Combined strategies for

NUMEROUS STRATEGIES ARE EVOLVING FOR CONTROL OF MYCOTOXINS, SOME CLEARLY MORE PRACTICAL AND EFFECTIVE THAN OTHERS. NOVEL APPROACHES COMBINING DIFFERENT STRATEGIES THAT COUNTERACT MYCOTOXINS THROUGH DIVERSE BIOLOGICAL AND DIETARY INTERVENTIONS SHOW THE GREATEST PROMISE. BY DIAN SCHATZMAYR

Born in 1972 in Villach, Austria, Dr. Dian Schatzmayr studied Food- and Biotechnology at the University of Natural Resources and Applied Life Sciences in Vienna. After a one-year course in Quality Management at the Institute of Technology (TGM) in Vienna, she joined the R&D-team of Biomin in 1997. In this post she worked on the biological detoxification of mycotoxins and did her PhD in this field at the Institute for Agrobiotechnology in Tulln. In 2001, she was appointed Product Manager of Mycofix.

Mycotoxins are toxic chemical products formed by fungal species, mainly those belonging to the genera *Fusarium, Aspergillus* and *Penicillium*, that colonise crops in the field or after harvest and thus pose a potential threat to human and animal health. There are hundreds of mycotoxins known, but few have been extensively researched and even fewer have good methods of analysis available. The major classes of mycotoxins, in terms of agricultural relevance, are aflatoxins, zearalenone, trichothecenes (e.g. deoxynivalenol, T-2 toxin), ochratoxin A, fumonisins and the ergot alkaloids. In farm animals a mycotoxin-contaminated diet may lead to substantial economic losses due to feed refusal, poor feed conversion, diminished body weight gain, immune suppression, interference with reproductive capacities and residues in animal products. Mycotoxins exhibit a great variety of biological effects in animals: specific tissue damage, central nervous system effects and digestive disorders, to name a few. However, mycotoxin-related losses in performance, reproductive disorders and immune-suppression, are of major concern. Even though recommended agricultural practices have been implemented to decrease mycotoxin production during crop growth, harvesting and storage, the potential for significant contamination still exists.

According to the Food and Agriculture Organisation (FAO), at least 25% of the world's crops are contaminated with mycotoxins, despite increased efforts of prevention. The significance of these unavoidable, naturally occurring toxicants to human and animal health are reflected in the increase in mycotoxin regulations and trans-global shipment of agricultural commodities and highlight the need to provide successful counteracting strategies.

NO SINGLE TREATMENT

Certain treatments have been found to reduce levels of specific mycotoxins. However, no single method has been developed that is equally effective against the wide variety of mycotoxins which may co-occur in different commodities. Moreover, detoxification processes that appear effective *in vitro* do not necessarily retain their efficacy when tested *in vivo* (i.e. in feeding trials). The efficacy of physical treatments (e.g. washing, separation, roasting, UV irradiation, solvent extraction) depends on the level of contamination and the distribution of mycotoxins throughout the

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grain. Subsequently the results obtained are uncertain and often connected with high product losses. Moreover, some of these physical treatments are relatively costly and may remove or destroy essential nutrients.

Chemical methods require not only suitable reaction facilities but also additional treatments (drying, cleaning) that make them time consuming and expensive. Only a limited number of tested chemicals are effective without diminishing the feed's nutritional value or palatability. Treatment of contaminated feed with ammonia was once the most attractive method. Although early studies showed this technique to be safe and effective, ammoniation has not been approved by the US Food and Drug Administration due to the potential toxicity and carcinogenicity of the resulting products.

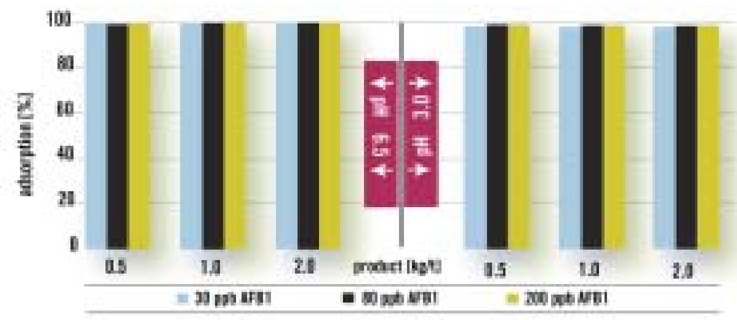
Over the course of several extensive research projects involving scientists from all over the world, a unique, continuously improved concept has been developed to successfully deactivate agriculturally relevant mycotoxins present in feed. The fourth generation (Mycofix® Plus, Biomin, Austria) is based on three different mycotoxin-counteracting strategies: (1) elimination of the toxin (adsorption), (2) elimination of the toxicity (biotransformation) and (3) elimination of toxin-related effects.

