MYCOTOXINS IN ANIMAL AND HUMAN NUTRITION

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1.- INTRODUCCIÓN

Mycotoxins are naturally occuring secondary metabolites of low molecular weight with diverse chemical structure, produced by fungi, mainly, of the genera *Aspergillus, Fusarium and Penicillium*. Mycotoxin production may occur during the growth of the crop (e.g., deoxynivalenol, zearalenol, fumonisin) or during the storage of feed or compounded feed (e.g., ochratoxin A, aflatoxin). Field contamination depends strongly on climatic conditions like rainfall, temperature, and humidity. For example, climatic conditions favouring aflatoxin contamination are high temperature, low rainfall and severe drought stress, while Fusarium molds producing toxins like deoxynivalenol (DON) and zearalenone (ZEA) are generally associated with cool and excessively wet growing season (Pinotti et al., 2016) Ochratoxin A producing fungi are usually invading the crops during field growth, but mycotoxin production occurs under unfavorable storage conditions (e.g., temperature, humidity, aeration, insects). While in warmer climates ochratoxin A production is mainly associated with *Aspergillus* species, in temperate climates the main ochratoxin a producing molds are *Penicillium* species (Marquardt and Frohlich, 1992). Climatic conditions and growth requirements of the different fungi are explaining the global distribution of mycotoxins, however, within each region mycotoxin occurrence and contamination level between years changes due to the annual weather fluctuation, often times most factors are beyond human control.

Mycotoxins can be acutely or chronically toxic to humans and farm animals, depending on the kind of toxin, the dose and exposure time. The economic impact of mycotoxins include loss of animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem (Hussein and Brasel, 2001). More than 400 mycotoxins have been identified, but due to their toxic potential, occurence and concentration in food/feed only several are of concern and several countries have set strict regulations on the allowable levels of each mycotoxin in feed and food (Schatzmayr and Streit, 2013). The most important mycotoxins are aflatoxins, ochratoxin A, and fusarium toxins like deoxynivalenol, zearalenone and fumonisins. This review provides information on occurrence, toxicity and metabolism of mycotoxins in animals and humans, and discusses some aspects of management of contaminated feed batches and the possibilities to prevent or to decontaminate mycotoxins in feed and foods.

2.- AFLATOXINS

Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*. These moulds need higher temperature (~ 25 °C) for growth and can be mainly found in tropic and subtropical areas of the world. It has long been classified as a main storage mycotoxin but preharvest aflatoxin contamination occurs, too. Chemically, aflatoxins are difuranocoumarin derivatives. There are four different aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) with AFB_1 being the dominant (~ 80%) one produced. Aflatoxins can be found in cereals (maize, millet, rice, sorghum, wheat) and oilseeds (cottonseed, sunflower, palm kernel, copra) and as well in cassava, tree nuts, and spices at concentrations up to 1 mg. Dietary exposure to AFB_1 in humans is associated with an increased incidence of hepatocellular carcinoma, especially in populations in which chronic infection with hepatitis B virus is a common occurrence (Williams et al., 2004). AFB_1 causes liver damage in all species depending upon the dose and is classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogen (carcinogenic to humans).
Aflatoxins are absorbed from the small intestine and once in liver, aflatoxin B_1 is activated by microsomal enzymes (cytochrome P450 system) to one of two reactive epoxides, AFB exo-8,9-epoxide and AFB endo-8-9-epoxide. While the endo-form of the epoxides is non-mutagenic, the exo-form is both toxic and carcinogenic. The AFB1 exo-8,9-epoxide reacts covalently with DNA to form adducts that presumably account for the cancerogenic effects. The reactive exo- and endo-epoxides may be detoxified by a number of pathways. The principal one is via glutathione S-transferase(GST)-mediated conjugation with reduced glutathione (GSH) to form AFB_1 exo- and endo-epoxide–GSH conjugates (Guengerich et al., 1998). Beside the epoxid forms several other metabolites are formed due to hydroxylation (AFM_1), hydration (AFB_2 alpha) and demethylation (AFQ1). These metabolites and other naturally occurring aflatoxins (G1, B2 and G2) are poorer substrates for epoxidation and, consequently, are less mutagenic, carcinogenic and toxic than AFB_1

(Wild and Turner, 2002) and excreted as such in urine, or in the form of glucuronyl conjugates or GSH conjugated (epoxid form) from bile in feces. However, although hydroxylation to AFM_1 in liver has been regarded as detoxification process, AFM_1 induced liver cancer in experimental animals, and its carcinogenic potency was estimated to be 2 to 10% of that of AFB_1 (Wogan and Paglialunga, 1974). Even though, there is inadequate evidence in humans for the carcinogenicity of AFM_1, the carcinogenicity of AFM_1 was classified by the IARC as a group 2B carcinogen due to its similarity with AFB_1 in structure, activity, and other relevant evidence. This is of importance as AFM_1 has been shown to be transferred into cows milk.

