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DETOXIFICATION METHODS OF AFLATOXINS. A REVIEW.

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ABSTRACT

Detoxification of aflatoxin contaminated foods and feeds is a current problem, as aflatoxins are highly carcinogenic and capable of passing unaltered through metabolic processes and accumulating in the tissues (seriously jeopardizing human and animal health). Although numerous detoxification methods have been tested, none seems able to fulfill the efficacy, safety, safeguarding of nutritional elements and costs requisites of a detoxification process. This paper critically reviews the main chemical detoxification methods and the latest approach to the problem using added sorbents capable of adsorbing aflatoxins.

KEY WORDS: Feeds, Foods, Aflatoxin, Toxicity, Detoxification, Sorbents.

INTRODUCTION

Any decontamination process must be technically and economically feasible if it is to be applied practically. The FAO requirements for acceptable decontamination process (1) stipulate that the procedure must:

1. destroy, inactivate or remove aflatoxins;

2. not produce nor leave toxic and/or carcinogenic/mutagenic residues in the final products or in food products obtained from animals fed on decontaminated feeds;

3. not significantly alter the important technologic properties;

and ideally,

4. destroy fungal spores and mycelium that could proliferate and produce new toxins under favourable conditions.

Analogous requirements have been set down in France and U.S.A., however the F.D.A. (2) requires additional data on the impact of the process to the environment. Many detoxification methods of aflatoxin contaminated foodstuffs have been recommended and include: mechanical separation of contaminated seeds, heat treatment, extraction using solvents, detoxification using chemical agents and added sorbents. This paper reviews chemical treatments and added sorbents.

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Treatment with Ammonia

Treatment with ammonia in the gaseous phase, in solution, or with substances capable of releasing it, achieved optimum results in detoxifying peanut, cotton and corn meals. It is legal in some of the North American states (Arizona, California, Georgia, Alabama) and French technology has been used to build some treatment plants in Senegal and more recently in France.

It has been widely demonstrated that the efficacy of detoxification with ammonia is positively correlated with the quantity used, reaction time, temperature and pressure levels and combination with formaldehyde (3). The mechanism of action induced by ammonia on the aflatoxin B_1 (AFB_1) molecule has been studied extensively and seems to have been clarified. It was observed that the AFB_1 molecular structure is irreversibly altered if exposure to ammonia lasts long enough. In contrast, if exposure is not sufficiently protracted, the molecule can revert to its original state. In fact, some tests aimed at verifying carry-over in dairy cows fed ammonia treated feeds (4,5) revealed percentages of AFB_1 metabolite elimination in milk (i.e. aflatoxin M_1 or AFM_1) between 10-20% compared with AFB_1 ingested. These percentages generally vary from 1-3%. This was explained by examining the detoxification reaction mechanism. The first step (reversible) consists of the opening of aflatoxin cyclic lactone (Fig. 1): in an acid environment (as in the stomach) the balance may shift towards the original products with consequent reformation of AFB_1. In this case the animal would ingest a greater quantity of toxin compared with the quantity revealed analytically, explaining the higher percentage of AFM_1 in the milk.

The products which can trigger of the AFB_1-ammonia reaction have diverse chemical characteristics. This variability may be determined by the presence of substances capable of interfering in the detoxification reaction and giving rise to not easily identified compounds in treated feed. Aflatoxin D_1 (AFD_1) is one of the well known reaction products identified as a non fluorescent phenol with molecular weight (MW) 286 which reveals the absence of the lactone carbonylic functional group with respect to the AFB_1 molecule (6) (Figure 1).

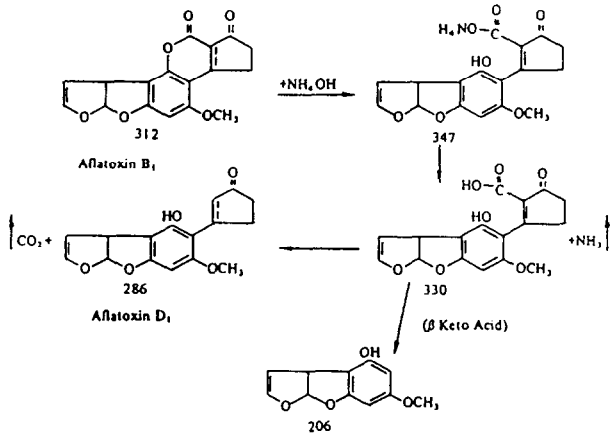

FIG. 1 - Ammoniation of aflatoxin (8).

