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Biotransformation strategies for effective mycotoxin deactivation

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ABSTRACT

Occurrence of mycotoxins is ubiquitous which is why it represents a worldwide problem for the animal industry. Even with the use of prevention techniques in the field or during storage, it is actually impossible to avoid their presence in agricultural commodities. Due to modern analytical methods and thanks to a growing interest in this field of research, more than 300 different mycotoxins have currently been differentiated. The toxicity of different mycotoxins brings serious risks upon humans and animals. Mycotoxicoses are animal or human diseases caused by mycotoxin ingestion, inhalation or skin-contact. In animals, these range from immunosuppression and performance effects to hepatotoxic, nephrotoxic, neurotoxic, dermal, carcinogenic, reproductive, teratogenic and gastro-intestinal effects depending on animal-, environmental- and toxin-related factors. The most applied method for protecting animals against aflatoxicosis is the utilization of clay minerals mixed with feed which are supposed to bind the mycotoxins efficiently in the gastro-intestinal tract. Binders are only very specific for aflatoxins but not for other toxins and that is why a novel strategy to control the problem of mycotoxicoses in animals had to be developed. It is the application of microorganisms capable of biotransforming mycotoxins into non-toxic metabolites. Biotransformation and biodegradation are mycotoxin-specific methods which rely in microorganisms and enzymes' capacity of metabolization or degradation of mycotoxins into less or non-toxic metabolites prior to their absorption in the gastro-intestinal tract. Some microorganisms have shown biotransformation capacity both in vitro and in vivo, representing effective tools for counteracting negative effects of mycotoxins in animal feed.
(Keywords: mycotoxins, biotransformation, BBSH, *Trichosporon mycotoxinivorans*, Fumzyme)

ÖSSZEFOGLALÁS

A mikotoxinok hatástalanítása biotranszformációval
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A mikroszkópikus gombatoxinok megjelenése elkerülhetetlen, s emiatt világszerte problémát jelentenek az állattenyésztésben. A szántóföldön és a tárolás során alkalmazott preventív módszerek ellenére lehetetlen megakadályozni a mikotoxinok jelenlétét az agrártermékekben. A modern analitikai eljárásoknak és a kutatások iránti egyre nagyobb érdeklődésnek köszönhetően, mára már több, mint 300 különböző mikotoxin meghatározása és elkülönítése lehetséges. A különböző gombatoxinok toxicitása komoly kockázati tényezőt jelent humán- és állategészségügyi szempontból is.

A mikotoxikózisok olyan állati vagy humán megbetegedések, amelyek a mikotoxinok megemésztése, belélegzése vagy a velük való bőrkontaktus következtében alakulnak ki. Állatok esetében immunszupresszív, teljesítmény csökkentő, hepatotoxikus, nephrotoxikus, neurotoxikus, bőrkárosító, rákkeltő, szaporodásbiológiai problémákat okozó, teratogenikus, és emésztési zavarokat okozó hatásúak lehetnek, melyek azonban nagyban függnek az állati és környezeti tényezőktől, valamint a mikotoxintól is. Az aflatoxikózis megelőzésére a leggyakrabban alkalmazott módszer az additív agyagásványok használata, amelyek az emésztőtraktusban feltételezhetően hatékonyan kötik meg a toxinokat. Ezek a toxinkötők csak és kizárólag az aflatoxint kötik meg, így a mikotoxinok okozta problémák hatékony megelőzésében új statégia kimunkálása vált szükségessé. Ennek egyik lehetősége olyan biotranszformációra képes mikroorganizmusok alkalmazása, melyek a káros mikotoxinokat nem toxikus metabolitokká átalakítják. A biotranszformáció és a mikotoxinok enzimatikus bontása mikotoxin specifikus módszer, melynek során még a felszívódás előtt kevésbé vagy egyáltalán nem toxikus metabolitok képződnek a káros mikotoxinokból. Néhány mikroorganizmus biotranszformációra képes in vitro és in vivo kísérleti körülmények között, s így ezek hatékonyak lehetnek az állati takarmányokban megjelenő mikotoxinok káros hatásainak lekűzdésében.
(Kulcsszavak: mikotoxinok, biotraszformáció, BBSH, *Trichosporon mycotoxinivorans*, Fumzyme)

INTRODUCTION

Mycotoxins are highly toxic secondary metabolic products of moulds mainly belonging to *Fusarium*, *Aspergillus*, *Penicillium*, and *Claviceps* species. Under certain conditions they produce mycotoxins, with the group of trichothecenes (e.g., deoxynivalenol, T-2 toxin), zearalenone, ochratoxins, aflatoxins, fumonisins and ergot alkaloids being the most prevalent. These toxins cause substantial economic losses in animal husbandry.