ADSORPTION ELIMINATES AFLATOXINS

The most well-known approach to detoxification of mycotoxins involves the use of nutritionally inert adsorbents with the capacity to bind and immobilise mycotoxins in the gastrointestinal tract of animals, thus reducing their bioavailability. In several independent scientific studies, hydrated sodium calcium aluminosilicates (HSCAS) have proven to be the most promising adsorbents. Mixed into feed they markedly diminish aflatoxin uptake by the blood and distribution to target organs, thus avoiding aflatoxin-related diseases and the carryover of aflatoxins into animal products. Unfortunately the efficacy of these adsorbing substances is limited against zearalenone (ZEA), ochratoxin A (OTA) and fumonisins (FUM) and totally ineffective for trichothecenes such as deoxynivalenol (DON), T-2 toxin and diacetoxyscirpenol (DAS).

However, today adsorption is not only an economically feasible, but a well-established and scientifically proven approach to prevent aflatoxicoses in farm animals. The efficiency of aflatoxin-adsorption mainly depends on the chemical properties of the adsorbent used. Several screening studies carried out in cooperation with Austrian universities in order to find the best adsorbents with regard to aflatoxin-deactivation and safe application showed that a synergistic blend of minerals afforded maximum, pH-independent activity at an inclusion rate as low as 0.5 kg/t without removing essential nutrients from the diet (*Figure 1*).

FIGURE 1 - AFLATOXIN B1 ADSORPTION. LEFT: 99-100% ADSORPTION AT PH 6.5 (INTESTINAL CONDITIONS). RIGHT: 98-99% ADSORPTION AT PH 3.0 (GASTRIC CONDITIONS).



BIOTRANSFORMATION BY BACTERIA

In the course of extensive research activities in the field of biological detoxification (1988 – 2004), "biotransformation" has been shown to be a unique practical method to successfully counteract less- and non-adsorbable mycotoxins. Defined as the enzymatic degradation of mycotoxins leading to non-toxic metabolites, biotransformation has been successfully applied since 1991. Continuous research finally led to the most recent development of patented microbial supplements able to detoxify all kinds of trichothecenes, zearalenone and ochratoxin A.

A safe bacterial strain (*Eubacterium sp.*) was found to have trichothecene-detoxifying activity and was named BBSH 797 after the research team that discovered it in July 1997. During its metabolism, BBSH 797 produces specific enzymes that eliminate toxicity of trichothecenes by selective cleavage of their toxic 12,13-epoxy group. Both *in vitro* and *in vivo* efficacy of the strain were scientifically proven *(Figure 2; Table 1)*.->

In the course of a several-year research project, the efficacy of the live yeast species *Trichosporon mycotoxinivorans,* named after its unique property to "eat" and thus detoxify both zearalenone and ochratoxin A (from the Latin, *vorans* meaning "eating" or "devouring"; patented as Biomin® MTV) was established.
Incubation experiments with the strain and subsequent cell culture studies at the University of Utrecht in the Netherlands proved successful in the degradation of 1 ppm ZEA. Additional *in vitro* studies with OTA-concentrations as high as 5 ppm revealed a complete detoxification within a maximum of 1 hour.

In vivo activity of *T. mycotoxinivorans* in piglets was investigated at the University of Gödöllö in Hungary. Addition of the yeast strain to the diet clearly improved weight development and feed conversion rate of animals (*Table 2*). Moreover, animal losses and cases of diarrhoea were lower in control and trial groups (A, C, D, E, F) than in the toxin group (B).

A feeding trial conducted at the University of Maribor in Slovenia revealed that the negative influence of high OTA-doses (1 ppm) on the performance of broilers could be totally neutralised by addition of *T. mycotoxinivorans*. The final weight of the trial group (toxin and yeast added) was on average 83 g higher than that of the positive control (toxin, no additive) and even better than the negative control.

TABLE 1- FEEDING TRIALS WITH PIGLETS (45 DAYS) AND BROILERS (36 DAYS), UNIVERSITY OF VETERINARY MEDICINE, VIENNA, AUSTRIA. MEAN VALUES.

