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Scientific Expert Panel

Evaluation of the exposure to mycotoxins on animal health and welfare

1. Introduction

This recommendation document is part of a set of recommendation documents developed by the IFIF Scientific Expert Panel and the IFIF Working Group (WG) on' Nutritional Innovation to Promote Animal Health'.

The IFIF WG was launched in 2017 with the objective to have '*animal nutrition solutions contributing to animal health and animal wellbeing* ***scientifically recognized, clearly understood,*** *and benefit from a proper regulatory* ***framework*** *to be valorized and implemented*'.

Nutritional solutions, now called nutritional strategies are aimed to support the concept of animal adequate nutrition.

Adequate nutrition is defined as '*the oral intake of animals of adequate levels of nutrients, substances, microorganisms, and other feed constituents, considering their combination and presentation, necessary to fulfill functions related to their physiological states, including the expression of most normal behavior, and their resilience capabilities to cope with stressors of various type encountered in appropriate husbandry conditions.'* Furthermore, the way to achieve adequate nutrition is described as follows:

- *Optimization of feed composition, manufacturing, presentation, and delivery to animals,*
- *Minimization of the exposure of animals to stressors in feeds,*
- *Coverage of the animal's requirements for maintenance, activity, growth, production, and reproduction,*
- *Support of digestion and physiological functions, body systems, and behavioral expression.*

The purposes of these recommendation documents are to provide:

- The developers of nutritional strategies with information on the way to evaluate the effectiveness of their strategy for a given purpose,
- The evaluation bodies in the different jurisdictions with an approach for the evaluation of the effectiveness of nutritional strategies for a given purpose.

Each recommendation document will focus on a specific purpose, in relation with microbiome, gut function, exposure control, immunity, physiology, and others.

The present recommendation document is focusing on the evaluation of the role of nutrition to mitigate the impact of exposure to mycotoxins on animal health and welfare.

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2. Scope

This document outlines how exposure to mycotoxins affects animal health and welfare, and hence production. The impact of mycotoxins on health and welfare manifests differently depending on the animal species and the level of contamination. This document is to highlight the link between exposure, health, and welfare, focusing on the main toxins, i.e., aflatoxins (AF), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM) and ochratoxin A (OTA) and T2-toxin. Due to the complexity and the very large number of toxins that affect animal health and welfare, the focus of this document is to highlight the importance of prevention, which starts with knowing and measuring the exposure limits of main toxins present in commonly used feed ingredients and complete feed for different species and classes of animals. The options and limits of mitigation strategies will be briefly covered.

3. Descriptions of endpoints

Mycotoxin control measures include prevention and mitigation. Prevention focuses on using concentration thresholds for key toxins as exposure risks. The most common and well-studied mycotoxins are AF, FUM, ZEN, OTA, DON, and T-2 toxin.

The statement “if there is feed, there will be mycotoxins” is mostly true under the natural cropping and feed storage conditions for the vast majority production settings in animal agriculture across the globe. Under such conditions, only the concentration of toxins and the type of mycotoxins differ (Santos Pereira et al., 2019) but the risk of mycotoxins on animal health, well-being and performance remains a concern.

Mycotoxins are the secondary metabolites of certain fungi. To date more than 1000 different toxins are identified although the function and impact of many remain uncharacterized.

Acute exposure to mycotoxins elicits a diverse range of well-characterized effects on animals, such as reduced reproduction, decreased digestion, and impaired immunity through neurotoxicity, hepatoxicity, and carcinogenicity. However, chronic exposure of animals to low doses of mycotoxins over a prolonged period can slowly erode the immune system and reduce the ability of the animal to defend itself, resulting in significant morbidity issues, loss of production efficiency, and massive economic losses (Xu et al., 2022). The US Food and Drug Administration (FDA 2023) and the European Commission (EC 2006) have recommended maximum levels of mycotoxins in various feed ingredients, considering the As Low As Reasonably Achievable (ALARA) principle.

