Scientific Report on the sensitivity and tolerance of some meat producing animal species to aflatoxinB_1 and on the possible transfer of the toxin to their products.

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Levels of aflatoxin B_1 in feed and effects on health of food producing animals

Aflatoxin B1 (AFB1) is a secondary metabolite produced by some strains of *Aspergillusflavus*and *Aspergillusparasiticus*that are microscopic fungi ('molds') invading, under favorable conditions of environment and substrate, agricultural products such as cereals and oilseeds.

AFB1 is particularly toxic because one of its metabolites, mainly produced in liver, is able to bind to cellular macromolecules (proteins, nucleic acids) and impair their function. Relatively low doses of this substance are in fact able to induce cellular necrosis and carcinogenesis. Consequently, as a precaution, maximum tolerance limits have been established also in Europe, not only in food for human consumption (Commission Regulation EU No 165/2010) but also in various raw materials intended for the manufacture of feeds for animals. In corn this limit has been set at 0.02 mg/kg (Commission Directive 2003/100/EC).

Regarding the effects on animal health and production AFB_1follows, as any toxic substance, the dose/response rule. This means that very low AFB_1 concentrations in feed cause very mild or negligible effects, while higher concentrations resultin progressively more serious effects. However, the response to a given concentration of AFB_1 in feed vary considerably based on the exposed animalspecies. This variability concerns also food-producing animal species, as a number of studies conducted at the turn of the seventies have shown. In these studies, feeds containing known concentrations of AFB_1 were administered to the animals for relatively long periods (weeks/months), and their performances and health conditions were compared to those of control groups not exposed to AFB_1.

In particular, a study presented by Gagné*et al*. (1968) tested various levels of AFB_1 in the diet of swine, exposing the animal from weaning to slaughter age, and showed that 0.233 mg/kg of AFB_1 in feed did not produce either effectson zootechnical performances or histopathological lesions. Some biochemical parameters (alkaline phosphatase, urea) were slightly altered instead, also in swine receiving 0.051 mg/kg of AFB_1in the diet.Histopathological lesions were detected only in groups exposed to AFB_1 concentrations of 0.615 mg/kg or higher.A study conducted by Arafa*et al*. (1981) in poultry has shown the different sensitivity of various bird species. Chickens were relatively resistant toAFB_1as opposed to turkeys and ducks, which are notoriously sensitive, especially when young. Indeed, exposition to 0.700 mg/kg of AFB_1in the dietdid not produce any effect in chickens while significantly reducing growth and causing some mortalityin poults and ducklings. In another study focused on hepatic lesions in poultry (Coker, 1979) the different sensitivity of the various species was confirmed.Ducks developed lesions already when exposed to 0.030 mg/kg of AFB_1in the diet, turkeys when exposed to 0.300 mg/kg and chickens only when exposed to 0.500 mg/kg. Helferich*et al*. (1986) evaluated the effects in steers of diet containing known concentrations of AFB_1. Two groups of steers exposed to diets containing 0.060 mg/kg and 0.300 mg/kg of AFB_1 , respectively, did not show alteration either of zootechnical performances or hematic components and enzymatic activities. However, in animals exposed to 0.600 mg/kg slight hepatic lesions and mild alterations of transaminases were recorded.

Presumably based on these and other studies carried on at that time, Professor Gary Osweiler published, in his book ‘Toxicology’(1996), the following table, which shows the response of various farm animal species to increasing concentrations of AFB_1in the diet. As can be seen from the table, diets containing up to 0.200 mg/kg of AFB1 are well tolerated by broiler chicks, feedlot cattle and adult swine.

TABLE 29-2. Relative Response of Animals to Dietary Aflatoxins*

Concentration (ppb)	Species	Violative residues	Decreased performance	Impaired immunity	Hepatic lesions	Clinical illness
50	Dairy cattle	+	−	−	−	−
100	Broiler chicks	−	−	−	−	−
	Feedlot cattle	−	−	−	−	−
	Piglets	−	−	−	−	−
	Adult swine	−	−	−	−	−
200	Broiler chicks	−	−	−	−	−
	Calves	−	±	−	−	−
	Feedlot cattle	−	−	−	−	−
	Piglets	−	±	±	±	−
	Adult swine	−	−	−	−	−
	Turkey poults	−	+	+	++	−
400	Broiler chicks	±	±	+	+	−
	Calves	−	±	−	−	−
	Feedlot cattle	+	−	−	−	−
	Piglets	+	±	±	+	−
	Adult swine	+	±	±	+	−
	Turkey poults	+	+	+	++	+
500	Broiler chicks	+	+	+	+	−
	Calves	+	+	±	+	±
	Feedlot cattle	+	−	−	±	−
	Piglets	+	++	+	++	−
	Adult swine	+	+	±	±	−
	Turkey poults	+	+	+	++	−
750	Broiler chicks	+	+	+	++	±
	Calves	+	+	+	+	±
	Feedlot cattle	+	±	±	+	−
	Piglets	+	++	+	++	±
	Adult swine	+	+	±	+	−
	Turkey poults	+	+	+	++	+
1000	All	+	+	+	++	+

*Estimates are based on extended feeding times (more than 2 weeks)
− = no effect; ± = variable effect; + = affected; ++ = severely affected.

