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# Dairy Cows Health Risk: Mycotoxins

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Additional information is available at the end of the chapter

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## Abstract

Mycotoxins are secondary metabolites of mycotoxigenic fungi affecting both human and animal health. Their production in plants is highly unpredictable and dependent on a variety of factors, as well as the stage of the culture and transportation, storage and processing of the raw materials. One of the risks for dairy producers is animal exposure to mycotoxins. The scientific literature shows nonspecific signs to appear in a herd, most often when mycotoxins are present in feed and worse, in milk. In general, ruminants are considered resistant to the action of most mycotoxins, attitude explained by the detoxifying role of ruminal microsymbionts and especially protozoa. The clinical examination performed on the dairy cows from the studied farm did not reveal the presence of any symptom characteristic to mycoses or to mycotoxicosis. Although considered resistant to the action of mycotoxins, research reveals the constant presence of mycotoxigenic fungi and the mycotoxins they produce in the fodder of dairy cows, many times in various combinations. The incidence of mycotoxins is unpredictable and influenced by numerous factors (climatic, of production, transport, processing and storage of fodder). The health of dairy cows is affected by the consumption of contaminated fodder.

**Keywords:** fungus, mycotoxins, dairy cows, milk, health

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## 1. Introduction

The notion of fungus refers to a large array of eukaryotic structures, unicellular or multicellular, as they are regulated through ISO 21527-1 and 21,527-2, which cancel and replace ISO 7698:1990, ISO 7954:1987 and ISO 13681:1995.

Molds are free of chlorophyll, mesophilic aerobic filamentous microorganisms which, on the surface of mycological agar medium, under some conditions (0.7–0.9 water activity ( $a_w$ ) and usually  $25 \pm 1^\circ\text{C}$  temperature), develop flat or fluffy spreading propagules/germs or colonies often with colored fruiting or sporing structures [1, 2].

The majority of microfungi are saprobiotic organisms that can be seen in all-natural media – earth, water, air. In nature, fungi can grow and invade any type of food, at any moment, if the conditions favorable for their growth are created. The latter are in general represented by: substrate humidity higher than 11.5%, relative humidity of the air of over 70%, oxygen presence of 1–2%, temperatures between 5 and 40°C, substratum pH of 4–8,  $a_w$  between 0.7 and 0.85, relatively low light intensity and plant stress under the action of unfavorable medium factors (action of damaging insects, climate factors etc.).

The direct action of fungi over live organisms is of tissular destructive levels and can be limited or generalized, determining diseases named mycoses. In the pathology of ruminants, the following types of fungi are generally involved: *Absidia*, *Alternaria*, *Aspergillus*, *Candida*, *Cryptococcus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Rhodotorula*, *Sporothrix*, *Stachybotrys*, *Trichoderma*, *Trichophyton*, *Trichosporon*. Of these, there are certain types which are recognized as having mycotoxigen potential: *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor* and *Rhizopus*. According to FAO/IAEA, mycotoxins are secondary metabolites of fungi, non-volatile, organic, developed by fungi in both food and fodder [3].

The optimal temperature and humidity for developing a species of fungi does not correspond to the optimal parameters mentioned to produce mycotoxins, which determines a disparity between the presence of a species of fungi and the mycotoxins developed at that moment in the respective substrate. Note that there can be situations in which we find in analyzed food or fodder either only fungi, fungi and the mycotoxin/mycotoxins they produce or only mycotoxins. Mycotoxins are developed through a secondary metabolic process, which differs to the primary metabolism through its random nature, the diversity of compounds developed and the specificity of the thalli involved. The metabolic chains involved in the production of mycotoxins respond to the signals received by the fungus from the outside medium, thus not being related to cellular growth.

The diseases resulting from the activity of mycotoxins are named mycotoxicoses. The acute forms have a rapid evolution and are produced due to the action of high doses of mycotoxin over an organism. The chronicle forms, much more common, imply a slow development of the infection.

In general, ruminants, compared to monogastric animals, are considered resistant to the action of most mycotoxins, attitude explained by the detoxifying role of ruminal microsymbionts and especially protozoa. Ruminal and intestinal microorganisms do not significantly degrade mycotoxins when the ruminant's food is rich in concentrated fodder, as an example, or when the quantity of mycotoxins ingested reaches over certain limits. Equally, rumen metabolites of the parent mycotoxins can become, after ruminal biodegradation, not just less toxic but, in some cases, also more aggressive than the initial substance. Even so, the clinical examination performed on the dairy cows from the studied farm did not reveal the presence of any symptom characteristic to mycoses or to mycotoxicoses at dairy cows. From this perspective, it is extremely useful the analysis of the quality of fodder in regards to their contamination with fungi and/or mycotoxins and the application of preventive measures for the health of the animals.

## 2. Mycotoxin occurrence and mycotoxicosis

Mycotoxins are secondary metabolites developed under increased temperature, humidity,  $a_w$ , pH and their presence in fodder cannot be detected organoleptically. Furthermore, the

differences in the conditions of production for fungi and the mycotoxins associated to them are significantly different.

In general, the favoring factors for the development of fungi and the production of mycotoxins can be divided into three main categories:

- Physical: relative humidity, substrata humidity, water activity, temperature, fodder integrity.
- Chemical: pH, substrata composition of nutritive substances.
- Biological: presence of some microorganisms and/or invertebrates.

In general, with  $a_w$  of 0.85 at 25°C which corresponds to approximately 14–16% humidity, fungi spores germinated within 5 to 12 days.