Mycotoxins affect poultry, pigs, ruminants, and aquaculture species. In pigs and poultry, exposure occurs as a result of feed produced from contaminated cereal grains and their co-products and vegetable protein sources. Poor harvest conditions for crops as well as inappropriate processing and storage of raw materials and feed increase the chance of mycotoxin contamination. Adverse environmental and housing conditions for the animals can exacerbate the severity of mycotoxin impacts on animals. In ruminant animals, such as dairy cows, mycotoxins can reduce feed intake and cause changes in the rumen ecosystem allowing toxins to reach the intestine. The exposure of aquaculture species to mycotoxins arises from the move to replace fish and fishmeal with vegetable protein sources using formulated feed. The risks associated with the raw materials, such as soybean meal, used in pig and poultry feed apply to aquaculture.

Through the adequate nutrition lens, the effect of mycotoxins on farm animals can be seen as the loss of productivity and reduced capacity to cope with other stressors since the affected animals suffer from gut health problems which hinder nutrient digestion and absorption and increase exposure to contaminants. However, the mechanisms by which mycotoxins exhibit their action are complex and varied but in general,

suppressed immunity and susceptibility to disease, damaged gut and poor nutrient absorption, disturbed gut microbiota and gut health, reduced feed intake and suboptimal performance, impaired reproduction, and breeder performance, and altered behavior and welfare issues. These effects do not usually happen in isolation; rather many of them occur in tandem. For instance, immunosuppression is a common mode of action of many mycotoxins (Pierron et al., 2016). Even at low concentrations, mycotoxins weaken immunity and hence make animals more susceptible to infections. The infections are often subclinical without clear symptoms but manifest in increased morbidity and unthrifty, leading to depressed animal performance. Likewise, mycotoxins like AF and OTA exert hepatotoxic effects on animals, whereas other toxins target specific organs, such as the kidneys or the reproductive system, leading to organ dysfunction and reduced animal welfare (Liew and Mohd-Redzwan, 2018). Mycotoxins can also cause leaky gut, which affects nutrient digestion and absorption (Gao et al., 2020). In addition, mycotoxins are thought to alter the gut microbiota through a direct effect on the organisms as well as changes in the digesta composition, such as increased mucus and gut secretion, leading to disturbance to the population equilibrium and potentially inducing dysbiosis (Guerre, 2020). Gut health issues can arise from such changes, which, in turn, may translate into production losses and welfare problems.

The impact of mycotoxins on animal health and welfare can only be measured for a specific parameter, such as weakened immunity, gut damage, or increased susceptibility to disease, by analyzing the relevant indices. Such measurements will still require the analysis of the toxin concentration that led to the manifestation of health or welfare issues.

Prevention, rather than treatment, is preferred but in the complex environment of feed and animal production, it is not possible to fully prevent the exposure of animals to mycotoxins. Therefore, post-harvest control measures are also important to alleviate exposure. The implementation of quality control measures for raw materials and the provision of good storage conditions are essential starting points for controlling the risk of mycotoxin contamination of feed. Moreover, there are numerous mitigation strategies to reduce the risk of animal exposure to elevated levels of mycotoxins (Hamad et al., 2023). The strategies vary from physical decontamination (sieving, drying, thermal treatment and cleaning of ingredients) to the use of chemical agents (alkali, acids, salts, reducing agents, oxidizing agents, and chlorinating agents etc.); from the use of inhibitors (mineral, microbial and cell wall binders) to biotransformation (enzymes or microbes that produce the necessary enzymes); and from ozone treatment to the application of carbon nanoparticles. These interventions, in general, see a reduction in the level of mycotoxins present in feed when animals consume it so that the toxins will not exhibit as large an impact on the animals as they can if left untreated. Indeed, many of these approaches can reduce the concentrations of some particular mycotoxins under set conditions. However, it is important to state that none of the approaches can eliminate or even cause a broad-spectrum reduction in all mycotoxin levels with equal efficiency.