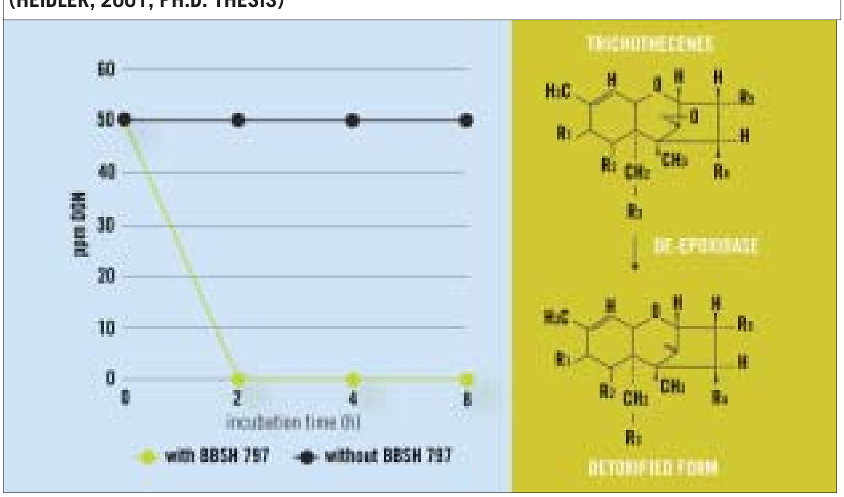
	Initial weight [kg]	Final weight [kg]	FCR	p
	Piglet trial, 2.5 ppm DON			
Control group	6.66	16.43^{a}	2.00	≤ 0.001
Trial group (+ BBSH 797)	6.76	23.62^{b}	1.62	
	Broiler trial, 10.5 ppm DON			
Control group	36.4	1.26^{a}	1.92	≤ 0.05
Trial group (+ BBSH 797)	36.4	1.44^{b}	1.87	

TABLE 2 - PIGLET TRIAL RESULTS WITH DIETS CONTAINING 500 PPB OTA. MEAN VALUESO

	A	B	C	D	E	F
T. mycotoxinivorans [CFU/g]	-	-	104	105	106	105
OTA [µg/kg]	-	500	500	500	500	-
Initial weight [kg]	10.23	10.20	10.24	10.23	10.23	10.25
Final weight [kg], 39d	32.65	30.78	32.07	31.53	33.81	33.10
ADWG [g]	457.7a	379.8b	445.4^{ab}	431.9^{ab}	476.7^{a}	465.5^{a}
FCR	2.44	2.35	2.30	2.31	2.13	2.24

a,b Values in the same row with different superscripts differ significantly ($p \leq 0.05$)

FIGURE 2 - IN VITRO DETOXIFICATION OF 50 PPM DON BY BBSH 797 WITHIN 2 HOURS. (HEIDLER, 2001, PH.D. THESIS)



ELIMINATION OF TOXIN-RELATED EFFECTS

The total number of mycotoxins is not known, but toxic metabolites of fungi could potentially number in the thousands. The number of mycotoxins actually known to be involved in diseases is considerably less, but even this number is difficult to assess, due to the diversity of their effects on animal systems.

Natural intoxications by mycotoxins are often more complex than can be related to those experimental studies utilising one mycotoxin. Therefore, natural responses may be the result of two or more toxins. The immune system, for instance, is not only a key target of the major classes of mycotoxins, but also of ergot and fescue alkaloids, citrinin, patulin and gliotoxin, to name a few. Hepato-toxic effects are not exclusively attributed to aflatoxins, ochratoxins and fumonisins, but also to sporidesmin (New Zealand, Australia: facial eczema), rubratoxins and phomopsins (Australia, New Zealand, South Africa, USA: lupinosis). All of these will produce significant liver damage when given to animals.

Finding successful detoxification strategies for agriculturally relevant mycotoxins is not an easy task; several years of intense research were necessary to develop the methods described above. However, finding respective strategies for minor classes of mycotoxins, that might act synergistically and contribute to various mycotoxicoses, is probably impossible. Thus, different methods are advised for non-adsorbable and non-degradable toxins.

A blend of scientifically studied and carefully selected plant and algae extract are have been studied that are able to eliminate toxin-related effects such as immune suppression, liver-damage or inflammation (*Table 3*). Herbs that support immune function are general immune-system-stimulators (immunostimulants). They

FIGURE 3 - MACROPHAGE CONSUMING PAHTOGENS

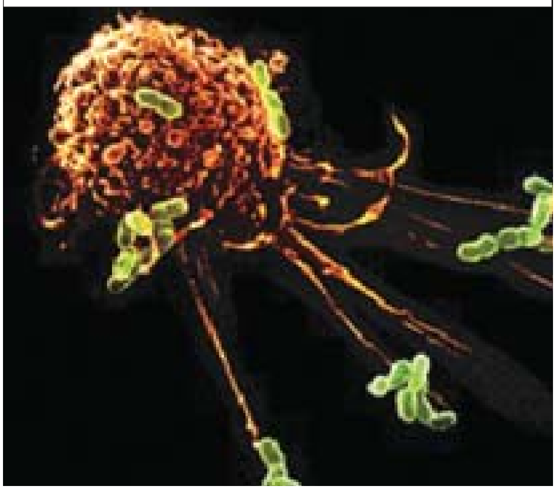


TABLE 3 - THE EFFECTS OF VARIOUS PLANT EXTRACTS.