In pigs, symptoms of aflatoxicosis are poor growth and reduced feed effciency (100-400 µg/kg feed) mainly due to a reduced feed intake, liver damage (friable yellow bronze liver) and immunosuppression (400-800 µg/kg), doses higher than 1 mg/kg feed result in icterus, coagulopathy anorexia and death. In sows, reproductive disorders and less vital new born piglets have been found at levels higher than 500 µg/kg feed. Aflatoxins can be transfered in utero from the sow to the fetus and are excreted in the milk and thereby affecting piglet performance (Hussein and Brasel, 2001). Poultry are sensitive to even low levels of AFB1, and among species of agricultural importance, the order of sensitivity is ducks > turkeys > Japanese quail (*Coturnix japonica*) > chickens. Domestic turkeys (*Meleagris gallopavo*) are one of the most sensitive animals known to AFB_1 due, in large part, to a combination of efficient hepatic bioactivation by cytochromes P450, and deficient hepatic glutathione-S-transferase (GST)-mediated detoxification (Monson et al., 2015). In poultry, the same effects as in swine occure, while in studies conducted prior to the 1980s 1.25 mg AFB1/kg diet were discovered as not having any negative effects on broiler performance. More recent studies showed reduction in growth performance at levels of 0.3 mg/kg feed. This may be due to the fact that modern genotype with high performance may be more sensitive to aflatoxin (Yunus et al., 2011). Ruminants are less sensitive to aflatoxin due to partial ruminal degradation of the mycotoxin. However at doses of 100 µg/kg feed, anorexia, depression, drop in milk production, weight loss, lethargy, ascitis, icterus, tenesmus, abdominal pain (animals may stretch or kick at their abdomen), bloody diarrhea, abortion, hepatoencephalopathy, photosensitization and bleeding may occur . The carry over of AFM_1 into the daily milk which ranges from 1-6% of the dose fed and is depending on milk performance is of high concern. Within the EU there is a strict regulation for AFM_1 in milk products (Jouany and Diaz, 2005). The limits for feedstuff of aflatoxins is set at 20 µg/kg and for milk at 50 ng/kg. The limit for feedstuff is much lower than the lowest observed adverse effect level therefore effects on animal productivity are obsolete. Carry over of aflatoxins into meat and eggs has been shown to be negligible. For laying hens a transfer ratio of 5000:1 for eggs has been found (Oliveira et al., 2000).

3.- OCHRATOXIN A

Ochratoxin A (OTA) is a secondary metabolite of some toxigenic storage fungal species of the genera *Aspergillus* and *Penicillium*. Chemically, OTA consists of a dihydrocoumarin moiety linked to phenylalanine via an amide bond. It has a widespread occurrence in foods and feedstuffs, and has been detected in cereal products, pulses, coffee, beer, grape juice, raisins and wine, as well as in cocoa products, nuts and spices. The concentration in feedstuffs ranges from 0-100 µg/kg feed. (Marquardt and Frohlich, 1992). Due to striking similarities between the porcine nephropathy caused by OTA and the Balkan Endemic Nephropathy including the development of urinary tract tumors in humans, OTA is assumed to be a causative agent in the development of the human disease as well, although other risk factors may be involved (Grollmann and Jelakovic, 2007). The IARC has classified OTA as a possible human carcinogen 2B. The mode of action of OTA is not clearly understood yet, and seems to be very complex. Inhibition of protein synthesis and energy production, induction of oxidative stress, DNA adduct formation, as well as apoptosis/necrosis and cell cycle arrest are possibly involved in its toxic action (Kőszegi and Poór, 2016).

The toxicokinetics have been reviewed by Ringot et al. (2006). Absorption of OTA into the systemic circulation is ranging from 40 % in chickens, 56% in rabbits, to 66% in pigs after oral administration. In blood, > 99% of OTA is bound to plasma albumin. This not only facilitates passive absorption of the non-ionized form but also delays elimination of OTA because filtration in the kidney is hindered which will increase the half live of OTA and accumulation of OTA in blood, especially in humans and pigs. However there are differences between species with respect to serum albumin binding affinity of OTA explaining differences in the elimination kinetics. In addition, OTA undergoes enterohepatic circulation as well as reabsorption along the nephron, increasing the exposure time and accumulation of OTA in liver and kidneys. Transfer of OTA via placenta is leading to OTA exposure of foetus. In rats, it has been shown that 0.1% of the dose may be transfered. Furthermore transfer into milk of monogastrics and humans has been shown.

