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**Decontamination and detoxification strategies for the
Fusarium mycotoxin
deoxynivalenol in animal feed and the effectiveness of
microbial biodegradation**

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3 1 **Decontamination and detoxification strategies for the *Fusarium* mycotoxin**
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5 2 **deoxynivalenol in animal feed and the effectiveness of microbial**
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7 3 **biodegradation – a review**
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Detoxification of deoxynivalenol

27 **Abstract**

28 Trichothecenes are a group of mycotoxins mainly produced by fungi of the *Fusarium* genus.
29 Deoxynivalenol (DON) is one of the most abundant and important trichothecenes in food and
30 feed and it is of a great importance among these contaminants because of its frequent
31 occurrence in toxicologically relevant concentrations worldwide. Since the toxin production
32 depends strongly on environmental conditions such as temperature and humidity, a *Fusarium*
33 toxin contamination can not be avoided completely. Therefore, exposure to this toxin is a
34 permanent health risk for both humans and farm animals. As cereal crops are commonly
35 contaminated with DON and animal diets consist mainly of cereals, it can be assumed that
36 animals are frequently exposed to DON contaminated feeds. Many strategies can be
37 undertaken to reduce the toxic effect of DON. In addition to the general necessity for
38 minimizing all risk factors that might influence the contamination of cereals with DON, such
39 as the so-called field toxins before harvest, several post harvest strategies can be applied to
40 counteract possible deleterious effects of this mycotoxin in farm animals. Another approach
41 for decontamination in feedstuffs is the use of adsorbent materials. Adsorbent materials may
42 bind mycotoxins in the gastrointestinal tract and reduce the absorption and systemic toxicity.
43 It was shown that some adsorbents are suitable to alleviate the toxic effects of specific
44 mycotoxins, but its efficacy against trichothecenes is practically zero. Therefore, alternative
45 strategies to reduce animal and human health risk are needed. The use of microbial additives
46 is a method, which uses microorganisms having the capability to detoxify mycotoxins by
47 metabolism or degradation prior to their resorption in the gastrointestinal tract. DON has been
48 reported to be completely transformed to de-epoxy-DON by ruminal and intestinal microflora.
49 *Eubacterium* BBSH 797 was capable of DON degradation and counteracted the toxic effects
50 of DON in animals. This review focuses on the efficacy of microbial feed additives in
51 ameliorating the toxic effects of DON. According to the results of experiments to date, it
52 appears that microorganisms are the main living organisms applicable for this mycotoxin
53 biodegradation. However, the use of this approach depends on its effectiveness from both the
54 practical and economic standpoints.

55 **Key words:** Trichothecenes, Deoxynivalenol, detoxification, microbial degradation, review

Detoxification of deoxynivalenol

59 **Introduction**

60 No region of the world escapes the problem of mycotoxins and a higher percent of the world's
61 crops are annually affected by mycotoxins (Lawlor and Lynch, 2005). Whether grain is
62 produced in temperate, sub-tropical or tropical climates, if rainfall and humidity are high
63 during the harvest season, infection of the grain by fungi is likely. Food and feed are usually
64 contaminated by more than one mycotoxin, because a certain strain of moulds may produce
65 different mycotoxins. Residues of some mycotoxins may also be found in food of animal
66 origin (meat, milk, eggs, and cheese) as the consequence of contaminated feed. Mycotoxins
67 are toxic compounds, and some of them are also mutagenic, genotoxic, carcinogenic, or
68 teratogenic. In mild climates, the most frequent toxicogenic moulds in food and feed are the
69 species of the genera *Fusarium* and *Penicillium*.

70
71 A complex of closely related species of the genus *Fusarium* is responsible for destructive
72 action and results in very economically important diseases of cereal crops (*Fusarium* head
73 blight, FHB1, of wheat and barley) and maize (*Fusarium* ear rot). In years with climatic
74 conditions that favour the development of the fungi, these infections can reach epidemic
75 proportions (McMullen et al. 1997). Diseases caused by *Fusarium* do not only severely
76 reduce yield, but also results in the contamination of grain with unacceptable high amounts of
77 mycotoxins, a problem of world-wide importance. A toxin class of particular concern to
78 human and animal health are trichothecenes, sesquiterpenoid epoxides, which are potent
79 inhibitors of eukaryotic protein synthesis. Depending on the concentration and the substitution
80 pattern of the trichothecene, translational initiation, elongation or termination are
81 preferentially inhibited (Cundliffe and Davies 1977). Animal exposure to DON (vomitoxin)
82 has numerous adverse health effects, with neural, gastrointestinal tract and immune system
83 being the most sensitive organs (Canady, et al., 2001). Low to moderate dose of trichothecene
84 cause gastrointestinal irritation or necrosis, haematological disorders, diarrhoea, vomiting and
85 feed refusal and decreased body weight gain. Whereas, the exposure to higher dose levels of
86 DON are mainly expressed as severe reduction in body weight, severe damage to the
87 haematopoietic systems in bone marrow, spleen, thymus and lymph nodes, and impaired
88 resistance to infection, particularly bacterial infection (Ueno, 1984).

