

How can we go further in managing the mycotoxins challenge in poultry?

Mycotoxins are secondary metabolites of moulds that can contaminate a wide variety of crops, such as cereals. In agriculture, their control is considered as a huge challenge because of their adverse effects on humans and animals. Although more than 400 mycotoxins have been identified only a few of them are of importance and considered as one of the main problems in the poultry industry.

by Dr Michel Magnin,
Scientific Advisor, MiXscience, France.
www.mixscience.eu

Like in other parts of the world, the European Union (EU) applies maximum levels for specific mycotoxins (aflatoxin B1, aflatoxin B1, sclerotia of *Claviceps* – ergot) in cereals and feed for poultry, in order to protect both animals and human consumers.

In 2006, several recommendations were published by EFSA regarding fumonisins B1+B2, Ochratoxin A, zearalenone, deoxynivalenol (DON) and since 2013 references are also available for the T2 toxin and its metabolite HT2. Such guidelines are very useful for limiting the exposure of birds to the toxic effects of mycotoxins but probably not sufficient.

Evaluation of the risk

It is important to understand why the list of molecules with regulatory limits or with maximum tolerated levels appears so limited compared to the 40-50 molecules (mycotoxins or their metabolites) which can be easily quantified by a well-equipped laboratory. When experts are consulted and have to propose contamination limits, they need well-established data and results of in vivo experiments found in the scientific literature.

Unfortunately these data are not available or not consistent enough for many mycotoxins which were recently studied or discovered, such as enniatins, beauvericin A, aurofusarin, or for which the analytical methods were improved, such as for some

Mycotoxins	Points of vigilance
Aflatoxin B1 (AFB1) + other aflatoxins + sterigmatocystine	Very high toxicity for ducks
T2 toxin + metabolites (including HT2)	Include MAS and DAS?
Deoxynivalenol (DON) + metabolites	Lower the value (<2000?)
Zearalenone + metabolites	
Fumonisin B1 and B2 (FB1 + FB2)	Lower the value for all poultry (10ppm?) and for ducks during fatty liver productions (<5ppm?)
Ochratoxin A + other ochratoxins	None
Alkaloids of ergot	Lower the value <0.5g/kg?

Table 1. Possible evolutions for the risk evaluation for mycotoxins with regulated or recommended values.

T2 or zearalenone metabolites (higher analytical sensitivity and lower quantification limits).

The relatively limited analytical tools 20 or 30 years ago, partially reduce the interest and the conclusions of relatively old toxicology studies because both identification and quantification of the global birds' exposure are difficult to estimate. For instance, it could be considered that it is the case for mono-, di- and tri-acetoxyscirpenol, cyclopiazonic acid, moniliformin, citrinin.

However, we have to take into account and evaluate all available data in order to decide if some raw material or feed could interfere with health or performance of poultry. In that way, Magnin et al (2015) recently discussed some possible evolution for the regulated or recommended limits applied in EU since 2013.

The proposals are summarised in Table 1; we have also mentioned some particular 'points of vigilance' after the publication of recent experimental studies or observations on mycotoxins in poultry. The interaction of DON and its metabolites or of fumonisin B1 with some functions or characteristics of the digestive tract (nutrient transport impairment, tight junctions weakness, modification of the mucin layer composition) the toxic effect of *Claviceps sclerotia* contaminated feed in Pekin ducks are data that could lead people to reconsider the contamination limits.

Taking into account not only the main toxin but also its main metabolites, presents a dual interest. For instance some DON metabolites (3- and 15- acetyl DON) could be less or more toxic than DON itself with similar and probably cumulative effects; it seems more relevant to consider the sum of the different molecules contents than DON contamination rate alone.

The second interest is that, in old experimental studies, fungal culture media or not purified extracted toxins were generally used by the researchers. These products were probably contaminated not only by the toxin but also by its metabolites; therefore the global contamination is probably more related to the observed effects on animals than DON content alone. The situation is more complex for mycotoxins without regulated or recommended levels in poultry feeds, i.e. Monoacetoxyscirpenol (MAS) or Diacetoxyscirpenol (DAS).

