WHY IT'S DIFFICULT TO DETECT HIGH LEVELS OF MYCOTOXINS IN CLINICAL MYCOTOXICOSIS?

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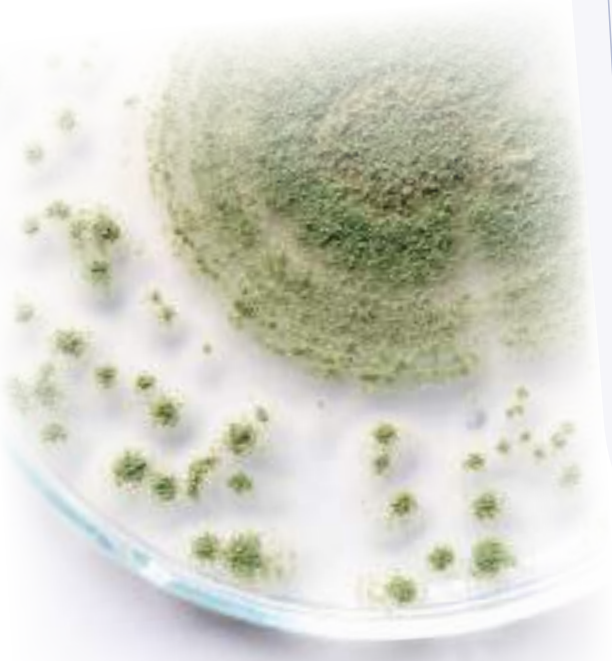
Traditionally, veterinarians and professionals who work in animal production have been trained to isolate or detect the causative agents of the clinical cases reported in poultry facilities.

Identification is often achieved by replicating the clinical signs of disease or poisoning observed in animals.



In the case of toxins, some feed factories often maintain experimental poultry sheds where they can test whether a nutritional ingredient or contaminant is causing the reported poisoning.

- Although **mycotoxins** are not living microorganisms, but rather metabolites produced by fungi when they grow, **we tend to try to conclusively detect which is the etiological agent.**
- In other words, to demonstrate that mycotoxins were indeed present in the feed consumed by the affected animals.

Unfortunately, it is not always possible to identify the mycotoxins that caused different symptoms or lesions in the animals after seeing a case that we consider typical.

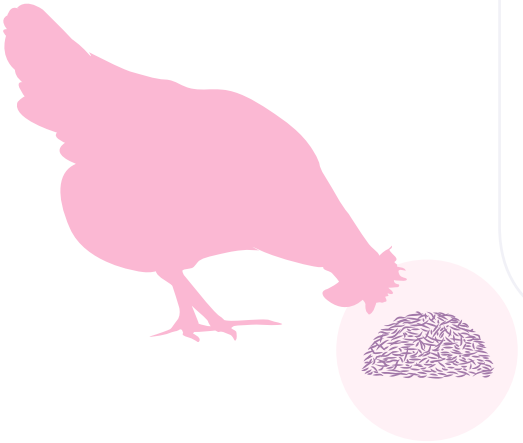
There are many reasons preventing us from reconfirming the relationship between what we see in the field and the presence of mycotoxins in the analysis, namely:

1. ***Irregular distribution of mycotoxins in grains and fee.***
2. ***Errors in taking samples.***
3. ***Laboratory techniques used to do the analysis.***
4. ***Presence of conjugated or masked mycotoxins.***

IRREGULAR DISTRIBUTION OF MYCOTOXINS

Unlike the protein or moisture content in corn or soybeans, mycotoxins are not evenly distributed. The main reason is that fungi don't grow everywhere, only in specific places.

- This trend can start in the field.
- Some grains can contain high levels of mycotoxins while others do not.



- At the level of **feed factories**, especially in the silos, fungi grow mostly where humidity is more prevalent, also known as "hot spots".
  - These points have been identified for decades and occur via the movement of moisture from the silo's exterior and, **mostly at night when ambient temperatures drop.**
  - The changes in temperature produce condensation on the internal walls of the silo, which promote the growth of fungi.
- The same phenomenon can occur in trucks, boats and other compartments where grain or feed gets stored.

SAMPLING

A correct analysis means determining the average contamination of within a batch of grain or formulated feed. If proper sampling procedures are not followed, analytical results are likely to underestimate the true mycotoxin concentration.

- That is, if only areas with low or no contamination are sampled, no real results will be obtained.
- False negative results can occur in tests, as a result of inadequate sampling and poor preparation of the sample to be tested.
- When few incremental samples are taken or the total lot sample is too small, it is much more common to "lose" a contaminated grain than to "find" it.

The chances of identifying mycotoxins in feed increase if they are taken from feeders located on farms, because that feed has been stored inside the silos for about 5 to 7 days and about a day inside the house once it is transferred to the hoppers and then to the feeders (if they are automatic)..

The length of time that the feed remains on the farms depends on the management in the company and the type of birds housed.

Unfortunately, even in clinical cases of mycotoxicosis where affected animals show typical lesions and where samples are taken in the right way and in the right place, we often do not identify mycotoxins during testing.

