Newsletter

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> EDITORIAL

Historically, veterinarians have had the role of controlling animal livestock diseases at the farm level. Nowadays, as countries begin to bring the serious diseases under control, the scope of such professionals normally increases to address production diseases of livestock, where control leads to more efficient production and/or better quality animal products (OIE, 2010). In order to perform such activity, vets must analyze the conditions on the field and whenever justified, search for potential causes for symptoms found which will lead to an accurate diagnosis.

In the case of mycotoxicoses, many questions are often posed.

Can we rely solely on the symptoms the animals are presenting to diagnose a mycotoxin problem? Usually mycotoxin analyses are a must, but what reasons might there be for the fact that the mycotoxin analysis report shows a low mycotoxin concentration and still, animals show severe mycotoxicoses symptoms?

Although the answer to these questions has been given in different, separate documents, this article intends to explain the motives which might lead to a discrepancy between observed symptoms and mycotoxin analysis. Hopefully this will also lead to a more critical interpretation of mycotoxin analysis reports.

Enjoy reading!

Inês Rodrigues

Mycofix® product line

You get into the poultry house and animals are not performing well and some of them have even died. Necropsy shows a pale, enlarged liver. Bingo! Aflatoxins are the problem!
At the sow barn, females are having reproductive problems and the abortion incidence has increased abruptly. There you go – zearalenone intoxication, without a doubt! However, be careful as things may not be what they look like!

Mycotoxin Risk Assessment - Converting suspicion into facts

Do you know that aflatoxicosis mimics infectious bursal disease, fatty liver syndrome, deficiency of linoleic acid, and malabsorption syndrome? (Atlas of Avian Diseases, USDA, 2010). And have you ever considered that abortions and infertility alone can be caused by Aujezsky disease, by *Brusella suis* or by perineal contamination?

Visual diagnosis vs. analysis of commodities/feeds

Actually, a correct differential diagnosis allows a practitioner to differentiate mycotoxicoses from poor nutrition, poor management, physical damage to tissues, and infectious diseases.
Visual diagnosis is a complex task and oftentimes erroneous as same symptoms can be caused by other etiologic agents. The best and most precise way to identify a problem involving mycotoxins is by analyzing commodities or finished feed for their presence. However, even when this is done and mycotoxin presence is confirmed, results must be cautiously interpreted. The reason for this relies on the fact that oftentimes the sampling process did not allow the withdrawal of what is commonly referred to as a representative sample.

Figure 1 – *Broilers with impaired feathering due to trichothecenes (notice the lack of feathers on the floor)*

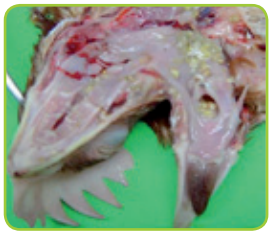

Figure 2 – *Necrosis of the tongue due to the presence of trichothecenes*
Source: The Lombardy and Emilia Romagna Experimental Zootechnic Institute (IZSLER)

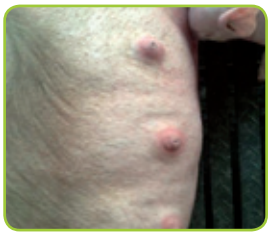

Figure 3 – *Red, swollen teats as a possible sign for zearalenone presence (Source: Everson Zotti, Brazil)*

Figure 4 - *Tail necrosis possibly caused by trichothecenes and ergot alkaloids presence (Source: Norbert Trattner, Belgium)*

Representative samples and the analysis of commodities/feeds

Testing for mycotoxins is a complicated process for which a sampling plan is of high importance. Sampling plans must be setup in accordance with the possibilities of the feed mill/farm; however, the essence of a correct procedure relies on 3 guidelines *(Figure 5)*:

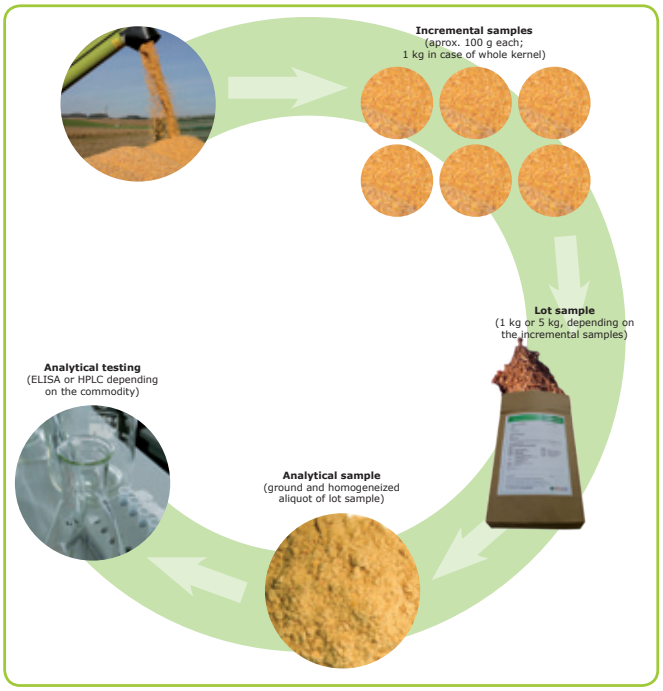

Figure 5 - *Scheme representing the sampling process*

1) From a large lot of bulk sample (e.g. from a truck), as many increments (points) as possible should be taken at a random fashion.
2) Each increment should have approximately 100 g. A minimum of 1000 g (1 kg) is recommended in case of whole kernel samples. However, the more the better (approx. 5 kg). This is called a "lot sample".
3) From this lot sample (1 to 5 kg), the entire collection is ground and homogenized) before weighing out an aliquot for the analytical testing, which will be 20 – 25 g according to the test method applied (sub-sampling). This step is usually done at the laboratory where samples will be analyzed.

Analytical techniques for the detection of mycotoxins have improved substantially in the last few years. However, even when using accepted test procedures there is variability associated with each of the above mentioned steps. The sampling step is widely recognized as the major contributor to the large variability in mycotoxin determination, especially in the case of *Aspergillus* produced mycotoxins, for which hot spots may be found in batches of most food and feed commodities (Krska and Molinelli, 2007) *(Figure 6)*.

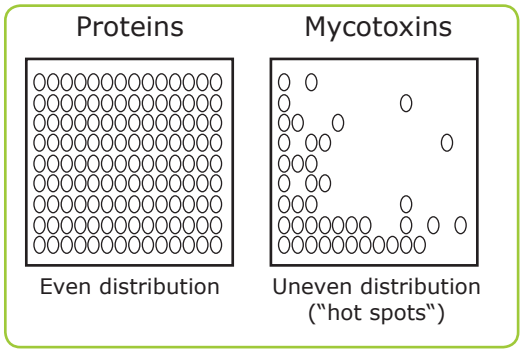

Figure 6 – *Representation of the heterogeneous distribution of mycotoxins vs. the homogeneous distribution of proteins*

As time and money are being spent for the analyses of mycotoxins, the extra time for proper sampling and sample preparation is crucial: the test results are meaningless if the analyzed sample is not representative of the lot (Romer Labs, 2005). A good sampling procedure will minimize the occurrence of false negatives (when contaminated particles are totally missed) and of false positives (when too many contaminated grains are collected). The latter though is not as common as false negatives because when sampling is done incorrectly it is much easier to "miss" the contaminated kernel than it is to "hit" too many of them.
So at the end, we have our representative sample of finished feed and we just send it out to the laboratory. But what testing method is to be used? Will ELISA and HPLC perform equally well for all commodities?

Different testing methods for the analysis of commodities/feeds

Why must HPLC be used to test finished feeds and ELISA test kits for commodities only? Rapid tests, such as ELISA, are used to determine the presence of a specific analyte(s) in a given matrix(es). They provide quantitative results in the calibration range and for validated commodities. If the matrix is different than that the ELISA test kit is validated for, for example finished feed rather than maize, then results are not reliable. Obviously, due to the different diet formulations it is impossible to validate an ELISA for all possible feed variations.
On the other hand, the advantages of using reference, quantitative testing, such as HPLC, allows the achievement of low detection limits, the possibility of testing very complex commodities (for instance finished feed and silage) and the analysis of several substances at once. *Table 1* presents an overview of advantages and disadvantages of both types of methods.