Metabolism of OTA is low. In liver and kidney hydroxylation and glucuronidation of OTA occurs. OTA can be enzymatically hydrolysed (e.g., by carboxypeptidase A, chymotrypsin) to the less toxic ochratoxin α (OTα) and phenylalanine by the bacterial microflora of the hindgut and rumen. This reaction is regarded as a detoxification process, as OTα is much faster excreted compared to OTA.

Pigs are the most sensitive species to ochratoxin. Battacone et al. (2010) estimated that a decrease in weight gain of 12% per mg OTA occurs in pigs. Kidney damage in pigs has been shown at concentration of > 200 µg/kg feed. Typical signs of poultry ochratoxicosis are reduction in weight gain, poor feed conversion, reduced egg production,

poor egg shell quality and nephrotoxicity. Numerous studies in poultry showed that exposure to levels of OTA of 0.5 mg/kg feed altered performance, including decreased feed consumption and growth rate and poor feed conversion efficiency according to Pozzo et al. (2013) concentration of 0.1 mg/kg of OTA has no deleterious effects in broilers.
Ruminants have been shown to be resistant to OTA. *In vitro* and *in vivo* results show extensive ruminal degradation (> 90) of OTA to the less toxic OTα. Only high doses which are not occurring under natural conditions result in signs of toxicity. However, surveillance of milk samples from Scandinavia showed OTA contamination of up to 58 ng/L, indicating that high levels of OTA may have been fed. Some studies indicate that the bioavailability of OTA is increased when high concentrate diets are fed to ruminants, due to changes in rumen pH, which seems to reduce the activity of OTA degrading microflora (Mobashar et al., 2010).

With respect to carry over into meat, milk and eggs following ingestion, it has been shown that the highest concentrations of OTA in slaughter animals can be found in blood serum, followed by kidney, liver, muscle tissue and fat. In contrast, in chicken the highest levels of ochratoxin A were found in the liver followed by the kidneys, whereas levels in other edible tissues are substantially lower (Marquardt and Frohlich, 1992). Transfer into eggs occurs only at high concentration (> 1 mg/kg feed). Carry over of OTA into milk of dairy ewes (Boudra et al., 2013) was about 0.02%. In own studies, with goats (Blank et al., 2011) carry over into milk was about 0.016% and was reduced to 0.008% when sodium bicarbonate was fed. The dietary contribution from food of animal origin is estimated to be less than 5% for humans.

4.- DEOXYNIVALENOL

Deoxynivalenol belongs to the trichothene B group and is frequently find in high concentrations (> to 5 mg/kg feed) in grains like wheat, rye, oats, barley. Similar to other trichothecenes, the primary toxic effect of DON is the inhibition of protein synthesis via binding to the ribosome (Shephard, 2011). Ingestion of highly contaminated feed by animals can lead to acute gastrointestinal symptoms such as vomiting (emesis), feed refusal and bloody diarrhoea. The most common effects of long-term dietary exposure of animals to DON are weight gain suppression, anorexia and altered nutritional efficiency. The acute effects of DON in humans are similar to those in animals. There is no experimental or epidemiological evidence for mutagenic and/or carcinogenic properties of DON (Pestka, 2007).

After rapid absorption, DON can be glucuronidated and sulfonated. Furthermore, in monogastric and ruminants, DON may be metabolized to a deepoxid form which seems to be mainly produced in the gastrointestinal tract by microbes. DON and its metabolites are

eliminated mainly by the kidney, however small parts are excreted via bile and feces (Dänicke and Brezina, 2013).

Pigs are the most sensitive species to DON, according to a literature evaluation of several feeding studies, feed intake decreased by 5% and weight gain by 7%. per mg/kg DON in feed Concentrations of 12 mg/kg feed caused almost complete feed refusal in pigs (Döll and Dänicke 2011, Dersjant-Li et al., 2003). Chicken are less sensitive to DON they respond only to DON at very high concentration of 5-10 mg/kg feed. Beside its growth reducing affect, DON has been shown to alter gastrointestinal structure by a reduction of microvilli which will result in a reduced absorptive area and a decreased sodium dependent glucose uptake, both effects resulting in a decrease in feed efficiency already at concentrations of 1 mg/kg feed. Furthermore, DON has been shown to suppress the antibody response to infectious bronchitis vaccine (IBV) and to Newcastle disease virus (NDV) in broilers (10 mg DON/kg feed) and laying hens (3.5 to 14 mg of DON/kg feed), respectively (Awad et al., 2013).

