Recent Advances on Microbial and Enzymatic Deactivation of Mycotoxins

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Introduction

Extensive research over the last couple of decades has made it obvious that mycotoxins are commonly prevalent in majority of feed ingredients. Mycotoxins can affect the animals either individually or additively in the presence of more than one mycotoxin, and may affect various organs such as gastro-intestinal tract, liver and immune system, essentially resulting in reduced productivity of the birds and mortality in extreme cases. It is commonly known that mycotoxins vary in their chemistry which results in vast differences with regard to their chemical, physical and biochemical properties. While the biochemical properties define the toxicity of mycotoxins, chemical and physical properties determine the methods that can be used to detoxify them. Realizing the great variety of mycotoxin structures, it is very obvious that there is no single method that can be used to counteract mycotoxins. Therefore different strategies have to be combined in order to specifically target individual mycotoxins without impacting the quality of feed. The best known method for mycotoxin deactivation is 'binding' with the use of binding agents, referred to as mycotoxin binders, adsorbents or enterosorbents. However, the adsorption efficacy of binding agents is limited to only a few mycotoxins, such as aflatoxins (AF), ergot alkaloids and some other fungal toxins, but for mycotoxins belonging to the group of trichothecenes (Huff et al., 1992; Kubena et al., 1993; Ramos et al., 1996; Scott, 1998; Huebner et al., 1999; Vekiru et al., 2007). These data are in line with results from the adsorption studies performed according to a standard protocol for mycotoxin adsorption using binder products sold in different markets around the world (**Figures 1 and 2**; Vekiru et al., 2007). While most of the clay minerals at 0.2 % inclusion level bound more than 85% of 200 µg AF/L at pH 3.0 as well as pH 6.5, they resulted in less than 25% adsorption rate for 1,000 µg Deoxynivalenol (DON)/L. On the other

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hand, organic materials such as yeast products (with ash content less than 15%) bound only less than 20% of AF and DON (**Figures 1 and 2**). Therefore, alternative approaches for efficient detoxification of mycotoxins are required.

Alternative Detoxification Methods

Enzymatic or microbial detoxification, sometimes referred to as "biotransformation" or "biodetoxification" utilizes microorganisms or purified enzymes thereof to catabolize the entire mycotoxin or transform or cleave it to less or non-toxic compounds. This approach has been known for a long time, even longer than the binder concept. Within few years after the discovery of AF, the first report on a bacterium capable of detoxifying AF by catabolization was published (Ciegler, 1966). Since then, many microorganisms were isolated from different habitats such as the gastrointestinal tract (GIT) of animals, soil, mycotoxin contaminated materials (e.g. grains) and insects feeding on such materials. However, only a few of these organisms were useful or further investigated for practical applications in animal nutrition.

Microbial Detoxification of Mycotoxins

One of the microorganisms which has been further developed into practical application is *Trichosporon mycotoxinivorans*, a yeast strain capable of detoxifying Ocharatoxin A and Zearalenone (Schatzmayr et al., 2003; Molnar et al., 2004; Vekiru et al., 2010). Application of this yeast in poultry diets has been proven to detoxify OTA (Politis et al., 2005). Another organism has been an anaerobic rumen bacterium BBSH 797 (Genus novus of family *Coriobacteriacae*, formerly *Eubacterium*) which was isolated and developed as a trichothecene detoxifying feed additive (Fuchs et al., 2002; Schatzmayr et al., 2006b). The BBSH 797 detoxifies trichothecenes by cleavage of the 12, 13 epoxide ring resulting in de-epoxy trichothecenes.

Enzymatic Detoxification of Mycotoxins

Several microorganisms, mainly aerobic bacteria but also yeasts, with Fumonisn (FUM) degradation properties were also explored and isolated in order to detoxify FUM (Schatzmayr et al., 2006a). However for various reasons, none of these microorganisms were useful as a mycotoxin deactivating feed additive. Therefore, the catabolic pathway of FUM degradation was investigated and the gene coding for the key enzyme of FUM detoxification (FUM*zyme*®) was identified, cloned and expressed in a yeast strain (Heinl et al., 2010; Hartinger and Moll, 2011). FUM*zyme*® (carboxyl-esterase) was further developed and tested in swine for gastrointestinal detoxification of FUM by cleaving the tricarballylic side chains of FUM leading to the non-toxic metabolite hydrolyzed FUM (HFB_1; Grenier et al., 2012; Grenier et al., 2013).

Evaluation of Alternative Detoxification Methods

While it is important to have guidelines which prove safety and efficacy of such additives under different *in vitro* and *in vivo* conditions in place, regulations for mycotoxin binders and deactivators have not been implemented in many parts of the world for various reasons. This negates the guarantee on the safety and efficacy of the product to the user. To overcome this unsatisfactory legal situation, recently the European Commission established a new group of technological feed additives for the reduction of mycotoxins in feed. In 2010, the European Food Safety Authority (EFSA) published a guidance document with stringent requirements - e.g. the binding capacity must be demonstrated; mycotoxin degradation products must be safe for target animals and consumers; minimum three *in vivo* studies with significant efficacy at the lowest recommended dose; relevant biomarkers of each individual mycotoxin have to be used to demonstrate the efficacy of the product, for the evaluation of mycotoxin deactivating products (EFSA, 2010).