Moreover, the effects of mycotoxins consist of:

- The reduction of the ingestion of food until it is completely refused by the animal.
- Reduction in the absorption of nutrients and the affliction of the metabolism.
- Alteration of the endocrine and exocrine systems.
- Suppression of the immune system.
- Reduction in the reproductive performances until the entire reproductive system is affected.

The symptoms are most often nonspecific, which makes the diagnostic difficult or even impossible. The difficulty in diagnosing mycotoxicoses is given as well by the occurrence of multiple mycotoxins, their uneven distribution in the fodder mass, the influence of certain factors linked to the animal, ration and climatic conditions.

## 2.1. Aflatoxins

Aflatoxins (AF) are mycotoxins produced in nature by fungi species of the *Aspergillus* (*A. flavus* and *A. parasiticus*) and more rarely *Penicillium* (*P. puberulum*, *P. citrinum*, *P. variable*) and *Rhizopus* types. The notion of aflatoxin in common languages refers to all its four representative forms: AFB<sub>1</sub> (C<sub>17</sub>H<sub>12</sub>O<sub>6</sub>), AFB<sub>2</sub> (C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>), AFG<sub>1</sub> (C<sub>17</sub>H<sub>12</sub>O<sub>7</sub>) and AFG<sub>2</sub> (C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>). *A. flavus* produces AFG<sub>1</sub>, AFG<sub>2</sub> as well as AFB<sub>1</sub> and AFB<sub>2</sub>. Aflatoxins B<sub>2</sub> and G<sub>2</sub> are dehydrogenated derivatives of AFB<sub>1</sub> and AFB<sub>2</sub>. From a mycologic point of view there is a large quantitative and qualitative difference regarding the ability of the different fungi species of producing these mycotoxins, about half of the *A. flavus* species producing aflatoxins [4].

The AF group comprises around 20 mycotoxins (e.g. M<sub>1</sub>, M<sub>2</sub>, B2a, AFL, AFL-M<sub>1</sub>, P<sub>1</sub>, Q<sub>1</sub>, H<sub>1</sub> etc.). The M<sub>1</sub> and M<sub>2</sub> metabolites are hydroxylated derivatives of aflatoxins B<sub>1</sub> and B<sub>2</sub> secreted in the animal milk following the consumption by the cows of food that has been contaminated with these [5].

At present, the kinetics of the transformations and the risk associated with the consumption and absorption of aflatoxins is well known, both at animals and at humans. The main conjugation way of aflatoxins is glucurono-conjugation, the resulting complex being eliminated

through the bile. The liver is considered the target organ for aflatoxins. At this level, the metabolization of mycotoxins takes place under the action of microsomal enzymes. The reaction products are eliminated from the organism through excretion products (feces and urine) and milk in unmodified form as well as metabolites.

The studies regarding the distribution and metabolism of AFB<sub>1</sub> marked with C<sup>14</sup>, in the organism of some animals, have demonstrated both the elimination in significant quantities of the mycotoxin from the organism in the first 24 hours, as well as the accumulation of the residual quantity in different organs (muscles, stomach, liver, heart etc.), accumulation conditioned by the dosage of mycotoxin ingested.

Naturally, ruminants seem to be more resistant to the action of aflatoxins compared to other species of animals, although the clinical signs of aflatoxicosis have been observed in cows, such as: the reduction in the ingestion of food, the decrease in the production of milk, the affliction of the hepatic function. The chronic exposure to the ingestion of aflatoxin determines an inefficient feeding, depression of the immune system and the reduction of the reproductive function [6].

The lipophilic mycotoxins with a small molecular mass like AFB<sub>1</sub> are absorbed in the digestive tract through passive diffusion. Aflatoxins, like other mycotoxins, induce severe hepatic dysfunction confirmed through biochemical tests in numerous studies [7, 8].

The pathologic modifications are more alarming in dairy cows, with high production, which are more sensitive to toxins. AFB<sub>1</sub> is a strong inhibitor of the protein synthesis which blocks *in vivo* the replication and transcription of DNA and inhibits the synthesis of RNA and proteins. The metabolic products of aflatoxin act on the chromatin inhibiting the transcription of genes and RNA polymerase, which as a result produces a decrease in the concentration of RNA and protein synthesis. *In vitro*, AFB<sub>1</sub> effectively couples with the DNA and causes irreversible mutations, which explains its incredibly strong carcinogenic effect. Approximately 90% of AFB<sub>1</sub> is present in blood, in plasma, being linked especially to albumins. Aflatoxins are oxidized in the liver with formation of very reactive molecules, capable of binding the nucleic acids or functional proteins. This hepatic bioactivation has a considerable importance for animal health due to the active metabolites that form *in situ*, at tissue level. The metabolization of aflatoxins at hepatic level takes place under the action of the microsomal enzymes, the most active of these being P450.

After the oral administration of AFB<sub>1</sub>, the metabolites are quickly found in urine and milk, while small quantities can be distinguished in feces which confirms the rapid absorption of AFB<sub>1</sub> in the digestive tract and hepatic metabolism [9].

In general, the ruminal degradability of AFB<sub>1</sub> is minor and the toxicity of the metabolic products is similar to that of the parent molecule. Aflatoxins affect the ruminal function through the reduction of ruminal motility, the capacity of digesting celluloses, of producing volatile fat acids and proteolysis [10].

The ruminal juice and, moreover, the bacteria population from the cow and sheep rumen does not have the capacity to convert aflatoxins in other metabolites except for AFM<sub>1</sub> which is found in large quantities in milk [11]. Auerbach et al. have observed that adding 9.5 ng AFB<sub>1</sub>/ml ruminal liquid did not alter *in vitro* the digestion of alfalfa and did not influence the production of volatile fat acids while, in another study, adding a dose of 1 µg AFB<sub>1</sub>/ml highlighted the lowering of the ruminal capacity of producing the acids [12].