It is generally conceded that the efficacy of these mitigation strategies against mycotoxins is difficult to assess in vivo due largely to factors such as the mycotoxin structure and its mode of action; age, breed, immunological, nutritional and health status of the animal in which the experiment is conducted; environmental and feed processing conditions; and cost and practicality of implementation. Direct assessment, such as the use of biomarkers and in vitro tests, is an advancing area of science and offers hope that rapid, on-farm tests for mycotoxin exposure of animals will become possible in the foreseeable future.

4. Parameters for the evaluation of the endpoint

There is a copious amount of literature showing the levels of the major mycotoxins, AF, DON, ZEN, FUM and OTA in key raw materials and feed and illustrating their health risks to farm animals.

An important measurable parameter for the endpoint to date is the concentration of mycotoxins. This is so despite the concentration threshold for different mycotoxins may differ depending on the criteria for determining the impact of the toxin, such as feed intake, growth depression or nutrient digestibility. In addition, further complexity arises because not only is the concentration of an individual toxin that matters but also the cumulative concentration of different toxins that have an additive, synergetic or antagonist impact on animals. As an example, Xu et al. (2022) summarized the concentration limits for the major mycotoxins set by the European Commission, FDA, and Canadian Food Inspection Agency. Setting such limits requires information on the animal species and their production or growth stage concerned, the type of feed or feed ingredients involved, and of course, the toxin or toxins in question. The information on concentration limits is quite dynamic as knowledge is gain continuously on the impact of mycotoxins on various aspects of animal health, welfare, and productivity. The FAO as well as numerous organisations publish up to date information regarding mycotoxin limits for different countries and jurisdictions across the globe.

However, this parameter - measuring concentrations, has its problems because analysis of concentrations does not guarantee the real impact of mycotoxins on animals. This stems from the fact that bioavailability of toxins can change drastically in different animals under various physiological and disease challenges and therefore the concentration limits set on certain toxins are not always reliable. Further errors associated with sampling from bulk storage and uneven distribution of mycotoxins can also hinder accuracy.

Thus, another parameter of assessment is the use of biomarkers. Indeed, a direct relationship between mycotoxin injection and its toxicity may also be determined using various biomarkers for mycotoxin exposure (Lauwers et al., 2019a,b). Biomarker assays can be done on tissues, blood, or body fluids. Despite their limitations at present, the reliability and ease of use have improved for mycotoxin biomarkers with limited, but increasing, number of commercial assays are available for on-farm application (Lauwers et al., 2019a).

5. Methods to measure the parameters.

For the measurement of mycotoxin concentrations, there are rapid tests and reference methods. Rapid analysis devices/kits are widely available and used, detecting a single mycotoxin or a limited range of mycotoxins in the field. Rapid test kits are immunochromatography-based tests or Enzyme-Linked ImmunoSorbent Assays (ELISA), both methods are using antibodies for the detection of mycotoxins. The main principle of immunoassays is the molecular interaction between target and biorecognition element, i.e., the antibody. So far, antibodies have been regarded with no doubt as the gold-standard recognition element in immunoassays and biosensors. The principle is comparable to the key and lock system (Wang et al., 2022; Singh and Mehta, 2020). Since mycotoxins are small molecules most immunoassays on the market are based on a competitive assay format.

Basically, the format or principle of an ELISA can be explained as follows. After a mycotoxin is extracted from a ground sample with solvent, a portion of the sample extract and a conjugate of an enzyme coupled mycotoxin are mixed and then added to the antibody-coated wells. Any mycotoxin in the sample extract or control standards is allowed to compete with the enzyme-conjugated mycotoxin for the antibody binding

sites. After washing, an enzyme substrate is added, and blue colour develops. The intensity of the colour is inversely proportional to the concentration of mycotoxin in the sample or standard. A solution is then added to stop the enzyme reaction. The method is detailed by Mohammadi et al., (2012).