The limit established for corn (0.02 mg/kg of AFB_1) is identical to the one for complete feedingstuffs for cattle (except dairy animals) and for pigs and poultry (except young animals), and it does not represent a safety limit threshold for cattle because it has been set up based on the ALARA (As Low as Reasonably Achievable) principle, which is usually applied to hazardous contaminants. In short, it was presumably considered that by applying the proper standards of cultivation and prevention, AFB_1 contamination of corn could be kept below 0.02 mg/kg and therefore, regardless of AFB_1 effects on animal health, there was no reason to tolerate higher concentrations. Also in US, at least in the first instance, the same conclusionwas reached. Indeed, in 1969, a limit of 0.02 mg/kg was established by the Food and Drug Administration (FDA) not only for corn but also for any ingredient and complete feedingstuff, taking into account the limits of the analytical methods and the goal of reducing as much as possible the exposition of man and animals to AFB_1. Afterwards, based on animal feeding studies conducted in the 1970's and 1980's, FDA

revised its action level in 1982 to 0.300 mg/kg for aflatoxins in cottonseed meal intended for use as a feed ingredient for beef cattle, swine, and poultry; in 1989 to varying levels for corn intended for use as a feed ingredient for subgroups of the same animals. In 1990, FDA issued guidance that aflatoxins in peanut products (i.e., peanuts, peanut meal, peanut hulls, peanut skins, and ground peanut hay) intended for use as a feed ingredient are no more toxic to these same subgroups of animals than is aflatoxin in corn.

Consequently, FDA action levels have been for many years the following:

- 300 µg/kg for corn and peanut products intended for finishing (i.e., feedlot) beef cattle;

- 300 µg/kg for cottonseed meal intended for beef cattle, swine, or poultry (regardless of age or breeding status);

- 200 µg/kg for corn or peanut products intended for finishing swine of 100 pounds or greater;

- 100 µg/kg for corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry;

- 20 µg/kg for corn, peanut products, and other animal feeds and feed ingredients, but excluding cottonseed meal, intended for immature animals;

- 20 µg/kg for corn, peanut products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known;

It should be emphasized that if these tolerance levels, after many years of being in place, are still accepted today in the United States, they have not caused problems to the animal health and production. Therefore, indications obtained from experimentations carried on in the seventies are corroborated.

On the basis of the above said information it can be concluded, from the animal health point of view, that also in our territory (at least in 'extraordinary circumstances') some small exceptions to the current limit of AFB_1 contamination of maize may be considered, for a controlled and specific use in feed.What still remains to be clarified is the possible exposure of the consumer to any AFB_1 residues in meat and offal of animals exposed to slightly higher levels of the toxin.

Consumer exposure to residues of aflatoxin in food of animal origin

Animal feeding studies conducted in the 1970's and 1980's gave also some information regarding the transfer of aflatoxin to products of animal origin. The paper of Keyl and Booth (1970) is a review of the experimentations performed until then in the various species and can be taken as a first point of reference.

1) In swine exposed to concentrations up to 0.810 mg/kg of AFB1 in the diet, the analysis of blood, spleen, muscle, fat, liver and kidney did not show any residue of aflatoxin. However, there was no indication of the detection limits of the applied analytical methods.
2) In beef cattle exposed to concentrations up to 0.700 mg/kg of AFB_1 in the diet no residues of aflatoxin were found either in meat or blood. In those exposed to 1.000 mg/kg traces were detected instead, both of AFB1 and its metabolite AFM1, but only in blood samples. These traces were no longer detectable after three days administration of the control ration. Even in this case, there was no indication of the detection limits of the applied analytical methods.
3) In broilers exposed for 8 weeks to 1.600 mg/kg of AFB1 in the diet, no residues of aflatoxin were detected in meat, liver and blood. The detection limits of the analytical methods were, in this case, indicated (3-5 µg/kg).