Moreover, *in vivo* studies showed the presence of AFM<sub>1</sub> in the ruminal content, which leads to the conclusion that AFM<sub>1</sub> produced in the liver can reach the rumen, through the rumeno-hepatic way [13]. At dairy cows, from the total of 4.52% aflatoxins detected in the organism, 1.55% was detected in urine, 2.79% in feces and 0.18% in milk, in the form of AFM<sub>1</sub> which represented 0.35% of the administered dose. At the sheep in lactation, from the 8.1% compared to the ingested dose, it was detected 6.4% in urine, 1.63% in feces and 0.1% in milk. After a period of 6 days from the administration, the aflatoxin was not detected anymore in milk, after 8 days in urine and after 9 days in feces [14].

The degradation of aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>) in the rumen is relatively reduced, with a proportion of under 10% at a quantity of ingested mycotoxin of 1–10 µg/ml which is less toxic [15].

AFB<sub>1</sub> is considered the most carcinogenic natural substance being recognized as the most aggressive mycotoxin, for all the animal species, including human. The carcinogenicity of aflatoxins is dependent upon: animal species, age, dosage ingested, duration of ingesting the mycotoxin and nutritional state [16].

#### 2.1.1. Aflatoxins and milk production

The transformation of AFB<sub>1</sub> from fodder in AFM<sub>1</sub> in milk is realized following a process of hydroxylation. AFM<sub>1</sub> and AFM<sub>2</sub> are metabolites (hydroxylated forms) of AFB<sub>1</sub> and AFB<sub>2</sub> found in milk. Other metabolites identified in cow milk are: AFM<sub>4</sub>, AFQ<sub>1</sub> and aflatoxicol [17].

At dairy cows, the absorption of AFB<sub>1</sub> in the digestive tract is rapid and complete, which explains its almost immediate transfer in the milk, under the shape of AFM<sub>1</sub> [9]. Although many researchers have concentrated their attention on the study of AFM<sub>1</sub> from public health reasons, its production represents between 1 and 3% of ingested AFB<sub>1</sub>, with an average of 1.7% [18, 19]. Other studies have reached the conclusion that certain quantities of AFB<sub>1</sub> in the food of dairy cows (13 mg impure AF/day, over 7 days) can induce the decrease in production, without the evident clinical sign of disease. The transfer rate of AFB<sub>1</sub> from fodder to milk has been variable, with values comprised between 0.3 and 2.2% [20]. Milk production and body weight returned to normal limits within the next 5–8 days from removing the contaminated fodder.

For preventing the risk of transmitting AFB<sub>1</sub> to the milk, the superior limit for the content of this mycotoxin in the fodder of dairy cows with a production of 20 kg milk/day, after ingesting 6 kg of contaminated fodder/day has been evaluated at 5 µg AFB<sub>1</sub>/kg [21].

It was administered to a lot of lactating cows, in their food ration, corn contaminated with 120 µg AF/kg fodder. As a result, it was discovered the apparition of reproductive disorders, health problems, as well as the decrease in milk production. After removing the contaminated corn from the ration, the milk production rose to 28% within 3 weeks [22].

Diaz et al. [9] affirm that AF appear in milk after approximately 12 hours from the oral administration of AFB<sub>1</sub>, the maximum quantity being registered after 24 hours from ingesting the aflatoxin.

The mathematical relationship between the ingested quantity of AFB<sub>1</sub> and the quantity found in the milk is:

$$\text{AFM}_1 \text{ (ng/kg milk)} = 10,95 + 0,787 X \quad (1)$$

where  $X = \mu\text{g AFB}_1 \text{ ingested/day}$ .

Similar studies have shown approximately 90% of  $\text{AFM}_1$  present in the blood can be determined in the plasma and is found afterwards in the milk and urine [23].

The FDA limits aflatoxins to no more than 20 ppb in lactating dairy feeds and to 0.5 ppb in milk; in Europe, the regulatory levels of  $\text{AFB}_1$  are 20 ppb for dairy feeds and 0.05 ppb in milk.

### 2.1.2. Aflatoxicosis

The aflatoxicosis is an acute or chronicle mycotoxicosis of mammals, as well as birds, produced by aflatoxins.

*Etiologic agent, toxicity.* Aflatoxicosis is induced by the aflatoxins produced by some species of fungi such as *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *A. ochraceus*, *Penicillium puberulum*, *P. citrinum* and *Rhizopus spp.* From these, the main aflatoxin producers are *A. flavus* and *A. parasiticus*. Mycotoxins, as well as their metabolic products, work on the act on the chromatin, inhibiting the synthesis of the nucleic acids DNA and RNA from the hepatic cells and, at the same time, on the cellular membranes and membranes of the different intracytoplasmic structures.

*Clinical signs.* Aflatoxins produce disorders at the level of the central nervous system, digestive system, cardiovascular system and hematopoietic organs.

Aflatoxicosis evolves under acute, sub-acute and chronic form.

*In acute form*, it can be observed clinically: inappetence, anorexia, nervous depression, ataxia, dyspnea, and melena, anemia, while in sub-acute form: hemorrhagic enteritis, subcutaneous hematoma and jaundice.

*In chronicle form*, it can be observed: reduction of growth, rough and pale hair coat, anemia, jaundice, slower growth, apathy, diminished appetite, teeth grinding, fixed stare, circular movement, ataxia, diarrhea, rectal prolapse at mammals [24]; reduced resistance to diseases and interferes with vaccine induced immunity in livestock [25].