As discussed previously, few scientific data are available. In Table 2 an evaluation scale is proposed for some toxins which are regularly analysed.

Measurement

The measurement of the real poultry exposure to mycotoxins hazard is a critical step starting with raw material or feed

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sampling due to the non-gaussian distribution of the toxin. Statistical methods exist mainly for official controls; practically there is no optimal solution.

A second difficulty is due to the improvement in the accuracy of analytical methods. ELISA analytical kits allow a large number of samples to be screened quickly and an easy survey of the quality of cereals and byproducts which are the main sources of mycotoxin contamination.

Unfortunately, the number of toxins, the types of matrix, the range of quantification are often very limited.

On the other hand, high performance liquid chromatography coupled to mass spectrometry is highly sensitive and quantifies easily a wide spectrum of toxins.

Guerre (2016) highlights that, due to the lower detection limits, the number of positive samples increases in the different surveys but it only reveals that contamination is quite common and not that the risk of mycotoxicosis increased.

Another consequence is that multi-contamination of raw materials appears as the most common situation. As reported by Speijers and Speijers (2004), it is quite difficult to predict the nature – additive, synergistic or antagonistic – of the effects of different mycotoxins when they are absorbed simultaneously by the birds. All figures are possible but only few combinations were studied. In practice, one must consider each toxin individually and compare the observed clinical or biological signs with those already described.

Any modification of the classical clinical picture could be due to a possible interaction among the different toxins but also to a change in animal response.

Management

Acute mycotoxin toxicity is generally not relevant for livestock and especially for poultry for which the median lethal doses (LD50) are relatively high, even for the most toxic molecules. For instance LD50 of aflatoxin B1 and ochratoxin A are around 0.5mg/kg of bodyweight in ducks. This

Mycotoxins	Evaluation scale
Acetoxyscirpenols (MAS +DAS)	+++
Nivalenol	+
Fusarenone X	+
Moniliformin	+
Tenuazonic acid	++
Cyclopiazonic acid	++
Fusaric acid	-
Citrinin	-
Trichothecenes group D (Roridine A, verrucarin A)	-
Patulin	-
Tremorgenic toxins (Penitrem A, verrucologen)	-
Beauvericin, enniatins, fusaproliferin	++

Table 2. Possible risk evaluations for mycotoxins without EU regulated or recommended values (+++ <0.05ppm, ++ <1ppm; + <10ppm, - >10ppm).

means that feed has to be contaminated with more than 6mg/kg of toxin to cause death of birds, which rarely occurs.

Repeated exposure to mycotoxins is a general figure but particular attention has to be paid to factors related to species and production stage, in the sensitivity to various toxins. Ducklings, adult ducks and turkeys are the most sensitive; while chickens, hens and quails are more resistant. More generally, young birds are more exposed to the effects of mycotoxins.

Fig. 1 shows an example of raw materials composition in the different phases of a feeding program.

Due to the evolution of the protein/energy ratio with older age the sum of cereals (wheat, corn) and their byproducts (distiller's dried grains) which are the most contaminated materials increases. A simulation of the exposure of turkeys was done with the following current on DON contamination levels: 500µg/kg for corn,

5000µg/kg for wheat and 8000µg/kg for DDGS. Fig. 2 illustrates the daily toxin intake related to the bodyweight. This simulation highlights the higher exposure of young turkeys (about +30%) compared to older turkeys.

This also reminds us that the first keys of toxin exposure management are the selection of raw materials and the feed composition. Cleaning, using airing or sieving could be interesting to easily eliminate a part of the toxins mainly present in dust or on the surface of the hulls. Recent studies report positive results with ozone decontamination of cereals containing high levels of different mycotoxins like T2 toxin or zearalenone, but results were more contradictory for DON.