89
90 In addition to the toxic effect of DON on farm animals, the role of this mycotoxin as a
91 virulence factor in plant disease has been proven (Desjardins et al. 1996, Bai et al. 2002).

Detoxification of deoxynivalenol

1
2
3 92 Therefore, many attempts have been made to find ways to detoxify contaminated feedstuffs in
4
5 93 order to cope with the problem. Different physical and chemical methods have been
6
7 94 recommended for detoxification of mycotoxin contaminated food and feed. Nevertheless,
8
9 95 only a few of them (e.g. destruction of aflatoxin by ammonia treatment) have been accepted
10
11 96 for practical use. Although preventive strategies to reduce the formation of mycotoxins are
12
13 97 applied, there is increasing awareness that it is sometimes very difficult to control mycotoxin
14
15 98 contaminations in the field and during storage. Therefore, detoxification strategies have been
16
17 99 developed to protect livestock animals against mycotoxin contaminated feed. Many
18
19 100 specialists are of the opinion that the best approach for decontamination should be
20
21 101 degradation by selected microorganisms, giving a possibility of removal of mycotoxins under
22
23 102 mild conditions, without using harmful chemicals and without substantial losses in nutritive
24
25 103 value and the palatability of detoxified food or feed.
26
27 104

26 105 According to results of experiments, it appears that microorganisms are the main organisms
27
28 106 applicable for mycotoxin biodegradation. A review of the literature revealed that DON is
29
30 107 degraded by *Eubacterium* sp. DSM 11798 which transforms DON into its metabolite DOM-1
31
32 108 the non- toxic de-epoxide of DON (Binder et al., 1997, 1998). Fuchs et al. (2002) also
33
34 109 reported that the *Eubacterium* sp. (DSM 11798) is capable of detoxifying *A-Trichothecenes*.
35
36 110 Additionally, Awad et al. (2006) found that *Eubacterium* BBSH 797 is beneficial in
37
38 111 counteracting the toxicity of DON in commercial broilers at the gut level. DON has been
39
40 112 reported to be completely transformed to de-epoxy-DON after incubating for 96 h with the
41
42 113 content of the large intestine of hens (He et al., 1992). Enzymatic detoxifications of
43
44 114 mycotoxins in feed additives are a promising approach for the reduction of mycotoxin
45
46 115 contamination of feed and food. Therefore, this review summarizes the current state of
47
48 116 knowledge in the field of DON detoxification with special reference to the microbial
49
50 117 degradation.
51
52 118

119 *Pre-harvest and post-harvest prevention strategies*

53 120 It is important for producers to realize that good agricultural practices (GAP) represent the
54
55 121 primary line of defense against contamination of cereals with mycotoxins, followed by the
56
57 122 implementation of good manufacturing practices (GMP) during the handling, storage,
58
59 123 processing, and distribution of cereals for human food and animal feed. A complementary
60
124 management system to consider in the future is Hazard Analysis Critical Control Point

Detoxification of deoxynivalenol

1
2
3 125 (HACCP) system, which is used to identify and control hazards within the production and
4
5 126 processing system. Emphasis should be placed on the fact that the planting, preharvest and
6
7 127 postharvest strategies for a particular crop will depend on the climatic conditions of that
8
9 128 particular year, taking into account the local crops, and traditional production conditions for
10
11 129 that particular country or region.

12 130
13
14 131 Therefore, it is understandable that much research has been conducted in an attempt to
15
16 132 salvage mycotoxin-contaminated products and to avert health risks associated with the toxins.
17
18 133 Whatever decontamination strategy is used, it must meet some basic criteria (Park, 1993;
19
20 134 Beaver, 1991; Pomeranz et al., 1990). The mycotoxin must be inactivated (destroyed) by
21
22 135 transformation to non-toxic compounds, fungal spores and mycelia should also be destroyed.
23
24 136 In order to prevent the formation of new toxins, the food or feed material should retain its
25
26 137 nutritive value and remain palatable, the physical properties of raw material should not
27
28 138 change substantially, and the process must be economically feasible. Principally there are
29
30 139 three possibilities to avoid harmful effects of contamination of food and feed caused by
31
32 140 mycotoxins: (1) prevention of contamination; (2) decontamination of mycotoxin-containing
33
34 141 food and feed; and (3) inhibition of absorption of mycotoxin from the digestive tract. The
35
36 142 theoretically soundest approach of prevention is without doubt the breeding of cereals and
37
38 143 other feed plants for resistance to mould infection and, consequently, mycotoxin production.
39
40 144 Particularly in breeding wheat and corn, significant improvement of resistance has been
41
42 145 achieved (Mesterhazi, 1989; Medianer, 1997). According to data reported in a review paper
43
44 146 (Medianer and Reinbrecht, 1999) in Germany, about 25% of the area under wheat production
45
46 147 is represented by resistant varieties. Despite progress in breeding, full resistance has not been
47
48 148 achieved. Another applied way of prevention is the inhibition of the growth of moulds and
49
50 149 their production of mycotoxins. First of all, well-selected harvesting, storage- and processing
51
52 150 methods may be a successful tool in the prevention of mould growth. A practical guide to the
53
54 151 prevention of mycotoxin contamination of grain and animal feedstuffs by fusaria was
55
56 152 developed and published by Trenholm et al. (1988, Figure 1).