Aflatoxins (Afla) are mainly produced by *Aspergillus flavus* and *A. parasiticus* on many different commodities, including cereals, figs, oilseeds, and others. Aflatoxin B_1 is moreover considered the main hepatocarcinogen in animals, although effects vary with species, age, sex, and general nutritional conditions (*Diener et al.,* 1987).

Trichothecenes constitute a large group of mycotoxins produced by various species of moulds, in particular those belonging to the genus *Fusarium*. The most prevalent mycotoxins of this group are deoxynivalenol (DON, vomitoxin) and T-2 toxin. An important issue is that some of these closely related compounds occur simultaneously (*Fuchs et al.,* 2004) and are proven to cause synergistic effects (*Weidenbörner,* 2001). Different types of trichothecenes vary in their toxicity though all of them have high to medium toxicity. They may cause haematological changes and immune suppression, reduced feed intake and skin irritations as well as diarrhea and haemorrhages of internal tissues. Pigs seem to be the most sensitive farm animals to this group of mycotoxins. Effects occurring at the lowest levels of trichothecenes were reduced feed intake and weight gain, as well as impairment of the immune system.

Zearalenone (ZEA) is also produced by *Fusarium* species and has strong hyper-estrogenic effects, which result in impaired fertility, stillbirths in females and a reduced sperm quality in male animals. ZEA is mostly affecting breeding animals which have a very sensitive reproductive system (*Hussein et al.,* 2001).

Ochratoxin A (OTA), which is produced by a number of *Aspergillus* and *Penicillium* species causes renal toxicity, nephropathy and immune-suppression in several animal

species, resulting in reduced performance parameters in animal production. OTA has also been detected in blood, animal tissues and milk (*Marquardt and Frohlich,* 1992).

Ergot alkaloids are produced in the sclerotia of *Claviceps* species, which are common pathogens of different agricultural commodities (rye, triticale, barley) and various grass species. The principal animals at risk are cattle, sheep, pigs, and chickens. Clinical symptoms of ergotism in animals include tail and ear necrosis eventually leading to gangrene, abortions, convulsions, suppression of lactation, hypersensitivity and ataxia (*Bennet and Klich,* 2003). As mentioned before in pigs a high level of toxin intake results in vasoconstriction and subsequently dry gangrene of hooves, ears and tails (*Bryant,* 2008).

Fumonisin B_1 (FUM B_1) can cause severe animal diseases such as equine leukoencephalomalacia (ELEM) in horses (*Marasas et al.,* 1988), and hydrothorax and porcine pulmonary edema in swine (PPE) (*Halloy et al.,* 2005). Besides their hepatotoxicity (*Gelderblom et al.,* 2001) and nephrotoxicity (*Edrington et al.,* 1995) they affect also the immune system (*Dombrink-Kurtzman,* 2003).

The unexpected high toxicity was attributed to undetected, conjugated forms of mycotoxins (deoxynivalenol-glucoside, zearalenone-glucoside) that hydrolyze to the precursor toxins in the digestive tract of animals (*Berthiller et al.,* 2009). As these masked mycotoxins are not detected during routine analysis, but are released during digestion, it seems likely that masked mycotoxins may contribute to cases of mycotoxicoses (*Binder,* 2007).

It is very difficult to set up valid and accepted levels of performance in animal production on a worldwide basis, and it is even more difficult to produce numbers and correlations that refer directly to the impact of hazards. A survey on worldwide limits and regulations for mycotoxins was published by the FAO (*FAO,* 2004).