Separating highly contaminated cereals from less contaminated products could allow the best quality in starter feeds to be introduced, which is the most critical period with the highest risk of exposure, and to use the poorest quality in feeds for finishing broilers or layers.

If a quick and accurate evaluation of the contamination level is possible by using rapid quantification tests, it is possible to formulate the feeding program with targeted maxi levels of toxins; for instance, targeted levels of DON could be 1000, 2000 and 5000µg/kg in starter, grower and finisher broiler feeds respectively.

Is it possible to manage the nutrient composition of feed for a better resistance of birds to mycotoxins exposure?

Some studies have shown positive effects of the modification of nutrient composition when feed is contaminated by mycotoxins. When methionine level is increased by 0.1% an improvement of biological and physiological responses of broilers to 250µg of aflatoxin B1/kg of feed was observed by Mamta Sharma et al (2014).

A similar result was reported by Ram Singh et al (2013) with broilers receiving a feed containing 1000µg/kg of aflatoxin B1 and 0.05% of supplementary methionine. Sapkota et al (2004) previously observed that methionine-hydroxy-analog (MHA; 0.6-1.6%) 300µg/kg of aflatoxin B1 in the feed.

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Fig. 1. Example of typical raw materials composition for turkey feeds in relation to their age (days).

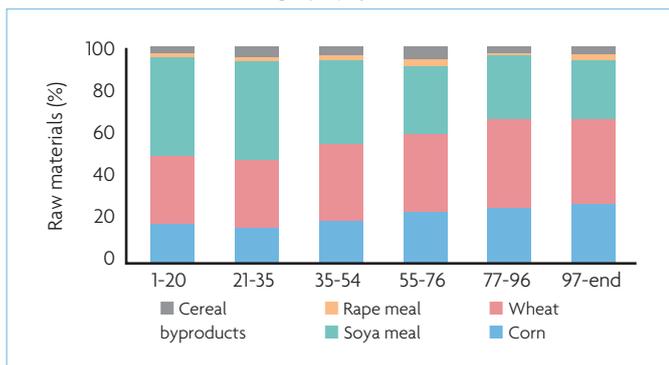
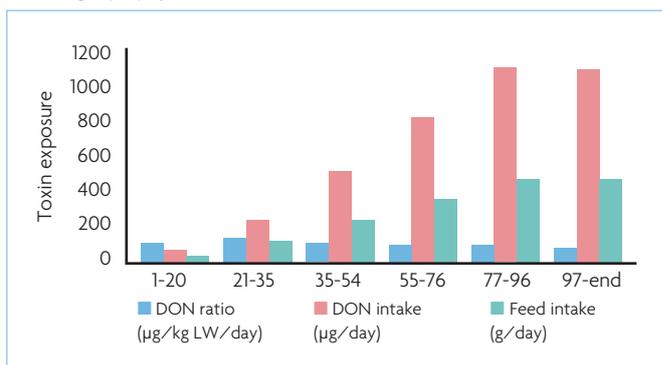


Fig. 2. An example of toxin exposure of turkeys in relation to their age (days).



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Kurkure et al (2004) showed that added levels of vitamin E and selenium (500mg and 100µg/kg of feed respectively) improved the performance and the immune response of broilers receiving a feed contaminated with 1000µg/kg of ochratoxin A from day old to six weeks of age.

The addition of 200mg of vitamin E and 1g of L carnitine per kg of feed either alone or combined, improved haematological and biological parameters in cockerels receiving 1000µg/kg of ochratoxin A in the feed from three days of age; it was not the case for 2000µg/kg of ochratoxin A.

Mycotoxin detoxification

The detoxification of mycotoxins, also sometimes referred as biotransformation, is a particular way of allowing the transformation of toxic molecules into less or no toxic metabolites which can be eliminated, before the absorption in the gut, in the faeces or through bile or urine if the metabolites are quickly absorbed.

Different types of enzymes appear as key tools for the detoxification of trichothecenes: de-epoxidases, acetylases and de-acetylases, oxidases.

Many micro-organisms are regularly identified for their ability to detoxify different mycotoxins of interest.