ANALYSIS TECHNIQUE USED

Results may vary depending on the type of test. Some tests are more specific than others with the ability to detect lower levels of mycotoxins (higher sensitivity).

HPLC (high precision liquid chromatography) has a higher sensitivity than Elisa (enzyme-linked immunoassay), that is, it is capable of detecting lower levels of mycotoxins in the sample.

In Elisa's case, the chance of false positives is higher than in HPLC.

QUICK, PRELIMINARY TESTS (SCREENING)

- This includes techniques such as Elisa, TLC (thin layer chromatography), and immunogenic tapes (immunostrips) that are characterized by reporting preliminary results in less time.
- Elisa's test is used to detect aflatoxins, T2 toxin, DON, HT-2, zearalenone, and fumonisins, among others.
- TLC is a technique that takes longer to perform than Elisa because it is necessary to clean the samples to obtain more accurate results.

RECONFIRMATION TESTS

- These are tests with greater specificity (less chance of presenting false positives) and sensitivity (capable of detecting very low levels).
- Among these is HPLC and lately it has been recommended to work in the technique known as liquid chromatography coupled to tandem mass spectrometry (LC / MS / MS).
- LC / MS / MS is highly advanced and simultaneously analyzes hundreds of metabolites and mycotoxins, something that cannot be done with HPLC or Elisa.

CONJUGATED OR MASKED MYCOTOXINS

They represent a new challenge that prevents us from determining the real concentration of these toxins in the feed that animals consume.

- They consist of derivatives of mycotoxins not detectable with conventional analytical tests because the chemical structure of the mycotoxin has changed during the growth of the plant in the field, before the grains were harvested.
- The agents that catalyze the development of these variations in the integrity of mycotoxins are enzymes produced by plants that commonly act in the detoxification process.
- Mycotoxins adhere to other nutrients such as sugars (glucose), fatty acids or amino acids.
- Generally, mycotoxins adhere to a more polar substance such as glucose and there is the possibility that these conjugates release their toxic precursors after hydrolysis occurs within the host.

Fungi that cause damage to plants at the level of fields or greenhouses are identified as phytopathogens.

It is important to clarify that not all fungi that grow on plants affect their well-being.

A metabolite of the T2 toxin is HT-2, which originates in plants as a defense mechanism to try to neutralize the toxic effect of this mycotoxin.

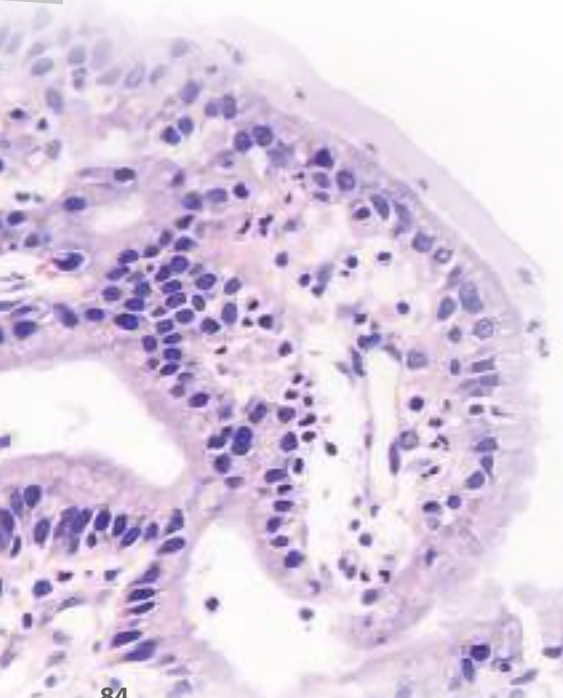
OTHER LABORATORY TESTS

After reviewing the factors mentioned in this article, we have no doubt that the detection of mycotoxins in ingredients or feed has certain limitations.

For this reason, **clinicians have resorted to other laboratory techniques to determine if mycotoxins are present in these clinical field cases.**

HISTOPATHOLOGY

The most useful technique used so far is **histopathology**, since it allows us to identify characteristic (non-pathognomonic) lesions in target organs caused by specific mycotoxins.



IDENTIFICATION OF MARKERS OR METABOLITES OF MYCOTOXINS

Another technique that may be used more frequently in the future, once it is more developed, is the **identification of markers or metabolites of mycotoxins.**.

With this test, the objective is to demonstrate that the animals were in contact with the mycotoxins and that as a consequence of this exposure, the host produces chemical substances indicative that the exposure occurred.

In recent years it has become very fashionable, but unfortunately it does not always offer consistent results, depending on the type of mycotoxin tested.

In our experience, compiled in scientific tests carried out in different countries, in some cases the relationship between markers and intoxication is clearly reported.

In other experiments the relationship is not observed.

We have observed this when evaluating the relationship between the levels of sphingosine and sphingonine, two markers associated with intoxication with Fumonisin, which inhibits the enzyme responsible for the metabolism of blood sphingolipids (fats).

Despite this variability in many markers, there is **an extremely reliable marker that has become a standard test worldwide and consists of the detection of M1 in cow's milk**, a metabolite of Aflatoxins B1 and B2.

Why it's difficult to detect high levels of mycotoxins in clinical mycotoxicosis?

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