In ruminants DON is metabolized to deepoxy DON which is regarded as nontoxic metabolite. Due to low recovery of doses fed to cows it has been emphasized that DON may be as well completely degraded in the rumen. There are some reports that feed intake may be reduced at levels of > 2 mg/kg feed. However, in the scientific literature signs of toxicity were only measured at concentrations which occur not under natural conditions (Gallo et al. 2015).

The carry-over of DON into edible tissues of pigs is rather low, especially for muscle and fat, which are most relevant as food derived from pigs. The maximum carry-over factors of 0.0043 and 0.0012 in muscle and back fat, respectively, show clearly that DON was diluted, rather than accumulated, in edible tissues of pigs. The carry-over rates expressed as the ratio between the excretion of DON or deepoxy-DON with milk and DON intake ranged between 0.0001 and 0.0002, and 0.0004 and 0.0024, respectively indicating low or no risk for humans (Dänicke and Brezina, 2013) .

5.- ZEARALENONE

Zearalenone (ZEA) is a mycotoxin produced by several Fusarium species including *F. graminearum* , *F. culmorum, F.equiseti and F. verticillioides,* which grow and invade crops in moist cool field conditions. It is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum and rye throughout various countries of the world and cocontamination with other fusarium toxins, such as deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, nivalenol, 4-acetylnivalenol may occur. Whilst zearalenon is primarily a field contaminant, toxin production may also occur under poor storage condition. (Zinedine et al., 2007)

Zearalenone mainly causes fertility problems in farm animals. Due to its similar structure to estrogen, zearalenone and its metabolites compete with the estrogen for binding to the receptor thereby affecting the reproductive tract and mammary glands. After absorption, ZEA is metabolized to α-zearalenol (α-ZOL) and ß-zearalenol (β -ZOL) by 3α- and 3ß-hydroxy-steroid dehydrogenases. ZEA and its reduced metabolites are conjugated with glucuronic acid and excreted via urine and partly via bile. Enetereohepatic recirulation may occur, however, excretion is very fast (Dänicke and Winkler, 2015).

Studies with subcellular fractions from the livers of pig, sheep, cattle, chicken and rat showed that pig liver was most active in converting ZEA into α-ZOL, whereas chicken liver produced mostly β-ZOL; cattle were poor reducers of ZEA, producing more β-ZOL than α-ZOL (Malekinejad et al., 2006). Since α-ZOL is 3-4 times more active estrogenically than ZEA, the production of this metabolite may contribute significantly to the estrogenic effects observed in animals with ZEA mycotoxicosis. Species differences in receptor binding may also contribute to the high sensitivity of pigs: When the relative binding affinity of α-ZOL to the estrogenic receptors from pig, rat and chicken were compared, porcine exhibited the highest and chicken estrogen receptor the lowest affinity (Fitzpatrick et al., 1989)

Pigs, especially, weaned and prepubertal gilts, are the most sensitive species to ZEA and concentration of 0.060 mg/kg feed have been sufficient to induce clinicals signs such as increase in mammary gland size, hyperemia edematous swelling of the vulva, increase of uterine and ovarian size possibly due to the not fully developed endogenous endocrine system (Döll and Dänicke, 2011). In cyclic sows, adverse effects often occurred only at commercially non-relevant concentrations of more than 1 mg/kg and manifested themselves as anestrous, reduction of uterine, placental and fetal weight with a consequent increase in the number of stillbirths and fewer piglets born alive and or piglets with less vitality (EFSA, 2011). In boars, fed diets with high concentrations (> 5mg/kg feed) of ZEA depressed serum testosterone, induced feminization, and suppressed libido (Diekman and Green, 1992). Poultry are relatively resistant to ZEA. For laying hens concentration of 0-800 mg/kg feed had no effect on laying performance, growth, and egg traits (Allen et al., 1981).

In ruminants, ZEA is converted by the rumen flora into its hydroxy-metabolite α-ZOL (approximately 90%) and to a lesser extend to β-ZOL (Gallo et al., 2015). Although α-ZOL has a higher estrogenic potency compared with the parent ZEA, its lower rate of absorption and its interconversion in the liver to the less potent β-ZOL might account for the low susceptibility of dairy cattle (Jouany and Diaz, 2005). However field observations of ZEA related reproductive problems in ruminants are not uncommon.

Due to the rapid biotransformation and excretion of ZEA in animals, secondary human exposure resulting from meat, milk and eggs is expected to be low, contributing only marginally to the daily intake. According to a literature review by Dänicke and Winkler (2016) the carry over rates varied from 0.004 to 0.295 in livers from various animal species, from 0.008 to 0.05 in bovine milk, from 0 to 0.021 in porcine muscle and amounted to 0.0007 in kidneys of turkey. Eggs from laying hens and adipose tissue from various animal species were virtually free from ZEA and respective metabolites after experimental oral exposure to ZEA.