Table 1 - *Overview on the advantages and disadvantages of rapid methods vs. reference testing methods*

	Rapid methods (i.e. ELISA)	Reference testing (i.e. HPLC)
Advantages	• fast • inexpensive • very reliable for raw materials (corn, wheat) • quantitative for the validated commodities	• reliable and quantitative for most commodities • result refers to the single toxins • necessary for legal issues
Disadvantages	• result can be a sum of similar toxins e.g. all Type B Trichothecenes	• more time consuming • relatively expensive

At this point a mycotoxin report should be available for the representative sample which was sent out to the lab. Results show the occurrence, at low levels, of Afla, ZON, DON and FUM. The normal thought would be: why are animals being impacted by such low mycotoxin concentrations?

Low mycotoxin concentration *vs.* synergistic effects

One of the factors which may explain the observation of symptoms even with low mycotoxin concentration is the synergism between mycotoxins. Overt symptoms due to mycotoxin consumption are not so common. Actually, most of economic impact is caused by immunosuppression and impaired performance due to the consumption of low levels of multiple mycotoxins. If one thinks that a single plant might be infected by several fungi and each fungus is able to produce multiple mycotoxins, the combinations in a finished feed containing several different commodities are immense. Several mycotoxins are known to interact amongst them causing synergistic effects. *Figure 7* presents the most common interactions between mycotoxins in poultry and pigs.

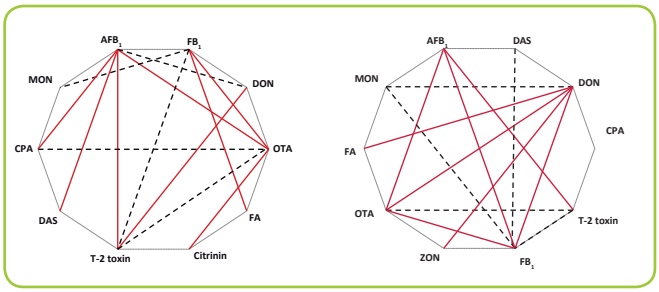

Figure 7 - *Additive (dashed line) and synergistic (red line) effects in poultry (left) and pigs (right). AFB_1 - Aflatoxin B_1; FB_1 – Fumonisin B_1; DON – Deoxynivalenol; OTA – Ochratoxin A; ZON – Zearalenone; FA – Fusaric acid; DAS – Diacetoxyscirpenol; CPA – Cyclopiazonic acid; MON – Moniliformin.*

Low mycotoxin concentration *vs.* masked mycotoxins

Feed is not necessarily safe just because the presence of well-known mycotoxins has been ruled out, as they might still be there in disguise. Mycotoxins can also occur in conjugated form, either soluble (masked mycotoxins) or incorporated into/associated with/attached to macromolecules (bound mycotoxins) as a product of plant, fungi, mammal metabolization or after feed processing (Berthiller *et al.,* 2009). Even if some of these compounds are resistant to the stomach acid conditions, they will be cleaved in the intestine into harmful molecules. The non-availability of measurement standards represents the major hindrance to the acknowledgement of these conjugated toxins. More than 50% of the amount of free mycotoxins (especially zearalenone and deoxynivalenol) is considered to exist in commodities in a masked form (Vendl *et al.,* 2010).

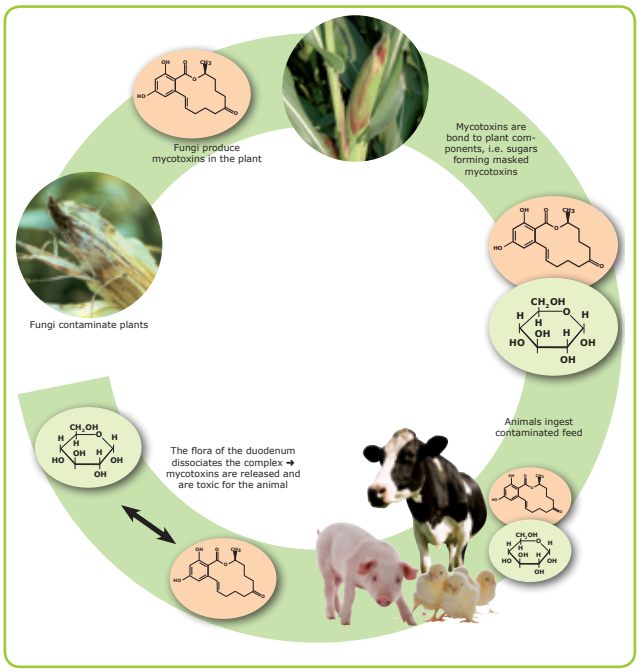

Figure 8 – *Scheme representing the example of the conjugate zearalenone-4-glucosyde formation in the plant and subsequent ingestion by the animal followed by the hydrolysis and release of the toxic compound.*