The toxic effects depend on: the dosage of mycotoxin ingested, the way of ingestion/administration, duration of exposure, nutritive quality of the ration, species and animal age. The calves, fresh cows or dairy cows in early lactation are most affected because their immune system is suppressed. Alongside *Aspergillus flavus*, *A. parasiticus*, producers of aflatoxin and *Aspergillus fumigatus* producer of tremorgens or viritoxin, fumagillin, encountered both in fibrous fodder, as well as silos, is considered as pathogen agent, being associated with the Mycotic Hemorrhagic Bowel Syndrome (HBS) at dairy cows [26].

Adult taurines and sheep are the least sensitive to the action of the mycotoxin. It is considered though that with the ingestion of at least 100 ppb aflatoxin/day, ruminants (both meat and dairy cattle) are exposed to the risk of the development of aflatoxicosis which debuts with: the reduction of food ingestion, diminished live weight, lowering of milk production, decline of the efficiency of the reproductive capacity [27, 28].

*Anatomopathologic modifications.* At the anatomopathological exam it can be observed: jaundice, generalized hemorrhage, hemorrhagic gastroenteritis, intestinal ulcers, hepatic necrosis, steatosis, hepatic fibrosis, hepatomegaly, cirrhosis, ascites, hydrothorax, thickening of the bladder walls and gall bladders [29].

*Histological modifications.* At the histologic exam, we can see perineural hemorrhage, perirenal edema, pioencephalitis with eosinophilic infiltrations and, in the case when it crosses the placental barrier, AF produces hepatic and digestive micro-lesions starting from the intrauterine life [24].

The most important action of the aflatoxins is considered to be the carcinogenic one, as well as the accusation of the preexisting tumoral formations. Neoplastic lesions are produced especially at hepatic level, but also at the level of the digestive tube, with lungs, kidneys metastases etc. Neathery et al. have observed that, in the situation when food is administered containing AFB<sub>1</sub> (0–5 ppm) and zinc (40–640 ppm), at calves, they showed some of the characteristic clinical signs of aflatoxicosis: reduction of food ingestion, lowering of live weight, reduction in pulse values and respiratory rhythm; no other anatomopathological modifications were seen in the liver or other organs [30].

*Presumptive diagnosis* is established based on the epidemiologic enquiry, clinical examination and anatomopathologic exam, while the certainty diagnosis is done on the basis of paraclinical examination (drop in vitamin D in the liver and the increase in alkaline phosphatase with return to normal before death) and of mycologic and mycotoxicologic examination of the fodder.

## 2.2. Ochratoxins

Ochratoxins are compounds developed by different species of *Penicillium* (*P. viridicatum*, *P. commune*, *P. purpurescens*) and *Aspergillus* (*A. ochraceus*, *A. alliaceus*, *A. melleus*, etc.) which contaminate cereal grain, vegetables and combined forage.

Ochratoxins A (OTA), B (OTB), C (OTC), D are a group of compounds in whose chemical structure L-phenylalanine is coupled through an amidic link with an isocoumarinic derivative. The production of these mycotoxins is frequent in molded or overheated fodder and is favored by the presence of oligoelements, a temperature of 20–28°C and a humidity of 18–19% at wheat, in the case of *P. viridicatum* or 22% in corn. In laboratory conditions, most strains of *A. ochraceus* produce OTB and OTC, while in natural environment conditions, the most frequent is OTA. Its precursors can also be seen in culture media [23].

OTA is passively absorbed, in unionized form, at the digestive tube level, especially at the level of the short intestine level, at a pH of 7.04. After OTA's penetration of the organism, the mycotoxin links itself to the albumines in the plasma and starts rapidly metabolizing, depending on the animal species. Among the organs, OTA's highest affinity is for the liver and kidney. OTA has the strongest inhibitor effect on animal growth, determining the excessive accumulation of glycogen in the liver of afflicted animals [31].

The halving time of OTA in studies on monkeys was 840 hours, or several days in the case of pigs [32] and 3 hours in chicken [23]. The most important transformation takes place at hepatic level, where OTA is metabolized through hydroxylation in 4-hydroxyochratoxin A, metabolite eliminated by the kidney [31, 32].



At the level of the digestive tube, where afterwards it is also absorbed, another important metabolite is formed, OT- $\alpha$ , which is finally eliminated through the digestive system or the kidneys. Other metabolites formed in the organism follow an enterohepatic circuit after which they are also eliminated through feces and urine [32].

Höhler et al. affirm that sheep fed with fodder containing various concentrations of OTA multiple values were recorded. Thus, at 2 ppm added OTA in food, there were significant concentration of OTA and OTA- $\alpha$  in the sanguine serum (10 and 3 ng/ml) and much higher concentrations for both mycotoxins at 5 ppm added OTA in food (80 and 15 ng/ml) [33].

The quantity of mycotoxin degraded by the intestinal and ruminal microsymbionts is dependent on the quantity of concentrates from food, the absorption of mycotoxins being higher at a larger content of starch in the food compared to fibers. From the total quantity of OTA ingested, approximately 70% of it is eliminated as OTA -  $\alpha$  (9% in feces and 61% in urine) compared to the underrated OTA form (1% in feces and 3.8% in urine) [9]. Recent studies have shown the ability of OTA to disturb the cellular signal and to influence the viability and proliferation of cells [34].