6.- FUMONISIN

Fumonisins are mycotoxins produced by various Fusarium species, primarily *Fusarium verticillioides* and *F. proliferatum*. These Fusarium species are common fungi associated with maize causing Fusarium kernel rot an important plant disease in hot climates. Fumonisins are mainly formed prior to harvest or during early stage of storage due to required high water activity. The predominant fumonisin in contaminated maize is fumonisin B_1 (FB_1) however several other structural analogs exist, differing in the number and placement of hydroxyl groups (Voss et al., 2007).

Fumonisins are mainly detected in maize and the concentration may reach up to 10 mg/kg however, much higher concentration are possible. Cooccurence of other fusarium toxins (DON, ZEA) is regularly observed. Equids and pigs are the most sensitive to fumonisin intoxication, developing species specific clinical symdroms as equine leukoencephalomalacia and porcine pulmonary oedema. In humans exposure to FB_1 has been associated with primary liver cancer and oesophageal cancer, which are frequent in certain regions of the world (such as Transkei region in South Africa) where maize is staple food. The occurrence of neural tube defect in children in some countries of Central America (such as Mexico and Honduras) has been connected with the consumption of FB_1-contaminated maize-based food. On the basis of the available toxicological evidence, the IARC has classified FB_1 in group 2B as a possible carcinogen to humans (Domijan, 2012).

Fumonisin are poorly absorbed (3-6%). Ruminal biotransformation as well as systemic biotransformation is low. Fumonisins are rapidly predominantly biliary excreted and only minor amounts are excreted via urine. Enterohepatic recycling of fumonisin may occur as bile duct cannulation reduced the elimination time of fumonisins (Voss et al., 2007).

In pigs, fumonisin toxicosis is characterized by pulmonary, cardiovascular and hepatic symptoms, hyperplastic esophagitis, gastric ulceration, hypertrophy of the heart and pulmonary arterie. Lethal dose for porcine pulmonary oedema and hydrothorax has been discovered at feed concentration > 12 mg/kg feed (0.6 mg/kg bw/day). First signs of

changes in the pulmonary system have been observed at 5 mg/kg feed over a period of 8 weeks (Voss et al., 2007). When pigs are exposed to fumonisins they develop also hepatic injury with necrosis and cholestasis. Affected animals become anorexic; they show signs of encephalopathy, loss of body weight, and hepatic nodular hyperplasia. These changes are associated to alterations in serum biochemical parameters, including an increase in circulating bile acids, elevated bilirubin concentrations, and increased values for liver enzymes in serum (Zomborsky-Kovács et al., 2002). In poultry, which are more resistant compared to pigs, signs of fumonisin intoxication (> 50 mg/kg feed) were reduced weight gain and hepatotoxicity. For example in a literature review (Dersjant-Li et al., 2003) per mg/kg of FB_1 pigs decreased weight gain by 0.72%, while for poultry only a decrease of 0.1% were observed.

In ruminants high doses (75 mg/kg feed) have been shown to reduce feed intake and milk production in Jersey cows, in Holstein Steers (94 mg FB1/kg feed; 253 d) mild histological changes in liver and bile and increased serum activities of liver enzymes were induced.

Carry over of fumonisin into edible tissue and milk has been reviewed by EFSA (2005) and considered to be of no major relevance. Feeding 2-3 mg of fumonisin for 24 days to pigs, resulted in low concentrations of kidney and liver (160 ng/g and 65 ng/g) and no fumonisins were detected in muscle and fat tissue. Higher dose of 100 mg FB_1 per animal per day for 5-11 d resulted in low amounts of 26 ng/g in muscle and 2 ng/g of fat tissue. In ruminants, after oral application of 1 or 5 mg/kg body weight no fumonisin were detected in milk. After feeding of 75 mg/kg feed for 14 days, no fumonisin residues were detected in milk. With the use of the isolated bovine udder technique, the carry over from blood-to-milk was estimated to be in the range 0.001-0.004%.

7.- COCONTAMINATION AND MASKED MYCOTOXINS

Most of our knowledge comes from experiments with exposure to a single mycotoxin, however, in practice co-contamination occurs due to the fact that different moulds may infest the plant or that the fungi is able to produce several mycotoxins like for Fusarium species In addition, the use of multiple feed ingredients, contaminated with individual mycotoxins, when combined, may lead to co-occurrence of all the mycotoxins present in the individual ingredients. In most cases, in vivo and in vitro studies showed that co exposure to mycotoxins in feed has additive or synergism effects, which corresponds to the observation that naturally contaminated feedstuffs have in most cases a higher toxicity than the pure mycotoxin. However most studies are *in vitro* studies using cell cultures and if *in vivo* studies were carried out, in these coexposure experiments acute or subacute levels of the mycotoxins were fed, thus, there is a scarcity of data relating to co-contamination and low levels of mycotoxin (CAST, 2003; Smith et al., 2016)