An analogous compound homogeneous for structure and molecular weight seems to form by reaction with sodium hydroxide at 100° C (7). In addition to the pathway leading to AFD_1 formation, the reaction can follow alternative pathways which can determine formation of other compounds. These include a series of non identified compounds with MW less than 200, a non fluorescent difuran phenol with MW 206 obtained after formation of a β-keto acid (8), a compound

with MW 256 formed by AFD_1 sublimation at temperatures between 220° and 340° C and a non identified compound with MW 236 produced after release of a CO_2 molecule (9).

AFD_1, AFM_1 and the chemical compound with MW 206 were biologically tested in order to evaluate the residual toxicity and mutagenic potential. Tests performed using salmonella microsomes, covalent binding index and chick-embryo development demonstrated that AFD_1 is much less toxic than AFB_1. However, the data reported in the literature vary: 450 fold less (10); 280 fold less (11); 130 fold less (12) and from 2.000 to 20.000 times less (13). In contrast, AFM_1's toxicity and mutagenic potential are similar to those of the original molecule as shown by trials carried out on ducks (14), rats (15) and trout (16), whereas mutagenic potential seems to be reduced in rat kidneys (17). Studies conducted on broilers (18,19,20), laying hens (21) and rats (22,23) fed ammoniated AFB_1-contamined feeds revealed no histologic alterations of target organs and improved performance parameters. A test on dairy cows (24) showed almost total disappearance of AFB_1 carry-over in milk (AFM_1 level was under 0.1 μg/l).

The disadvantages of ammonia treatment are mainly related to the need to build special plants as ammonia corrodes metal and becomes explosive in the air at mixtures over 15% in volume. In addition, some effects on the chemical and qualitative characteristics of the feeds cannot be overlooked, these being the undesirable brown color of the treated feed, the increase in total nitrogen and non protein nitrogen together with a marked reduction in nitrogen solubility and diminished content of some amino acids (cystine, methionine and especially lysine), which is the main drawback of ammonia treatment.

Treatment with Sodium Bisulfite

Sodium bisulfite treatment is a valid AFB_1 detoxification strategy (25,26,27). Although it is less efficacious than ammonia detoxification it overcomes some of the typical disadvantages of ammonia methods and also has much lower costs. In addition, sodium bisulfite is already commonly added to food and drinks, such as wine, fruit juices, jams and dried fruit, where it acts as an enzymatic degradation inhibitor, an antioxidant and bacteriostatic agent. More light needs be shed on the mechanism of action of sodium bisulfite on the AFB_1 molecule. The main reaction product has been isolated and identified as a sulfonate, called 15α-sodium sulfonate or aflatoxin B_1S (AFB_1S) (28,29), which forms by insertion of $NaHSO_3$ at the double bond of furofuranic ring, depriving the AFB_1 molecule of main DNA molecule reaction site, thus reducing mutagenic potential.

Treatment with Calcium Hydroxide

It has been demonstrated that calcium hydroxide is able to reduce contamination levels when used alone (28) or in combination with formaldehyde. A test on peanut meal (31) showed that this association enhanced AFB_1 detoxification efficacy. The elevated efficaciousness of calcium hydroxide combined with formaldehyde has been verified by in vitro tests (32) and on dairy cows (33). Satisfactory results have also been observed in association with monomethylamine (34). Some authors (35,36) have studied the efficacy of nixtamalization, a traditional practice widely used by South American populations to prepare typical corn tortillas consisting of cooking the corn in boiling water supplemented with calcium hydroxide. This type of treatment achieved corn meal detoxification even if it did not reach safe levels for human consumption. Higher doses of calcium hydroxide reduced the typical organoleptic characteristics but furnished significant results concerning G_1 (AFG_1) and G_2 (AFG_2) aflatoxins, whereas B_2 aflatoxin (AFB_2) and particularly AFB_1 were more stable. The validity of cooking in calcium hydroxide has been verified at rising doses (37) which revealed proportionally increasing levels of AFB_1 destruction (up to 46%). However, the studies also showed the reversibility of the reaction in acid environments and the

greater mutagenic potential of the reaction product compared with the original molecule. Only hazy data are available on the chemical modalities of the molecule's neutralization reaction. Although treatments with sodium, potassium or calcium hydroxides determine only slightly lower detoxification percentages than achieved with ammonia treatments, they present the following advantages:

1. Low cost. Calcium hydroxide is the cheapest alkali and is listed in the mineral and chemico-industrial products that may be used in animal feeds.

2. Easy application. It is a powder and so can be readily mixed with the feed to be detoxified. Furthermore, it does not require specially built plants using sophisticated technology.