Low mycotoxin concentration *vs.* interacting factors

Another factor which may explain why in some situations clinical symptoms are observed in animals at low mycotoxin presence is the interaction with other factors.
The effects of mycotoxins depend on several animal-, environmental- and toxin-related factors *(Figure 9)*. Young animals are in general more susceptible to the effects of mycotoxins. Animals inserted in a hostile environment characterized by, for example, high temperatures, poor ventilation, high humidity, crowding, and viral and bacterial challenges are more susceptible to the effects of mycotoxins.

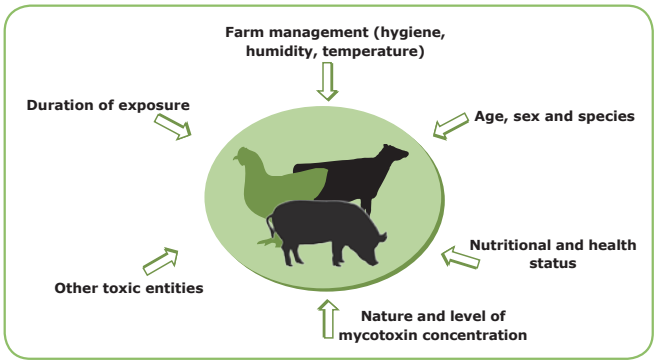

Figure 9 – *Some interacting factors which influence mycotoxin effects in animals*

Low mycotoxin concentration *vs.* feed quality fluctuation

Fungal growth and mycotoxin production vary greatly with the conditions on the field and during storage (temperature, humidity, insects, amongst others) *(Figure 10)* and most of them cannot be

controlled by humans. Therefore, it is impossible to assure a low mycotoxin status of the commodities/feed throughout the whole year. If animals present symptoms, it is very probable be that the highly contaminated feed has already been consumed some time earlier, so by the time the representative sample is sent to the lab and results are available, these might no longer reflect the contamination the animals ingested.

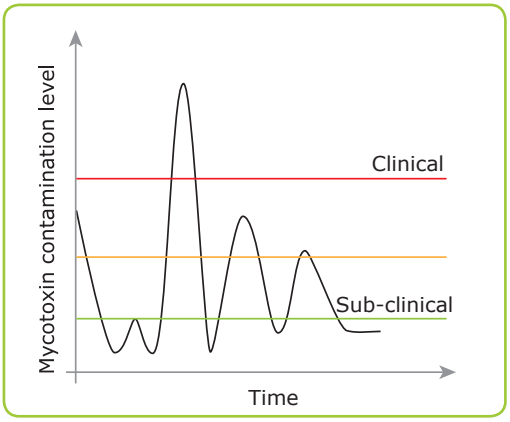

Figure 10 - *Scheme representing usual feed quality fluctuation in a farm. Symptoms may vary from sub-clinical to clinical depending on the mycotoxin level.*

As a summary, before blaming mycotoxins for problems in a farm:

- A careful study and diagnosis must be made, taking into attention that there might be different etiological agents for the same symptom.
- Mycotoxin analysis of a representative sample of feed components or finished feed should be performed.

After confirmation of the presence of mycotoxins with the adequate technique and if only low levels are found, it is crucial to consider:

- Mycotoxins may interact amongst themselves and their individual effects are increased.
- Several factors may interact and therefore increase the susceptibility of animals to mycotoxins.
- Feed quality has great variations within the year; therefore low contamination levels at one particular period most probably will not reflect the situation throughout the whole year.

All in all, routine analyses of commodities allow people to understand the risk incurred throughout the year, or in other words, which periods of the year are more critical in terms of mycotoxin contamination and therefore require a proactive mycotoxin risk management.

References available upon request!

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