These data could lead to the conclusion that there are no traces of AFB_1 in meat and offal of swine, broilers and beef cattle exposed to diet containing relevant concentrations of the mycotoxin. However, taking into account the progressive refinement of analytical methods over the years, it is worth to refer also to some slightly more recent publications.

1) Jacobson *et al*. (1975) exposed, for one month, 4 groups of feeder pigs to concentrations of AFB_1 in the diet between 0 and 0.400 mg/kg. They applied an analytical method for the detection of AFB_1 residues in liver, muscle, blood and kidney with a limit of quantification (LOQ) of 0.12 µg/kg. In pigs exposed to 100 mg/kg the maximum average level of AFB_1 was measured in liver and kidney (0.23 µg/kg), where residues of AFM_1 (0.18 µg/kg) were also detected. Levelsof AFB_1 higher than 1 µg/kg were detected only in pigs exposed to the highest concentrations, with a maximum of 3.33 µg/kg in the blood of a subject exposed to 400 mg/kg of AFB_1. In pigs receiving feed containing 100 mg/kg of the toxin, trace of AFB_1 were detected also in blood and muscle (0.19 and 0.17 µg/kg, respectively).

2) Helferich*et al*. (1986) exposed 3 groups of steers to 0.060, 0.300 and 0.600 mg/kg of AFB_1 in the diet, for periods ranging from 2 to 5 months. They measured residues of AFB_1 and AFM_1 in liver, muscle and fat (biopsy samples) applying an analytical method with a limit of detection (LOD) of 0.25µg/kg. AFB_1 and AFM_1were not detected in the muscle or fat of any of the animals. Some variable levels of residues of both toxins were found in liver, with a maximum of 0.62 µg/kg (AFM_1) in steers exposed to 0.060 mg/kg and of 2.76 µg/kg (AFM_1) in steers exposed to 0.600 mg/kg. In biopsy samples no residues of AFB_1were detected after 5 month of exposition to 0.300 mg/kg, thus indicating a metabolic adaptation of the animals and the low tendency of AFB_1 to accumulate.
3) Hussain*et al*. (2010) exposed, for one week, some groups of broiler chickens of different age (7, 14 and 28 days)to very high levels of AFB_1 in the diet (1.600-6.400 mg/kg). They applied an analytical method with a LOD of 0.025 µg/kg to detect residues of AFB_1 in liver and muscle. In groups exposed to the lowest dose, which was still very high, the highest levels of detected residues were 3.60 µg/kg in liver and 1.63 µg/kg in muscle.

Based on these more recent experimentations we can assert that AFB_1 has little tendency to be transferred to the muscle (meat)of exposed animals. Organs like liver and kidney do usually present minimum levels of AFB_1 and/or AFM1 residues, which are proportional to the exposition levels. If concentrations of AFB1 in the diet are kept under 0.100 mg/kg we can expect that in swine, beef cattle and broilers residue levels will be under 1 µg/kg also in liver,notwithstanding thatthis organ, being the main site of AFB1 metabolism, is more prone than others to bring residues of the toxin.

Altogether, these data confirm for aflatoxin the consideration made by Fink-Gremmels (2006) for all mycotoxins, namely that the overall contribution of animal-derived foods remains significantly below the overall exposure level to mycotoxins from plant-derived foods.
In this respect, one may consider that the current European legislation, although in observance of the ALARA principle, has set a tolerance level of 2 µg/kg for AFB_1 in cereals intended for human consumption.

Conclusions

Based on the literature consulted, it is possible to assert the following. The exposition of adult beef cattle, swine or broilers to AFB_1 concentrations in the diet up to 100 µg/kg does not cause damage to the animal health and to their performances, and can generate,mainly in liver and kidney,the presence of very low residues. These residues are presumably below 1 µg/kg, which is half of the current European limit for AFB_1 in cereals intended for human consumption.

Note

Analogous considerations regarding the transfer of AFB_1 to meat and offal can be found in a document(Transfer of aflatoxins to milk, eggs, meat and offal - Opinion No. 009/2013, of 4 March 2013) recently published by the Federal Institute for risk assessment (BfR) of Germany. In that document it is shown that when food producing animals (beef, pigs turkeys and laying hens) are exposed to AFB_1 concentrations up to 0.200 mg/kgin feed,the calculated levels of AFB_1 residuesin edible tissues are well below the national tolerance level of 2 µg/kg [VerordnungüberHöchstmengen an Mykotoxinen in Lebensmitteln (Mykotoxin-Höchstmengenverordung – MHmV) vom 2. Juni 1999, BGBI. I S.1248 (Germangovernmental regulation)]

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