Effect	Active constituents	References[1]
Stimulation of	polysaccharides, alkaloids	Goel et al. (2002); Keplinger et al. (1994);
immune system		Mungantiwar et al. (1999); O'Neill et al. (2002);
		Rossi (1993); Sohni et al. (1996); Wagner (1985);
		Wenigmann (1999); Williams et al. (1997)
Liver protection	flavo(no)lignans	Agarwal et al. (1994); Gujral et al. (2002);
		Handa et al. (1990); Hruby et al. (1992);
		Letteron et al. (1990); Rastogi et al. (2000, 2001);
		Salmi and Sarna (1982); Trivedi and Rawal (2001)
Anti-inflammatory	flavonoids, triterpene	Blumenthal et al. (1998); Della Loggia et al. (1994);
activity	saponines, carotinoides	Glowania et al. (1987); Leung and Foster (1996);
		Shipochliev et al. (1981); Weiss (1988);
		Zitterl-Eglseer et al. (1997)

[1]Details available on request

increase resistance by mobilising "effector cells" which act against all foreign particles rather than just one specific type. Immune-stimulating extracts have been selected using different in vitro test systems. Numerous preparations of plant and algal origin were compared in a macrophage activation assay. Macrophages (*Figure 3*) are one of the major cells of the unspecific immune system responsible for consuming invading microbes (i.e. for phagocytosis of pathogens). Thus, substances which are able to enhance the activity of macrophages lead to enhanced phagocytic activity and subsequently to a strengthened immune system. A synergistically acting blend of plant and algal extracts finally gave the best results (*Figure 4*). The immune stimulating effects of these substances were further confirmed in a lymphocyte proliferation test.

The liver-protecting effect of some plant derived substances was demonstrated in a broiler feeding trial carried out at the National University of Colombia.
A total of 144 chicks were fed a commercial starter mash ration which contained the hepato-protective additive and/or two hepato-toxic substances: pyrrolizidine alkaloids and aflatoxin B1 (200ppb).
A clear difference (52.5 g) in body weight gain was observed between the toxin and the trial group (*Table 4*). Feed intake and relative liver weights followed a similar trend, indicating that the birds completely overcame the adverse effects caused by the hepato-toxic substances.

TABLE 4 - BROILER FEEDING TRIAL (21DAYS) WITH LIVER PROTECTING SUBSTANCES.

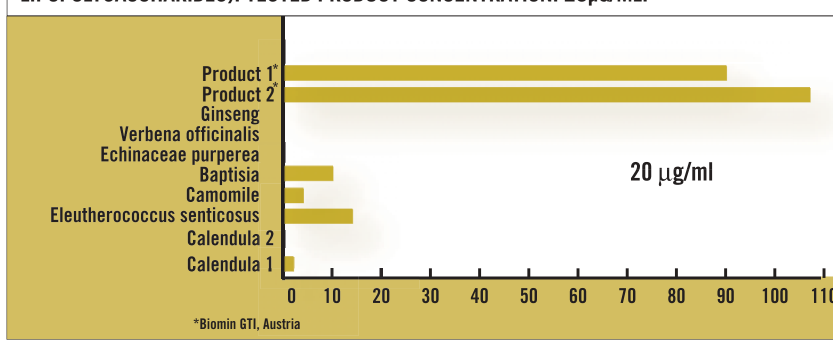
	Control group	Toxin group	Trial group
Final weight gain[1] [g]	594.5 ± 33.4	566.5 ± 58.0	619.0 ± 49.1
Total feed intake[1] [g]	835.2 ± 23.6	786.5 ± 92.8	842.3 ± 95.7
Relative liver weight[2] [g/kg]	28.2 ± 4.2	32.4 ± 2.8	30.2 ± 2.8

[1] values are means ± S.D. of 4 replicate pens per treatment

[2] values are means ± S.D. of 10 liver weights per treatment

Control group: no toxin, no additive; Toxin group: + toxin, no additive; Trial group: + toxin, + additive.

FIGURE 4. ACTIVITY OF HD11 MACROPHAGE CELL LINE (CONTROL = 20 NG/ML LIPOPOLYSACCHARIDES). TESTED PRODUCT CONCENTRATION: 20µG/ML.



CONCLUSION

The isolation and characterisation of microorganisms that are able to bio-transform mycotoxins in the intestinal tract of animals is a major breakthrough in successful mycotoxin control. The biological methods described above may become the technology of choice, as enzymatic reactions offer a specific, irreversible, efficient and environmentally friendly way of detoxification that leaves neither toxic residues nor any undesired by-products. Research teams working in this field are convinced that combinations of selected adsorbing agents and bio-transformation methods will ensure an effective control against mycotoxins taken in with contaminated feeds. Selected plant and algae extracts that counteract effects of non-degradable and non-adsorbable toxins complete the system. <-