OTA was determined in quantity of 2.2  $\mu\text{g/kg}$  in the oat assay and in amount of 3.2  $\mu\text{g/kg}$  in the bran assay. In the case of this mycotoxin, the results obtained revealed a quantity of 0.1 ng/ml in blood serum, 0.018 ng/ml in milk and 0.009 ng/ml in urine. Although it can be observed that from the 5.4  $\mu\text{g/kg}$  ingested OTA, 1.8% were transferred in the blood serum, 0.3% in milk and 0.1% in urine, the conclusion regarding the conversion rate is uncertain, as different studies regarding the absorption and excretion of OTA and OTA- $\alpha$  at ruminants have shown the major influence of the type of food on the metabolites transfer to blood, milk and urine. The protozoa population at the rumen level is largely influenced by the type of alimentation of the ruminants. For example, the transformation of OTA in OTA-  $\alpha$  is favored by feed rich in starch more than one rich in fibers [23, 35]. In a study on sheep, Blank et al. administered OTA through wheat contaminated with mycotoxins, at a base ration of 70% concentrated feed and 30% silo. The study showed that a large part of the OTA quantity remained undegraded and was detected in the sheep serum (from 1.5 to 18  $\mu\text{g OTA/kg BW/day}$ ), regardless of the OTA level in the food, while the quantity of mycotoxin excreted in the urine remained almost constant (6–8% of the ingested dose), regardless of the food dose. Alongside OTA, small OTA- $\alpha$  were detected in the serum (from 0.5 to 1.6  $\mu\text{g OTA-}\alpha\text{/kg BW/day}$ ), directly proportional with the of the quantity of OTA in the food [36].

An important aspect of the metabolization of OTA in the organism is represented by the renal absorption at the level of the proximal tubes (2/3) and at the level of the distal tubes and collector channel (1/3). This phenomenon takes place due to the disturbance of pH homeostasis at cellular level of the nephron walls, which affects the acid–base transepithelial transport and determines the acidification of urine. The latter favors the reabsorption of OTA leading to the accumulation of the mycotoxin in the organism through the reduction of the elimination rate [37, 38].

Protozoa are considered organisms with a major role in degrading OTA to OTA- $\alpha$ . Other factors that can significantly influence the metabolic rate of OTA in the ruminant organism are: the animal age, genetic structure, health of the ruminal microsymbionts, alimentary ration structure.

The quantity of mycotoxin degraded by the intestinal and ruminal symbionts is dependent on the quantity of concentrates from the food, the absorption of mycotoxins being higher at a higher content of starch in the food compared to fibers. The inhibitor effect of OTA on the bacterial growth was only observed in the case of gram-positive bacteria, in general under a neutral or lower than 7 pH [9]. Müller estimated through *in vitro* studies that adult cows are capable of degrading 33 to 72 mg OTA/day, while sheep are capable of 3 up to 7 mg OTA/day. The food composition influences the structure of the ruminal microsymbionts and, implicitly, the capacity of degrading OTA. When the mycotoxin is found in a large quantity in the food, the microbial detoxification capacity is reduced and the symptoms of the ochratoxicosis appear. Moreover, the OTA metabolism in the rumen is much reduced in the case of the digestion of an increased quantity of concentrated fodder compared to an alimentation richer in fiber [39].

### 2.2.1. *Ochratoxicosis*

Due to the detoxifying capacity of ruminal symbionts for OTA, ruminants appear to be more resistant to the action of ochratoxins compared to monogastric animals. This capacity is the more evident the healthier the population of microsymbionts, especially protozoa. An alimentation rich in concentrated fodder affects the level of ruminal pH, consequently affecting the protozoa population and, implicitly, the capacity of metabolizing mycotoxins, in this case OTA. Moreover, after the administration of a dose similar to the one naturally found in fodder, OTA and OTA- $\alpha$  were not detected in milk, which is explained by the degradation of the ochratoxin in the rumen by the microsymbionts.

From the clinical signs of ochratoxicosis at ruminants, we distinguish the development of the pulmonary edema and the damaging of the animal health up to its death at OTA concentrations of over 3 ppm/kg in the fodder. At the same time, studies have shown that OTA does not cross the placenta barrier in the case of the oral administration of mycotoxin in reduced quantities (0.38 mg OTA/kg), although this was detected in the cow milk, as well as in ilk from other animals (pig, rabbit, rat).

### 2.3. Zearalenone

Zearalenone (ZEA) is a mycotoxin produced by fungi species of the *Fusarium* (*F. graminearum*, *F. tricotum*) and is most commonly found in cereal grain.

From a chemical point of view, zearalenone ( $C_{18}H_{22}O_5$ ) is a lactone of the resorcilic acid, with a structure similar to steroid hormones [5].

An experimental study followed the administration of 385–1925 ppb ZEA during 7 weeks did not show a change in milk production, nor the presence of ZEA residues in milk, serum, urine or tissue. Both ZEA and its metabolites are absorbed at intestinal level, covering the enterohepatic cycle [40].

$\alpha$  and  $\beta$ -zearalenol are derivatives of ZEA that are eliminated from the organism through feces and urine and, to a lower degree, through milk. From these,  $\alpha$ -zearalenol is considered the metabolite with the strongest estrogenic activity. Another metabolite of ZEA, which only develops in reduced quantities in animals, is zeranol, substance with a strong anabolic effect [41].

### 2.3.1. Toxic effects at ruminants

The symptoms of ZEA toxicosis are: uterus hypertropia, swelling of the vulva and mammary glands, decline in the ovulation rate and disturbance of the heat cycle, conception rate is low at dairy cows, the estrogen effect of ZEA being owed to the link of the mycotoxin to the cytoplasmic estrogen receptor.

It was discovered that the milk production decreased, infertility and hypoestrogenism appeared in the case of cows that consumed fodder contaminated with ZEA or with other fungi of the *Fusarium* type. Coppock et al. have shown that the effects of ZEA over the reproductive apparatus (vaginitis, vaginal secretions, mammary gland enlargement) at dairy cows can be strengthened through the synergic action of 600 ppb ZEA and 440 ppb DON in food; the consumption of food decreases which leads to the reduction of milk production, cases of diarrhea, increased infections of the reproductive tract and the entire reproductive activity is compromised. In general, it is considered that 400 ppb ZEA in the food is the maximum concentration for which the reproductive activity of dairy cows is not affected [42].