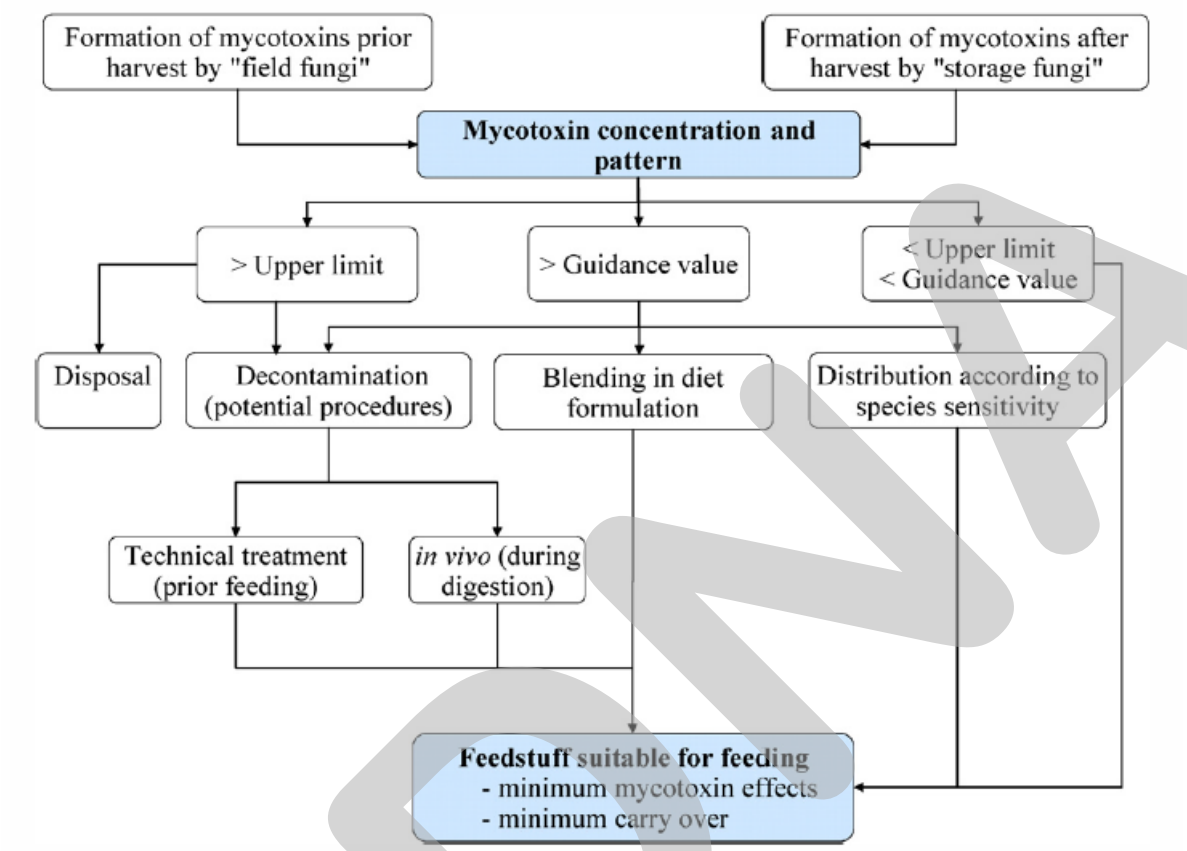
In the last decades, it was found that plants as well metabolize mycotoxins possibly as a detoxification process and that these metabolites are not determined by standard analysis of the parent form. ZEA may be for example glycosylated or sulfated resulting in zearalenone-14-glucoside (ZEA-14-Glc) or zearalenone-14- sulfate (ZEA-14-Sulf) and DON may be glycosylated to DON-3 glucoside (DON-3-Glc). It has been shown in cereals that the relative proportion of ZEA-14-Glc or ZEA-14-Sulf to parent ZEA may be up to 30% (Schneweis et al., 2002). Similar values have been reported for DON-3-Glc in wheat, glucosides may account for up to approximately 20% of the total DON contamination (Berthiller et al., 2013). For fumonisins, it has been shown that a significant proportion of fumonisins escapes routine analysis (Dall'asta et al., 2009) possibly due to covalent bond formation between the tricarboxylic moiety and hydroxyl groups of carbohydrates, fatty acids or the amino groups of amino acids upon heating or by physical entrapment of the mycotoxins into the structure of macromolecular components (such as starch).