There have been numerous studies on the transformation of trichothecenes by micro-organisms (strains of Eubacterium, Butyrivibrio, Selenomonas) isolated from rumen fluid but also from the environment.

Cserhati et al (2013) screened strains of Rhodococcus for their ability to transform aflatoxin B1, T2 toxin and zearalenone. Abrunhosa et al (2014) isolated a strain of Pediococcus parvulus able to detoxify ochratoxin A, while Pérez et al (2013) found different strains of lactobacillus detoxifying alternariol and alternuene, two toxins of Alternaria fungi.

Bacillus bacteria seem to be a type of micro-organism highly efficient against mycotoxins, either by a detoxification

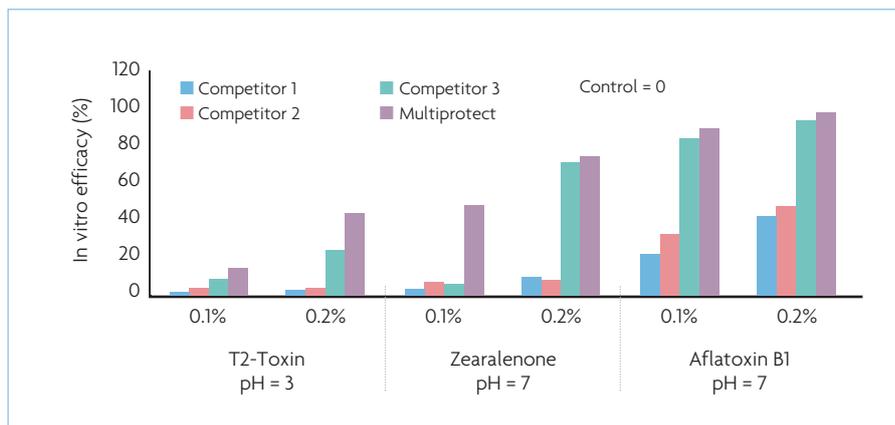


Fig. 3. In vitro efficacy (%) of commercial binders on different mycotoxins (T2-toxin, zearalenone and aflatoxin B1) at pH 3 and pH 7.

process (i.e. Bacillus amyloliquefaciens with a carboxypeptidase) or by a binding capacity.

Mycotoxin adsorption

The best known and currently applied method to prevent animals from the effects of mycotoxins is adsorption or 'binding'. The 'binder' is generally a clay (smectite or bentonite, zeolite); sometimes yeast cell walls or yeast cell wall extracts are used.

Some combinations of these products and the addition of algae are also possible. Exact mechanisms are often not well known. For clays, the adsorption is linked with cationic charges at the external and interlayer surfaces and in the layers.

Adsorption capacity of clays to bind different mycotoxins is easy to test in vitro. Some protocols are recommended by EFSA in EU like isotherms approach. Very good sorption results are obtained with planar molecules like aflatoxin B1. It is more difficult to find a clay able to bind significant quantities of several toxins.

Fig. 3 points out the results of the in vitro binding of aflatoxin B1, zearalenone and T2 toxin at pH 3 and 7 using different commercial products.

As demonstrated several times,

Multiprotect, a combination of different ingredients including a specific smectite source also selected to ensure maximal safety, presents the widest binding spectrum.

Bioprotection

Even if it is possible to bind large quantities (40-95%) of different toxins, some of them have no or too low affinity for the binders (i.e. DON or Ochratoxin A).

In that case, it is important to combine a potent binder with some other ingredients like antioxidants (vitamin E, selenium, polyphenols) and/or with gut and liver protectors, as done in Multiprotect.

The cell toxicity of several toxins, particularly Trichothecenes A and B, is quite important on enterocytes, including the secondary impairment of gut tight junctions, as the first defence barrier, and liver cells in charge of the transformation and detoxification. To prevent damage linked with cell toxicity, some other compounds, such as choline, betaine, and carnitine, could be quite useful. ■

References are available from the author on request