Mycotoxin test strips are one-step lateral flow immuno-chromatographic assay for the quantitative screening of mycotoxins in samples. The test is based on a competition immunoassay format. The sample extract migrates through the conjugate pad containing mycotoxin specific monoclonal antibodies conjugated to colloidal gold nanoparticles. In the case, if the sample is positive for a specific mycotoxin, the mycotoxin will bind to the nanoparticle-antibody complex (conjugate) and migrate into the detection zone. In the competitive format, the test line consists of immobilized analyte molecules (specific mycotoxin) conjugated to a protein carrier. Any unbound mycotoxin antibody will be captured in the test zone and will form a visible line. As the mycotoxin concentration in the sample increases, the mycotoxin will be captured by the antibody gold particles and reduce the interaction of antibody-gold particle with the test zone analyte, resulting in a reduced signal at the test line. The colour intensity of the line is therefore inversely proportional to the concentration of mycotoxin in the sample. The control line indicates proper flow through the strip and should always be visible in the control zone, irrespective of the presence/absence of mycotoxin. The mycotoxin test strips are measured using a lateral flow device reader, which quantifies the concentration of mycotoxin in the sample.

Rapid test kits are easy to use and usually such assays do not require specialised laboratories. But there are limitations. For instance, lateral flow devices are widely available that allow for rapid on-site measurement of toxins. In fact, there are rapid test kits for the major six mycotoxins. Results can be received within several minutes and the test can be by people who have only some basic trainings. Additionally, some kits are now on the market that use water or buffer for extraction of toxins, negating the need to use organic solvents, making it safer to use and easier to dispose of the used reagents. Furthermore, there is no need to send samples to a laboratory. One limitation of lateral flow devices is that they cannot be used to assess finished feeds or silages. They give no structural information. This is because the different components in the matrix affect the flow in different ways and the accuracy of results.

Near infrared spectrometry (NIRS) for the analysis of mycotoxins is a new trend, relying on the passive detection of mycotoxins through matrix changes in the sample. NIRS detection of mycotoxins is an indirect method for the detection of mycotoxins and is at an early stage of development. But as a fast and non-destructive method, the potential of using NIRS in the field of mycotoxin detection is attracting more attention and resources to improve its accuracy and reliability in the future.

While well-established rapid methods as ELISA (enzyme-linked immunosorbent assay) and lateral flow devices give fast, reliable, and quantitative results at low costs for well-known mycotoxins related to certain feed ingredients, they are less sensitive than reference methods and do not allow measurement of masked and modified mycotoxins as well as more complex matrices such as silage and finished feed.

Most advanced reference analysis methods for concentration analysis are based on high performance liquid chromatography (HPLC) and tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS). LC-MS/MS technique allows simultaneous analysis of multiple mycotoxins at once. As such, it gives a complete picture of mycotoxins, including fungal metabolites, plant toxins and metabolites as well as bacterial toxins and metabolites(Steiner et al., 2020; Sulyok et al., 2020).

For direct assessment of mycotoxin exposure, there are biomarker assays. The field is developing rapidly in concert with the advent in bioinformatics, analytical techniques, and precision instruments. Biomarkers can

be based on biological responses in protein, enzyme or gene expression levels induced by mycotoxins or measurements of mycotoxins themselves or their metabolites in a urine, faeces, serum, tissues, and blood sample. Proper measurement of biomarkers requires in-depth knowledge in sampling, mycotoxin characteristics, production and physiological status of the animal, and the right equipment.

For instance, a biomarker assay using a small amount of blood samples has been developed to test multiple mycotoxins in pigs and chickens (Lauwers et al. 2019a).

6. Conclusions

Prevention is the key approach in determining the impact of mycotoxin exposure of animals, involving measurement of mycotoxin concentrations in feed ingredients. However, post-harvest measures are also important, such as the use of mycotoxin binding agents and potentially the application of enzymes to target specific toxins in complete feed. In terms of measurements, there are commercially available rapid kits specifically designed to screen either a single mycotoxin or a number of key mycotoxins. There are also commercial services offering the detection and quantification of dozens of mycotoxins in a simple run. Such services are based on techniques using LC-MS/MS.

7. Abbreviations

AF: aflatoxins

DON: deoxynivalenol

ZEN: zearalenone

FUM: fumonisins

OTA: ochratoxin A

MS: mass spectroscopy

LS: liquid chromatography

PCR: polymerized chain reaction

8. References

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