The bacterial strain BBSH 797 and the purified FUM*zyme*® were the first ones for which dossiers were submitted to EFSA to get the approval as mycotoxin biotransforming agents in animal feeds. A comprehensive set of toxicity assays had to be performed for both components such as Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD 408; 1998), Acute Dermal Irritation/Corrosion (OECD 404;

2002), Acute Eye Irritation/Corrosion (OECD 405; 1997), Skin Sensitization (OECD 406; 1992), Mammalian Erythrocyte Micronucleus Test (OECD 474; 1997), Bacterial Reverse Mutation Test (OECD 471; 1997), In Vitro Mammalian Chromosome Aberration Test (OECD 473; 1997) and Tolerance test in target species at 100-fold dose level. Furthermore, *in vitro*, *ex vivo* and *in vivo* experiments were also carried out. The *in vitro* experiments comprised buffer tests and different cell based assays to prove the reduction of toxicity of the metabolites formed (Schatzmayr et al., 2006b). For the *ex vivo* experiments, pieces of different parts of the intestine were collected and inoculated with the bacterium and the enzyme, respectively, together with the mycotoxins for a short period, before samples were taken and analyzed for residual toxins and the non-toxic metabolites. Feeding experiments were conducted to demonstrate the mode of action of the bacterium and enzyme and biomarker analyses were performed to investigate the effect in animals. It has to be noted that performance parameters alone are not sufficient for this class of additives to prove effectiveness. In case of trichothecenes and the group's main representative DON, the biomarker of exposure is the mycotoxin itself (DON) and/or its metabolite (DOM-1) in blood serum, urine or faces of the animals. Also for FUM, the biomarker of exposure is FUM itself and the metabolites in the GIT of poultry while the biomarker of effect is the SA:SO ratio. Compared to a group of poultry only receiving mycotoxin contaminated diet, both, BBSH 797 and FUM*zyme*® were able to significantly reduce the biomarkers of exposure as well as the biomarkers of effects when added together with the mycotoxins (unpublished data). Based on these results provided to EFSA, individual approvals were issued for BBSH 797 (EFSA, 2013) and FUM*zyme*® (EFSA, 2014).

In summary, although binders or enterosorbents successfully eliminate the risk of certain mycotoxins such as the AF, they do not work comprehensively on all of the mycotoxins relevant to the poultry industry. Biotransformation has been one of the proven approaches for the detoxification of the non-adsorbable mycotoxins that can't be bound by using mycotoxin binders. Latest microbial and enzymatic deactivation technologies are used to alter their molecular structure into non-toxic metabolites which are excreted.