A secretory activity of the mammary gland was observed at heifers that consumed fungi contaminated corn in the pre-puberty period. The administration during three estral cycles, at a heifer lot, of 250 mg purified ZEA, determined the reduction of the conception rate with 62% while at the control lot, the rate of conception was reduced by 87% [43].

Signs of hyperestrogenism were shown in cows that consumed fodder contaminated with 1 mg ZEA/kg fodder, over 5 days, while at sheep that received small doses of up to 24 mg ZEA/day/animal administered through fodder during the same period did not produce any evident clinical effects over them, after the breeding period [42].

### 2.3.2. Transmission of zearalenone to milk of dairy cows

In general, it is considered that the transfer of ZEA and its metabolites in milk is very low [44].

Many researchers associate the reduced milk production, low fertility and hyperestrogenism at cows with the presence of ZEA in cereal or hay. Shreeve et al. ascertained that dairy cows fed with a ration containing 385–1982 µg ZEA/kg fodder, over 7 weeks, had a normal production of milk and there were no cases mycotoxin residues in milk, urine, serum or tissue [45]. In a 2004 study regarding the contamination with aflatoxin, ochratoxin and zearalenone, wheat and barley bran samples which were administered as a supplement to the food of dairy cows were analyzed. At the same time, determinations were done regarding the mycotoxin transfer in blood, milk and urine for the cows that consumed the contaminated feed. The results obtained showed the absence of aflatoxins AB<sub>1</sub>, AB<sub>2</sub>, AG<sub>1</sub> and AG<sub>2</sub>, as well as the absence of ZEA (values under 5 ppb in fodder and under 1 ppb in serum, milk and urine) in the fodder samples analyzed (values under the detection limit of 0.1 ppb). In a significant proportion, of approximately 90%, ZEA is transformed in  $\alpha$ -zearalenone whose toxicity is very high and, in a smaller proportion, in  $\beta$ -zearalenol. As in the case of OTA, protozoa are 9 times more active than bacteria in the degrading of ZEA [23]. The transformation at ruminal level of ZEA in zearalenol, together with the reduction in polarity, affects the absorption and excretion rate of the toxin thus, [23] in accordance with many other studies, reducing the elimination rate of ZEA and its metabolites in milk.

## 2.4. Trichothecene

Trichothecenes are a group of 43 mycotoxins (DON or vomitoxin, NIV, DAS, T-2 toxin etc.) with a similar chemical structure, developed by species of fungi from the following types: *Fusarium* (*F. graminearum*, *F. sporotrichioides*, *F. culmorum*, *F. poae*), *Myrothecium* sp., *Phomopsis* sp., *Stachybotrys* sp., *Trichoderma* sp. and *Trichothecium* sp. [23].

From a chemical point of view, trichothecenes are derivative compounds of a tetracyclic sesquiterpene nucleus containing the epoxy- stable group in positions 12 and 13 and double C-C link in positions 9 and 10 [5].

Trichothecenes are metabolized *in vivo* in four ways: hydrolysis at the ester group level, hydroxylation, epoxy reduction and conjugation in the digestive tract, liver and other target organs of the animal organism [46]. The metabolization of trichothecenes is relatively simple, the halving time in the plasma varying between several minutes and several hours, depending on the mycotoxin. Within 24 hours of the oral administration, in the digestive tract of bovines were found both parental compounds and their metabolites, free and glucorono-conjugated [47].

In general, the DON, T-2 toxin and DAS mycotoxins do not accumulate in significant quantities in the organism, regardless of the administration method, since their metabolic compounds are eliminated from the organism within days. In certain situations though, there can be accumulations of the lipophilic trichothecenes, T-2 toxin and DAS, at the skin and fat tissue level. *In vitro* incubation in ruminal fluid of the DON mycotoxin, for 48 hours, determined its partial conversion into deepoxy-DON, metabolite non-toxic for ruminants.

Charmley et al. administered a ration of contaminated wheat and corn to 18 primiparous cows, formulated in order to induce a daily consumption of 0.59 mg, 42 mg and 104 mg DON. The authors saw that an increased concentration of DON in the ration did not affect fodder consumption or milk production. There were however modifications of the fat percentage in the milk and of the fat production, for the cows that received 42 mg of toxin daily. The authors did not observe the transfer of DON or deepoxy-DON in milk [48].

Ruminal microsymbionts can degrade DON resulting in the formation of 12,13-de-epoxide-oxinivalenol (DOM-1). Côté et al. ascertained, following the administration of a ration with 66 mg DON/kg fodder, the presence of the DOM-1 metabolite in amount of 30 µg/l in milk and the absence of the parental mycotoxin [49]. In a study on lactating sheep, Prelusky et al. administered 880 mg DON/kg fodder, for 3 days and highlighted the presence in the milk of 220 µg/l mycotoxin, of which the majority was DOM-1 [44].

In a study done in North Carolina, Whitlow et al. found a significant decrease in the production of milk at cows that consumed concentrated fodder contaminated with 0.8 mg DON/kg DM. Such a result can be explained through the synergic effect of mycotoxins associated with DON even though these were not identified. The presence of DON residue in the animal tissue was not identified in this study [50].

As is the case for other mycotoxins, studies regarding the adding of DON to fodder did not reveal the same toxicity compared to the food naturally contaminated with DON [51]. This is explicable due to the multiple interactions between mycotoxins in fodder, under natural conditions.



