the blood samples. The average values of the blood biochemical parameters show a strong increase of FA (46 U/l) accompanied by hypercalcemia (13.2 mg/dl), hypermagnesemia (4.4 mg/dl), proteinemia (7.25 g/dl) and azotemia (21 mg/dl) at the lower limits of the normal range. These values may show complex metabolic disorders that can be attributed to the mycotoxicoses from the forages, without, however, confirming it.

It is established that FA is involved in the hydrolysis of the organic phosphates at an alkali pH. Since there are several isoenzymes that can originate from the liver, bones, kidney or intestines, we must first determine the origin of this increase in order to make a correct interpretation of this pathological phenomenon.

The fact that this hyperphosphatasemia is accompanied by hypercalcemia (13.2 mg Ca/dl) and hypermagnesemia (4.4 mg Mg/dl), elements with direct implications on bone metabolism, make us believe that this increase of FA originates mainly from the bones and, secondary, from the liver. The hepatic origin of the hyperphosphatasemia is not due to the lack of bone FA elimination, but to its retention by the cells lining the bile ducts. When the cows eat improper forages contaminated with mycetes and mycotoxins, we can expect the disturbance of the hepatic bile system which, among other, produces a severe hyperphosphatasemia.

Glycaemia was close to the physiological limit (50 mg/dl), and likewise were the total proteins (7 g/dl) and the albumin (3 g/dl). Urea was below the physiological limit for this animal species (30 mg/dl).

The low GPT activity must not be discussed because this enzyme is intracellular and therefore only its higher values have a pathological significance. The low values are normal and furthermore show the cellular integrity of the specific organism, which prevents the passage of the enzyme towards the exterior, in the blood. Therefore, the low GPT activity, as well as the low GOT activity, are normal. Furthermore, GPT activity in cattle has no relevance, which is why its determination is not part of the current biochemical panel for this species, as also supported by this determination [8].

Table 2 shows the results of the biochemical panel of the urine samples.
Table 1. Biochemical parameters of the blood samples

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>Glycaemia mg/dl</th>
<th>Total proteins g/dl</th>
<th>Albumin g/dl</th>
<th>GOT IU</th>
<th>FA U/l</th>
<th>Total Ca mg/dl</th>
<th>P mg/dl</th>
<th>Mg mg/dl</th>
<th>Urea mg/dl</th>
<th>GPT IU</th>
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<td>7.21</td>
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<td>7.23</td>
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</tbody>
</table>

Limit of variation: 41.6-44.4 7.07-7.42 3.53-3.85 18-62 23-69 9.3-17.1 5.7-6.9 2.1-6.7 12-30 5.4-18.6

Average value: 43 7.25 3.63 40 46 13.2 6.4 4.4 21 12

Reference limits: ±5.84 7-7.6 3-4 10-170 8-11.5 5.56 ±1.56 2.05 ±0.25 10-45 4.5-60

Table 2. Biochemical parameters of the urine samples

<table>
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<tr>
<th>Urine sample</th>
<th>Glycosuria</th>
<th>Proteinuria mg/dl</th>
<th>Urobilinogen</th>
<th>pH</th>
<th>Bilirubin</th>
<th>Density</th>
<th>Red cells</th>
<th>Ketone bodies</th>
<th>Nitrites</th>
<th>Leucocytes</th>
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</table>

Limit of variation: UD-30 Normal 7-8 1.021-1.039 UD UD UD

Average value: UD-30 Normal 8 1.030 UD UD UD


*UD – Undetected, considered negative

Table 2 data shows a slight pH increase, to the higher limit of the physiological values. Urine alkali character can be due to the presence of salts (sodium bicarbonate, acetates, lactates, nitrates) which make the urine turn alkaline; it may also be due to a metabolic alkalosis [9] or to pyelonephritis or to bacterial infections of the urinary ducts.

Normally, the glomerular membrane doesn’t allow the elimination of plasma proteins, except for some physiological states when transformations occur (new-borns, parturition and oestrus). In some renal disorders (acute renal failure, glomerulonephritis, chronic nephritis, pyelonephritis, nephritic syndrome), as well as in the renal neoplastic metastases and parasitoses, proteinuria was also detected [9]. In this case, proteinuria values of 30 mg/dl and density values exceeding 1.030 may indicate a possible pathological process undergoing in the urogenital apparatus, as also supported by the alkaline urine. In our investigation the modification of the blood and urine biochemical parameters can be a consequence of the consumption of forages contaminated with mycotoxins, without being in a position to confirm this aspect, however.

Blood biochemical panel has been performed by many authors with the purpose to determine the
implications of the dietary mycotoxins on animal health using paraclinical examinations.