In vitro and in vivo data showed that these so called "masked" or "modified" mycotoxins can be potentially reactivated by cleavage of the conjugate and liberation of the native toxin in the digestive tract of animals. For example, ingested DON-3-Glc is nearly completely hydrolyzed in pigs, but only partially absorbed. Results by Nagl et al. (2014) indicate that the cleavage predominantly occurs in the digestive tract. The proportion of urinary excreted metabolites after oral DON-3-Glc administration was reduced by a factor of 2, indicating a lower bioavailability of DON-3-Glc in comparison to DON. A recent risk evaluation by EFSA (2014) concluded that for farm animal species and pets the exposure to the sum of modified and parent toxins does not exceed the lowest observed adverse effects level. However, for humans the risk evaluation revealed that combined exposure to parent and modified mycotoxins for high consumers (95^{th} percentile) may exceed the tolerable daily intake of zearalenone 2.2 fold in humans and for fumonisin the provisional maximal tolerable dietary intake for children 2.5-3 fold.

8.- MANAGEMENT OF MYCOTOXIN CONTAMINATED FEEDSTUFFS

As mycotoxin contamination of feed is unavoidable, the question arises how to handle contaminated batches at farm or feed mill level (Figure 1). For this decision the legal limits and guidance values for feedstuffs within the EU should be taken into account. After analysis of the feed, it has to be decided if the mycotoxin levels exceed upper limits (AFB1, ergot in unground cereal grains) or guidance values for critical concentrations (DON, ZEA, OTA, Fumonisin), which could cause adverse effects on the health and performance of animals or result in unacceptable mycotoxin residues in food of animal origin. If the upper legal limit of the feedstuff is exceeded, only disposal or subjection to a different use (e.g., ethanol production) rather than feeding can be considered.

Figure 1.- Principles of mycotoxin management in animal nutrition (Döll and Dänicke, 2011)



However, mycotoxin concentrations higher than guidance values (below legal limits) allow further options besides decontamination; e.g., such contaminated feedstuffs can be fed according to the species-specific susceptibility which is reflected by the differences in the corresponding guidance values (e.g., pigs *vs* ruminants), however, this is only an option for non-specialized animal farms. Another option would be to include contaminated feedstuffs only up to the limit that the guidance values are not exceeded in the final complete feed. However, the availability of uncontaminated feedstuffs may be low in some years, especially farmers relying on feeding their own cereal grains, might have restrictions in blending when a high contamination level and a high proportion of the concerned cereal grain have to be achieved. Thus, prevention and the need for developing efficient decontamination procedures for mycotoxins is highly demanded.

9.- PREVENTION AND DECONTAMINATION

Agricultural practices have been shown to reduce mould infestation and mycotoxin production in the field. For example, as summarized by Jouany (2007) the risk for Fusarium infections and mycotoxin production in the field can be reduced by crop rotation (avoiding maize as the previous crop); avoiding intense rotations of Fusarium fungi host

crops (maize, wheat, barley and oats), deep tillage (ploughing under or removing of harvest residues), choice of resistant/less susceptible varieties/hybrids, fungicid application at proper time, avoiding over/under nutrition after harvest, and delay of harvest beyond the use-specific maturity date. In some areas modelling of mycotoxin risk at the field levels have been developed. Such models could assist farmers in controlling mycotoxin contamination through agro-management (e.g., timing of fungicide application) at an earlier stage. Field management practices that reduce the risk of aflatoxin development include use of resistant varieties, crop rotation, well-timed planting, weed control, pest control (especially control of insect pests) and avoiding drought and nutritional stress through fertilization and irrigation. Measures to stop the infection process by controlling the aflatoxin causing fungi in the field are achieved through use of pesticides and atoxigenic fungi to competitively displace toxigenic fungi, and timely harvest (Hell and Mutegi, 2011).

To reduce fungi growth and consequently mycotoxin production during storage cereals should be dried in such a manner that damage to the grain is minimized and moisture levels are lower than those required to support mould growth during storage (generally less than 15%). This is necessary to prevent further growth of a number of fungal species that may be present on fresh grains. Aerate the grain by circulation of air through the storage area to maintain proper and uniform temperature levels to avoid condensation. The use of suitable, approved preservative (e.g., organic acids such as propionic acid) may be beneficial (Magan and Aldred, 2007).