Treatment with Formaldehyde

Formaldehyde is a compound which is moderately efficacious in attacking and neutralizing the AFB_1 molecule, even if no data on its reactions mechanism are available. Studies showed its enhanced efficacy in association with ammonia (3) and calcium hydroxide (31). In contaminated milk samples addition of 0.5% formaldehyde reduced 1.1 μg AFM_1 to 0.05 μg (38).

Other Treatments

Other substances that seem active against aflatoxin include some oxidants, such as sodium hypochrolite, potassium permanganate, hydrogen peroxide and sodium borate. No important studies have been conducted on these treatments.

Addition of Sorbents

One of the most important approaches aimed at reducing the risk of aflatoxicosis or in limiting decrease in animal performance and toxic metabolite carry-over in milk, meat and eggs, is the use of clays in contaminated feeds to reduce aflatoxin absorption in the intestine. Some in vitro tests (39) showed that various absorbing materials classifiable as aluminas, silicas and aluminosilicates are capable of binding aflatoxin in solution. Extraction using various solvents at different temperatures and pH showed a release which varied in intensity in function of the type of material used. The same authors observed that hydrated sodium calcium aluminosilicates (HSCAS) were particularly efficacious in binding aflatoxin. Definitive information on the molecule AFB_1 fixing modalities cannot be furnished yet, even if chemico-physical adsorption phenomena may occur. The AFB_1 molecule may easily remain imprisoned within the typical complex stratified-reticular structure of the HSCAS. However, the stability of this adsorption in particular pH conditions, such as those in the stomach, should be verified.

Nevertheless, numerous experimental studies have demonstrated the validity of this binding action. Trials conducted on chickens, turkeys and swine fed AFB_1 contaminated feeds and added HSCAS revealed the absence or reduction of typical intoxication phenomena, e.g. increase of the relative weight of vital organs (40,41,42, 43,44,45,46), decrease in body weight or reduced body weight gains and feed conversion (39,41,42,47,48,49,50), brittle bones (51) and metabolite deposition in tissues (52). Some authors (53) obtained successful results by adding HSCAS to contaminated growing lambs' feed. The same authors (54) conducted a detoxification test by adding

HSCAS to dairy cows' feeds characterized by AFB_1 micro contamination level (200 μg AFB_1/Kg) obtaining a significant reduction in the carry-over and confirming the efficacy of the treatment even at the low contamination levels frequently found in practice.

Analogous detoxification trials have been performed using zeolites, bentonites and modified phylloaluminosilicates. A micronized zeolite (55) was tested as an aflatoxin sorbent in feeds for weaning piglets and it induced a marked reduction in mortality rate and increase of feed consumption and body weight. In contrast, a study on dairy cows (56) did not detect any zeolite induced reducing action on carry-over, while a test on broilers of domestic fowl (57) showed the total absence of beneficial effects determined by addition of zeolite.

These contrasting data can be ascribed to the different experimental methods and, above all, to the type of zeolite used. In fact, in synthetic zeolites, as opposed to natural ones, the pore size distribution varies very little and is generally concentrated within a narrow diameter range. If the size of the pores is compatible with those of the aflatoxin molecules (Table 1), conspicuous adsorption occurs. Vice versa, adsorption can be easily nil because no intermediate sized pores are present.

TABLE 1

Molecular Sizes of Aflatoxins (58).

Aflatoxin	Molecular Size (Å)
B_1	5,18
B_2	5,18
G_1	6,50
G_2	6,50

A test on bentonite as an aflatoxin sorbent conducted on dairy cows (59) revealed a 33% carry-over reduction , while in vitro trials on trout feed (60) achieved adsorption of 70% the AFB_1 present in the feed. An in vitro test (61) demonstrated the efficacy of a commercial product (Mycobond) made of chemically modified phylloaluminesilicate combined with multilayered montmorillonite and detected the formation of an inert, stable complex capable of preventing absorption of mycotoxins in the intestine.

CONCLUSIONS

This review furnishes the following conclusions:

Although the main chemical detoxification methods are very efficacious, they do not seem able to fulfill all the requirements, especially those concerning the safety of reaction products and the safeguarding of the nutritional characteristics of the treated foods and feeds. In addition, chemical treatments determine substantial increase in costs which often cannot be met by the breeders.

Addition of sorbents is a simple low cost effective detoxification method which seems to offer good prospectives, even if the vast amount of data available and the contrasting results have not clarified the mechanism of action, nor the materials that can guarantee better results. Therefore, more detailed studies should be performed to detect any possible reactions between the structural characteristics of the sorbents and their adsorption capacity in order to select the most efficacious.

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