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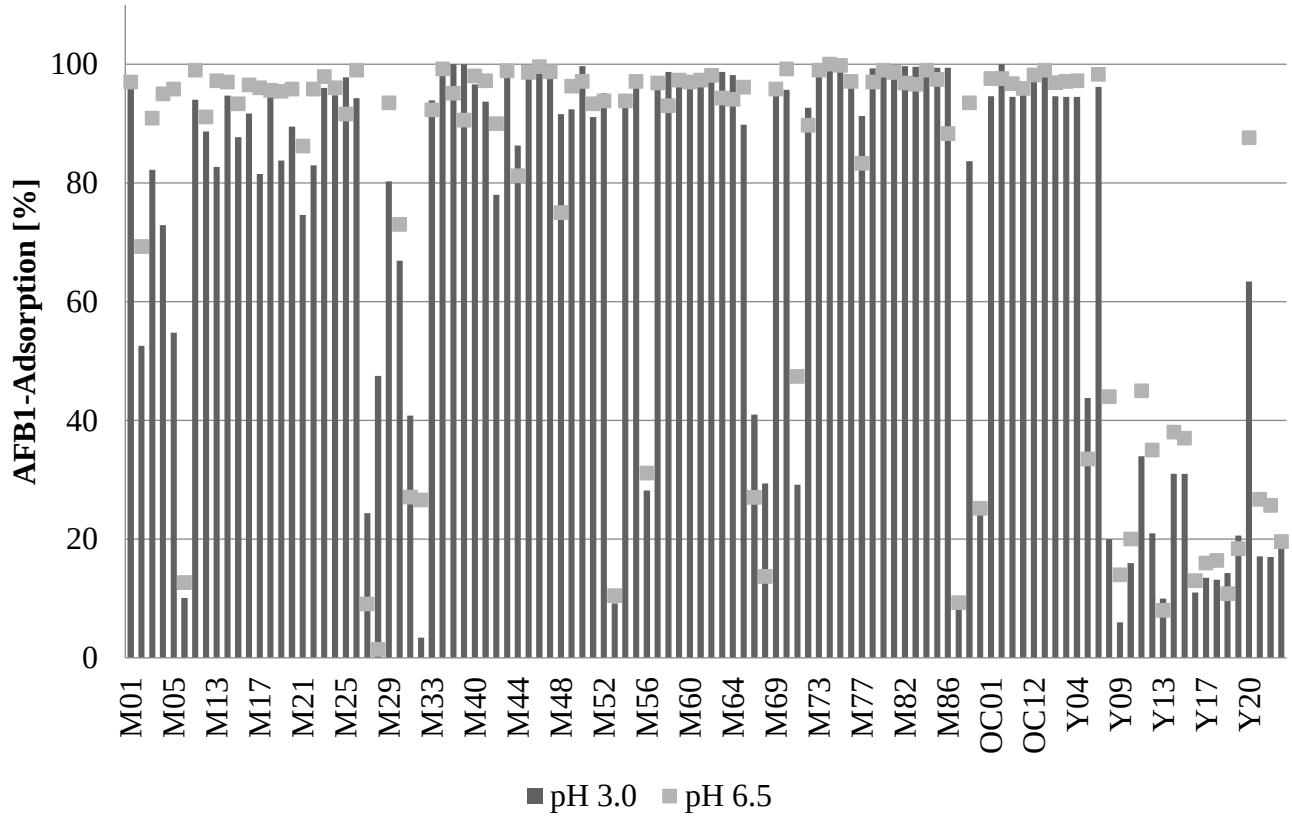
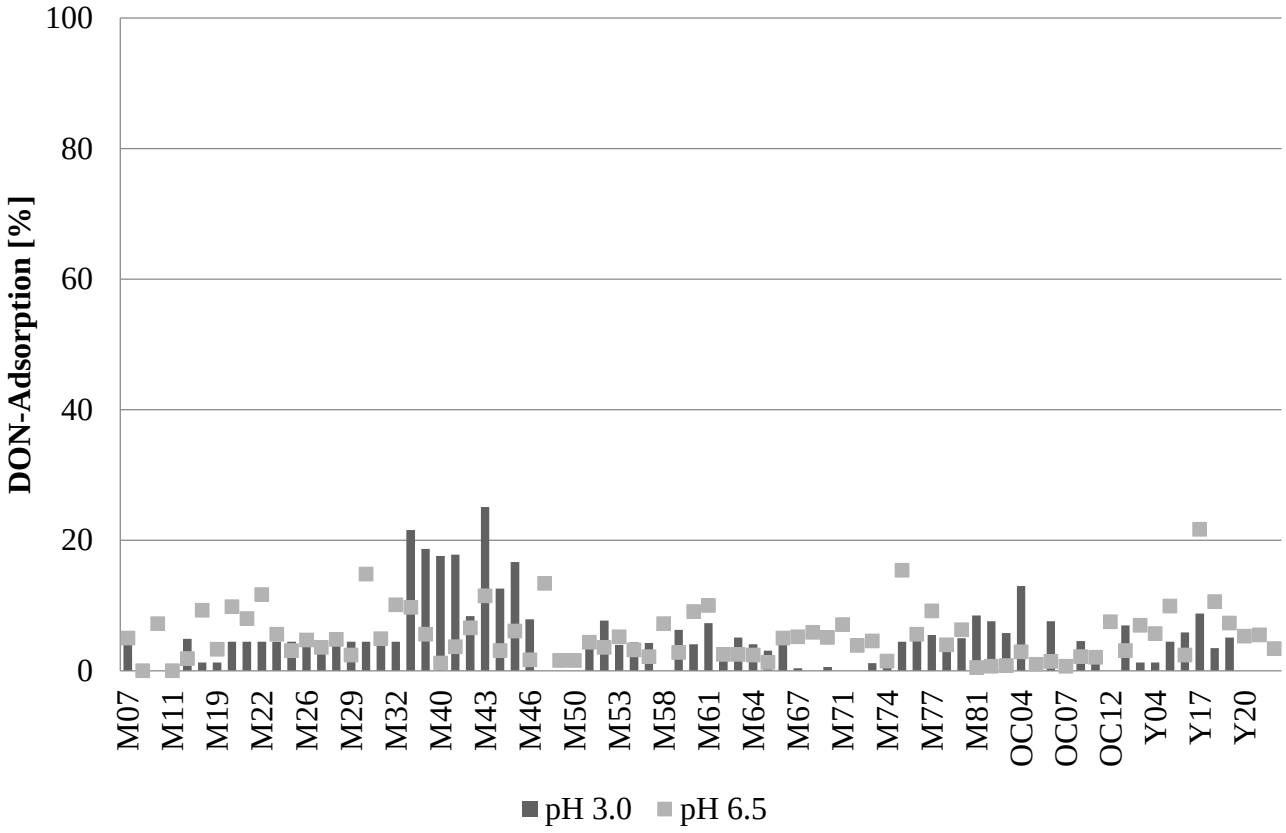
Figure 1. Adsorption capacity of mycotoxin binder products of different origins at pH 3.0 and pH 6.5 on Aflatoxin.[1]



[1]M: Mineral; OC: Organoclay; Y: Yeast

Figure 2. Adsorption capacity of mycotoxin binder products of different origins at pH 3.0 and pH 6.5 on Deoxynivalenol (DON).[1]



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