Mycotoxin survey 2010

Mycotoxins are, more frequently than not, present in animal commodities and feed. That is the conclusion from an annual survey on the presence of mycotoxins in raw materials and animal feed (*Biomin,* 2011). Out of the more than 3.300 samples tested during the 12-month period of 2010, a striking 78% were positive for mycotoxin presence.

A total of 1731 samples were analyzed from central Europe – including Hungary for the most important mycotoxins in terms of agriculture and animal production: aflatoxins, zearalenone deoxynivalenol, fumonisins and ochratoxin A. Samples tested were diverse, ranging from cereals such as corn, wheat and rice to processing by-products, namely soybean meal, corn gluten meal, dried distillers grains with solubles (DDGS) and other fodder such as straw, silage and finished feed.

In central Europe, DON was the most prevalent mycotoxin (60% positive samples), followed by FUM (32%) and ZON (26%). From all samples tested positive for DON in central European regions, an average contamination as high as 967 ppb was analyzed.

Deactivation of mycotoxins

So far, no single adsorbent was tested to be effective against most types of mycotoxins (*Huwig et al.,* 2001). *Vekiru et al.* (2007) were screening 61 bentonites for their ability to adsorb AFB_1. According to the evaluated chemisorption index (Cα) AfB_1 was in general strongly bound to bentonites indicating and adsorption process due to chemisorptions. Hydrated sodium calcium aluminosilicates (HSCAS) for example resulted in almost total protection against aflatoxicosis (*Kubena et al.,* 1988; *Doerr,* 1989; *Ramos and Hernandez,* 1996), but its efficacy against zearalenone and ochratoxin was very limited

(*Bursian et al.,* 1992; *Huff et al.,* 1992; *Bauer,* 1994) and against trichothecenes practically zero (*Kubena et al.,* 1990, 1993, 1998; *Patterson and Young,* 1993).

An alternative strategy for combating mycotoxins in feedstuffs is detoxification of mycotoxins by biotransformation using microorganisms or enzyme systems. *Binder et al*. (1998) were the first who described a novel strain of *Eubacterium* sp. (*Eubacterium* BBSH 797) with a capability to biotransform DON to DOM-1. Eubacterium BBSH 797 was isolated from rumen fluid and is capable of detoxifying DON by enzymatic reduction of the 12,13-epoxy-group to a diene, resulting to the known metabolite de-epoxy- deoxynivalenol (DOM-1). Another microorganism, a novel yeast strain, characterized as *Trichosporon mycotoxinivorans* was discovered to have a very high capability to degrade both ochratoxin A and zearalenone. *Trichosporon mycotoxinivorans* (MTV) was shown to cleave OTA into phenylanine and the non-toxic OTA metabolite (OTα) *in vitro* (*Molnar et al.,* 2004; *Schatzmayr et al.,* 2003; *Schatzmayr et al.,* 2006/1). Besides biotransformation of OTA *Trichosporon mycotoxinivorans* also transforms ZON into the non-estrogenic metabolite ZOM-1 (*Vekiru et al.,* 2010). *Sphingopyxis* MTA 144 is a bacterium identified by *Schatzmayr et al.* (2006/2) and is capable of biotransformation of fumonisin B_1 into non-toxic metabolite 2-keto-HFB_1 (*Hartinger et al.,* 2011). The carboxylesterase FumD (Fumzyme) is encoded as a part of a gene cluster of *Sphingopyxis* sp. MTA 144 and enables the bacterial strain to degrade fumonisin B_1 (*Heinl et al.,* 2010) into non-toxic metabolite hydrolysed FB_1 (HFB_1) (*Hartinger and Moll,* 2011).

CONCLUSIONS

The isolation and characterization of microorganisms that are able to biotransform non-adsorbable mycotoxins into non-toxic metabolites in the intestinal tract of the animals is a major breakthrough in successful mycotoxin control. The biotransformation agents described above may become the technology of choice, as enzymatic reaction offer a specific, irreversible and very efficient way of detoxification that leaves neither toxic residues nor undesirable by-products. The elimination of adsorbable mycotoxins, such as aflatoxins and ergot alkaloids can be achieved through adsorption while selected plant and algae extracts that counteract effects of non-degradable mycotoxins complete the picture for successful control of mycotoxins.

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