### 2.4.1. *Micotoxicosis produced by trichothecenes*

Trichothecenes produce a large variety of gastrointestinal disorders such as: vomiting, diarrhea, dermic inflammation or irritation, abortion, hemorrhages and immunosuppression.

Immunosuppression generated by trichothecenes is realized through a complex mechanism that makes the animals more sensitive to pathogen agents.

Deoxynivalenol (DON) or vomitoxin has a reduced impact on dairy cattle, clinical signals being associated between DON contamination of fodder and reduced performances in dairy herds, especially the reduction of milk production. A Canadian study on 18 first-lactation cows during mid-lactation, showed that the production of milk reduced with 13% or 1.4 kg when the cows consumed food contaminated with DON 2.6–6.5 ppm [48]. Meat cattle and sheep tolerated a diet with 21 ppm DON without visible effects on the health state or production [52].

Among the general effects of DON on the organism, we mention: inhibitor of protein synthesis, affliction of the gastrointestinal tract, immune system depression.

T-2 toxin is found in a relatively lower proportion in fodder compared to other trichothecenes, under 10% and, in general, data related to its effect on ruminant health are reduced. T-2 toxin reduces ingestion, lowers production and affects reproduction; depending on dosage and duration of toxin ingestion, it results in gastroenteritis, ulcers and death [53].

The hemorrhagic syndrome can be either absent although gastrointestinal injuries are produced as presented by Weaver et al. or present, combined with reduced ingestion, milk production and absence of estrus cycles in cows [43, 54].

## 2.5. Fusariotoxycosis

Fusariotoxycosis is a mycotoxicosis that manifests itself through a complex of clinical symptoms and injuries to the digestive and genital apparatuses, central and hematopoietic nervous system and of the blood, provoked by different toxins from some *Fusarium* species.

*Etiologic agent.* Fusariotoxycosis is produced by the mycotoxins ZEA and DON developed by *F. graminearum* as well as other *Fusarium* species such as *F. nivale* and *F. tricinctum*.

Contamination sources are represented by cereals contaminated with *Fusarium*, most affected being corn grains. Development of *Fusarium* fungi is favored by high temperatures of 24–27°C, while the development of ZEA is favored by lower temperatures, of 12–14°C.

This ecologic characteristic explains the higher incidence of the mycotoxicosis in autumn or fall, when humidity is high and low and high temperatures alternate [29].

*The toxic form* begins at 5–6 hours from the consumption of contaminated fodder, with 1–4 days of evolution. It is manifested through salivation, chills, accelerated pulse and breathing, rumen hypotonia, teeth screeching. Clinically, we can also observe: loss of appetite, deviation, photophobia, arrhythmia, cutaneous hyposensitivity, exophthalmia, diarrhea, paresis, paralysis of the hindquarters.

*The estrogenic form* is rarely seen at taurines and is manifested through parturition and puerperal complications, metritis consequence of retained placenta, uterine involution, abortions,



heat cycle disruption, vaginal edema and prolapse, hypertrophy of mammary glands, etc. These clinical forms appear after consuming fodder contaminated with more than 24 ppm of ZEA [29].

Other species of *Fusarium* produce through the mycotoxins they develop various clinical manifestation in cattle. Thus, *F. nivale* develop the mycotoxins nivanelon, fuzarenon and BT butenolid, same as *F. tricinctum*, producing after being ingested by dairy cow's peripheral vasoconstriction and gangrene injuries of extremities due to ischemia; *F. tricinctum* develops the toxin T-2, F-2 and DAS. The toxin T-2 has inflammatory action over teguments and, in large quantities, can lead to skin necrosis. The consumption of fodder that contain the toxin T-2 leads to clinical manifestations such as loss of appetite, vomiting, severe dysentery, drop in coagulability of the blood and other signs of gastroenteritis [55].

*Anatomopathological modifications in toxic form.* At the necropsy exam hemorrhagic injuries can be seen, as well as catarrhal and sometimes hemorrhagic inflammation of the rennet, intestines and, as blood modification, leukocytosis with neutrophilia and eosinophilia.

*Presumptive diagnosis* is established on the basis of epidemiologic enquiry, clinical exam and anatomopathological exam, while the certainty diagnosis is established on the basis of para-clinical examination.

### 3. Combined mycotoxins

105 samples of fodder were analyzed, of which 75 samples of concentrated feed (cereal grains, wheat and maize bran, peas, sunflower and soybean meal) and 30 samples of fodder feeds from 5 family dairy farms in Southern Romania. The mycotoxicologic analysis was performed by the ELISA immunoassay test for AF, OTA, DON, ZEA and T-2.

In the 105 feed samples analyzed, in descending order, OTA was identified in a proportion of 63.80% (67 samples), T-2 in a proportion of 40.90% (43 samples), AF, ZEA and DON in a proportion of 39.0% (41 samples). By mycotoxin categories, in descending order, the maximum admissible limit in the 105 feed samples analyzed was exceeded in proportion of 40.95% for T-2 (43 samples), 33.30% for ZEA (35 samples) and 9.52% (2 samples) for OTA.

According to categories of feed, in descending order of the 30 analyzed fodder feed samples, the following were determined: 66.60% (20 samples) OTA, 36.60% (11 samples) ZEA, 33.30% (10 samples) DON, 26.6% (8 samples) T-2; in the concentrated feed analyzed, OTA was identified in proportion of 62.66% (47 samples), AF in proportion of 54.60% (41 samples), T-2 in proportion of 46.60% (35 samples), DON in proportion of 41.30% (31 samples) and ZEA in proportion of 40.00% (30 samples).