Decontamination or deactivation of mycotoxins is a difficult task not only because the substances are relative stable, but also due to the diverse chemical structure of the mycotoxins, one cannot expect to deactivate different mycotoxins with the same method. Most mycotoxins are moderately heat-stable, varying degrees of destruction can be achieved with the use of high-temperature processing. Most of the data indicate that baking, frying, roasting, extrusion and microwave heating caused reductions in mycotoxin levels varying from 50-90% in different food materials. It is important to note, however, that the amount of reduction is highly dependent on cooking conditions, such as temperature, time, water content and pH, as well as the type of mycotoxin and its concentration in the food or feed matrix (Kabak, 2009). For example, as previously indicated for fumonisins the reduction by heat in fumonisin content maybe just an increase in modified fumonisins not determined by standard analysis. Gamma irradiation (> 10 kGy) in high doses have been shown to reduce mycotoxin content in different commodities however the loss of toxicity was only shown for aflatoxin (Calado et al., 2014). However, irradiation and excessive heat are known to have detrimental effects on the nutritional value (amino acids, vitamins, fatty acids) of the product. In addition, in most studies only the analytical disappearance was determined, but not the loss of toxicity after treatment.

Cereals processing before milling (cleaning, gravity separation washing, sorting, debranning) reduces the mycotoxin amount in flour for human consumptions but increase the mycotoxin content in the byproducts destined for animal feed. The high mycotoxin repartitioning in byproducts may indicate a higher concentration of toxins in the outer part of the kernel. For products from bioethanol production like dried distillers grains enrichment of most mycotoxins of up to 3.0-3.5 times compared to the original product has been observed (Pinotti et al., 2016).

Chemical methods, like the ammoniation process have been shown to reduce aflatoxin toxicity and may reduce carry of AFM1 when ammoniated feed was fed to cows, however, ammoniation did not reduce fumonisin toxicity in spite of a 45% reduction in FB_1 content (Scott, 1998). Treatment of DON contaminated feed with sodium bisulfite has been shown to convert DON into DON-sulfonates and to reduce toxic effects of DON in pigs, however, the DON sulfonates have been shown to be unstable in the presence of alkali, which will lead to reformation of DON (Dänicke et al., 2005). However, most of the chemical methods are impractical and do not generally meet the requirements for ideal detoxifying agents, especially as regards to safety and palatability.

However, methods using an additive which is simply mixed to the feed are more practical. Inert adsorbents (clays, yeast cell wall, activated carbon) which prevent absorption in the gastrointestinal tract of the animals and increase elimination of the mycotoxin via feces have been extensively tested. The efficacy of the adsorption appears to depend on the chemical structure of both the adsorbent and the mycotoxin. The most important feature for adsorption is the physical structure of the adsorbent, *i.e.* the total charge and charge distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbed mycotoxins, like polarity, solubility, shape and charge distribution, also play a significant role 1]. Numerous studies have shown that clay (bentonite) are very efficacy in binding aflatoxin and reducing its toxicity and carry over, but not for other mycotoxins. At the time, there is only one binding agent registered within the EU as feed additive for the reduction of the contamination of feed by AFB_1 for ruminants, pigs and poultry. Nevertheless, some of these adsorbent have been reported to bind other nutrients, such as vitamins and minerals, and consequently impairs the nutritional value of feed (DeVreese et al., 2013).

Microorganisms (bacteria, fungi, yeasts) or specific enzymes have the ability to degrade mycotoxins to nontoxic metabolites. This is underlined by the low sensitivity of ruminants to some mycotoxins (e.g., DON, OTA) mainly due to extensive microbial degradation to non toxic metabolites. As feed additives with so called biotransforming action, a microorganism strain DSM 11798 of the Coriobacteriaceae family which counteracts DON toxicity by de-epoxidation (EFSA FEEDAP Panel, 2013) and a fumonisin esterase which fully or partial hydrolyse FB_1 (EFSA FEEDAP Panel, 2014) have been registered for the use pigs.

As reviewed by Surai and Dvorska (2005), numerous studies have shown that increasing the levels of selenium, methionine, carotenoids, and vitamin supplementation in food/feed can be beneficial in reducing adverse effects of mycotoxins (e.g., reducing oxidative stress caused by mycotoxins). The addition of such feed additives may be advisable to use as a therapeutic treatments, but cannot be regarded as safe, as bioavailability and carry over of the mycotoxin remains unchanged.

10.- CONCLUSIONS

Mycotoxins are still a constant threat to animal and humans, prevention of mycotoxin contamination, monitoring, surveillance and legislation can reduce the exposure to animal and humans to mycotoxins but no complete eradication can be achieved. In animal nutrition, more research is needed how low mycotoxin concentrations and co-contamination affects animal performance, especially in pig production as pigs are the most mycotoxin sensitive species. With respect to decontamination methods more practicable and inexpensive procedures are needed and as well their efficacy when these methods are combined or applied simultaneously. With respect to consumer protection mycotoxins in animal products are of no concern, the contribution of animal products to dietary mycotoxin intake in comparison to plant derived foods is usually lower than 5% due to their low carry over into animal products.

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