In an experimental study on Holstein dairy cows in mid lactation, the cows were given, through fistulization, 13 mg impure AFB1 (AFB1 and other metabolites produced in culture by A. parasiticus), for 7 days. The following blood biochemical parameters have been determined on a daily basis for each animal: triglycerides, cholesterol, bilirubin, total protein, creatinin, uric acid, enzymatic activity of the transaminases glutamyl-oxalyl-acetate (GOT), alkaline phosphatase (FA) and gamma-glutamyl tanspeptidase (GGT). Blood NO2 has also been determined.

It was observed that the values of the investigated parameters remained unchanged during the experiment. Serum glucose in the blood of animals treated with impure AFB1 was 9% lower throughout the treatment, alteration accompanied by the decrease of the milk yield. In a similar group of dairy cows which received pure AFB1, no changes of glucose, cortizol and insulin concentration were noticed.

Consecutive to the experimental administration to a group of cows, for 6 weeks, of a forage contaminated with AFB1 concentrations ranging between 0.008 – 0.080 mg AFB1/kg live weigh/day, some parameters such as the total serum protein and the albumin to globulin ratio didn’t change significantly due to the ingested mycotoxin; the higher dietary concentration of mycotoxin above the level of 0.020 mg AFB1/kg live weigh/day increased the serum ALP. AFB1, AFM1 and aflatoxicol were not detected in the liver, kidney and urine of calves with chronic aflatoxicosis. Different values of AF in the tissues and urine have been detected in the animals which received a single dose of 0.8 mg AFB1/kg live weigh and 1.8 mg AFB1/kg live weigh.

Truckess et al. conducted an experimental study on the absorption and distribution of AFB1 in dairy cows organism. The administration of a single oral dose of 0.5 mg AFB1/kg live weigh in two dairy cows revealed the presence of aflatoxicol (AF Ro), AFB1 and AFM1 in the milk, plasma and erythrocytes, after one hour, the peak concentrations being detected 12 and 60 hours after the intake of the contaminated forage. The ratio of metabolite concentration was 1:10:100 for AF Ro : AFB1 : AFM1. AFB1 concentration in the liver, kidney, urine, bile and ruminal fluid of the animal which died was 5.1; 3.3; 4.1; 1.6 and 320 ng/g.

On the other hand, the experimental feeding of a group of cows with wheat contaminated with Fusarium spp. containing 8.21 mg DON /kg DM and 0.09 mg ZEA /kg DM determined, consecutively to the higher intake of OM, the increase of serum AST, GLDH and GGT, values conditioned by the amount of ingested dietary mycotoxin [12]. Cote et al.[13] have reported that about 20% of the DON ingested by dairy cows is found in the urine and faeces as deepoxi-DON (96%) and DON (4%), while in the milk it was detected as deepoxi-DON in a concentration in excess of 26 ng/ml.

The experimental administration of 1.25 mg ZEA/kg oats to a group of heifers didn’t induce clinical modifications in the animals, or pathological histological alterations of the reproductive organs in the same animals. The absence of the estrogenic clinic signs has been also reported by other authors, most probably due to the influence of the ruminal flora in ZEA degradation [14]. The effect of toxin T-2 produced by Fusarium spp. on the immune system in cows has been evaluated by Buening et al.[15]. The daily administration of 0.6 mg T-2/kg/day was associated to the significant depression of the lymphocyte response to mitogen and the significant reduction of the chemotaxis activity of the neutrophils.

The biochemical blood tests revealed a strong neutropenia [16] in the calves treated with a single dose of 0.25 mg toxin/kg live weight. The indicators of a depressed immune status such as IgG, IgM and serum globulin, had higher values in the calves treated with 0.2 mg T-2/kg live weight per day, administered as gel-coated capsules for 28 days [16]. The calves which consumed less than 0.8 mg T-2/kg live weight per day, equivalent to the ingestion of 2 kg corn contaminated with 2 ppm toxin/50 kg-live weight animal, per day, didn’t produce any observable change in these parameters [16]. In other studies, the experimental administration at sub-toxic level of T-2 in cattle induced, at a concentration of 0.3 mg T-2/kg per day, a non-significant increase of B lymphocytes and to no effects on T lymphocytes; the vales increased, however, upon administration of 0.5 mg T-2/kg per day.
Sharma et al. [17] observed a lower activity of the peripheral lymphocytes in the sheep and calves treated with T-2 infected forages. The sheep and calves treated with T-2 produced by Fusarium spp. had a lower activity of the peripheral lymphocytes and displayed leucopenia [17].

4. Conclusions

The biochemical parameters of the 10 blood samples reveal a high value of FA (46 U/l) accompanied by hypercalcemia (13.2 mg/dl) and hypermagnesemia (4.4 mg/dl), with proteinemia and azotemia being at the lower limit of the normal range, which shows the occurrence of complex metabolic disorders which can be attributed to the dietary mycotoxins, but without confirming this aspect, however.

The biochemical parameters of the 10 urine samples reveal a pH value at its physiological limit [18] associated to proteinuria, 30 mg/dl, and to a density in excess of 1.030, which suggests the existence of an unspecific pathological process in the urogenital apparatus.

References