Of 105 analyzed feed samples, in decreasing order, 29.50% (31 samples) had two mycotoxins, 27.60% (29 samples) had three mycotoxins, 23.80% (25 samples) had one mycotoxin, 9.25% (10 samples) had four mycotoxins, 5.71% (6 samples) had no mycotoxins, and 2.85% (3 samples) had five mycotoxins.

Of the 25 samples with a mycotoxin, in decreasing order, the incidence was 9.52% (10 samples) for OTA, 4.76% (5 samples) for T-2, 3.80% (4 samples) for AF and ZEA and 1.90% (2 samples) for DON.

Of the 31 samples with two mycotoxins, in decreasing order, the incidence was: 6.66% (7 samples) for AF + OTA combination, 5.71% (6 samples) for OTA + T-2 combination, 4.76% (5 samples) for OTA + DON and AF + T-2 combination, 3.80% (4 samples) for OTA + ZEA combination and 0.95% (1 samples) for ZEA + DON; ZEA + T-2; DON + T-2 and AF + ZEA combination.

Of the 29 samples with three mycotoxins, in decreasing order, the incidence was: 6.66% (7 samples) for OTA + ZEA + DON combination, 3.80% (4 samples) for OTA + DON + T-2; AF + OTA + DON and OTA + ZEA + T-2; 2.85% (3 samples) for AF + ZEA + T-2 and 1.90% (2 samples) for AF + OTA + ZEA; AF + DON + T-2 and AF + OTA + T-2 combination.

Of the 10 samples with four mycotoxins, in decreasing order, the incidence was: 3.80% (4 samples) for AF + OTA + ZEA + DON + T-2 combination; 2.85% (3 samples) for AF + OTA + DON + T-2 combination; 1.90% (2 samples) for OTA + ZEA + DON + T-2 combination, and 0.95% (1 sample) for AF + ZEA + DON + T-2 combination.

Of the three samples with five mycotoxins, the incidence was 2.85% (3 samples) for AF + OTA + ZEA + DON + T-2 combination.

#### 4. Prevention and treatment

Prevention of mycotoxin contamination of feed should start from the field, especially since the climatic condition indicate possible crops contamination with mycotoxigenic fungi: drought or heavy rain, aggression of harmful insects, other stress situations for plants (application of treatments, for example). A mycological and mycotoxicological analysis of feed at this stage would be indicated and would provide accurate and important information for subsequent prevention actions (during storage and feeding animals).

Also, the fungal growth and mycotoxin production conditions are not the same. Thus, *Aspergillus spp.* grows at a higher temperature and lower  $a_w$  compared to *Fusarium spp.*; the production of AFB<sub>1</sub> and AFB<sub>2</sub> by *Aspergillus flavus* in corn, for example, is favored by heat and drought stress associated with warmer climates and, furthermore, is enhanced by insect action both before and after harvesting. In fact, *Fusarium spp.* is one of the genres of fungi that develops both pre-harvest and post-harvest. On the other hand, fungal growth and association with Alimentary Toxic Aleukia has the best conditions at 25–30°C while the production of mycotoxins by *Fusarium* is not favored under this conditions; the opposite effect, strongly mycotoxigen, although fungi do not have a high growth rate, is observed at near-freezing temperatures [56]. Similar situations can be also observed when applying fungicides that reduce the growth of fungi but not the mycotoxins [57].

During the storage period, maintaining optimum conditions: less than 14% humidity in the feed, creating optimal conditions for silage - quickly reducing pH and elimination of oxygen, microbial or enzymatic silage additives, may partially inhibit the development of mycotoxigenic fungi or reduce their ability to produce mycotoxins. However, if contaminated feed is still in the animal feed, a dilution of it with healthy fodder is recommended. A number of mycotoxin-adsorbing agents can also be used as food supplements: sodium and calcium aluminosilicates, bentonites, montmorillonites, zeolite, some organic polymers (polysaccharides, glucomannans, peptidoglycans, etc.), activated carbons, yeast cell walls, micronized fibers, bacteria. As

mycotoxin-biotransforming agents: gram-positive anaerobic and aerobic bacteria, gram-negative aerobic bacteria, fungi, yeast, enzymes (e.g. *Flavobacterium aurantiacum* for aflatoxins, *Eubacterium* BBSH 797 and LS100 for trichothecene, and for OTA and ZEA, *Trichosporum myco-toxinivorans*; protease A, pancreatin etc) [58]. Galvano et al. have shown that an increase in the level of some nutritional parameters in food – protein, energy and antioxidants, mineral and vitamins can be beneficial to animal health by mitigating the harmful effects of mycotoxins [59].

## 5. Conclusions

The increase and diversification in the production of fodder, particularly cereal, through new technologies has direct consequences on the change in their chemical composition and, implicitly, over the growth and development of fungi before the harvest, during the transportation or during the storage of fodder. Moldy feed has reduced palatability, which certainly determines reduced ingestion and implicitly a drop in milk production and, afterwards, in corporal weight. There are unanimously accepted losses of 5–10% of the performances of milk cows under the condition that they ingested fungi contaminated fodder, irrespective of the latters' contamination with mycotoxins. Mold growth and mycotoxin production are strongly linked with the action of certain predisposing factors such as extreme weather conditions (draught followed by rain, for example), the favoring action of harmful insects and can be produced in the field, during transport, processing or even while the fodder is administrated to the animal. The risk of affecting the health of ruminants due to the action of mycotoxins is much larger compared to that of the action of fungi. Among the mycotoxins that can affect the health of milk cows and, implicitly the reduction in production, aflatoxins are certainly the most aggressive. The risk is proportionally higher with their metabolites, as aggressive as them, reaching the milk production and affecting human health.

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