53 153
54
55 154 Agro-technical methods may also help to combat maize and wheat ear diseases. The sowing
56
57 155 time determines the harvesting time, which can have a substantial influence on the appearance
58
59 156 of disease. This is particularly important when late FAO maize hybrids are used, because they
60
157 are readily contaminated by moulds during wet autumns. Mould contamination is more
158 pronounced if wheat is grown after maize or vice versa or in which wheat is grown each year

Detoxification of deoxynivalenol

1
2
3 159 in the same field (McMullen et al. 1997). Multi-field crop rotation with rape, sugar beet,
4
5 160 sunflower or soybeans reduces the incidence of infection. Ploughing fields after harvesting
6
7 161 reduces the carryover of trash and risk of mycotoxins, and where possible, select a
8
9 162 recommended variety of seed resistant to *Fusarium* head blight. Aerate grain during storage
10
11 163 and use clean and dry storage facilities. Fertilization with nitrogen increases plants' sensitivity
12
13 164 to moulds (Reid et al., 2001), and balanced fertilization based on soil analysis is required.
14
15 165 Removing old seed heads, stalks, and other debris that may serve, or may potentially serve, as
16
17 166 substrates for the growth of mycotoxin-producing fungi.

17
18 167
19 168 However, even the best management practices can not eliminate the risk of contamination
20
21 169 over years are in favour of mycotoxins. Climatic conditions, such as temperature and
22
23 170 humidity, are not under human control, but they may be crucial in contamination with
24
25 171 moulds. Fungicides applied before blossoming decrease contamination with *Fusarium* and the
26
27 172 related production of mycotoxins (Cvjetkovic and Jurjevic, 1997). Delayed harvest
28
29 173 particularly favours contamination with *Fusarium*. Therefore, early harvest followed by
30
31 174 drying of the corn can help avoiding increased mycotoxin contamination. During harvest it is
32
33 175 of utmost importance to use appropriate harvesting equipment that is correctly adjusted to
34
35 176 avoid damage of the kernel, since those damaged kernels might be predisposed to infection
36
37 177 during storage.

37
38 178
39 179 Obviously damaged and high moisture parts should be removed from the harvest as they pose
40
41 180 a great risk of contamination. Mechanically damaged and shrivelled grains are regularly
42
43 181 contaminated by moulds, and mouldy grains can partially be removed by separators (Miller,
44
45 182 2001). The humidity of grain and the relative air humidity are very important in processing
46
47 183 and transport (Rilley and Norred, 1999). The kernel must be desiccated as soon as possible,
48
49 184 and optimal humidity maintained. It is particularly important not to allow increases in
50
51 185 humidity after desiccation, as they strongly favour contamination. It is well accepted that
52
53 186 grains should be dried to 15% humidity or lower before storage. The final safe moisture
54
55 187 content depends on the crop and the climatic conditions under which the commodity is stored.
56
57 188 Avoiding mycotoxin contamination depends primarily on moisture control. If the product is
58
59 189 dry and also kept dry further on, no deterioration of mycotoxin contamination will take place.

58
59 190
60 191 During long-term storage, the kernel is exposed to oscillations in temperature and humidity,
192
and insects of species *Sithophilus*, *Tribolium*, *Trogoderma*, *Oryzaephilus* can be vectors for

Detoxification of deoxynivalenol

1
2
3 193 mould contamination (Korunic, 1990). Insect and rodent control is important not only because
4
5 194 their activity in stored products creates favourable microclimates for fungal growth but also
6
7 195 because they damage kernels and therefore predisposes these kernels to fungal manifestation.
8
9 196 Adequate Storage should be without delay, good and clean storage facilities, insect control,
10
11 197 moisture of grains and humidity control, temperature control, insect and rodent control,
12
13 198 chemical preservatives, antifungal agents (acids, mould inhibitors) and the hygiene in silos
14
15 199 may decrease the growth of toxicogenic moulds (Willson and Abramson, 1992). Antifungal
16
17 200 agents should be used to supplement good management practice rather than as a substitute for
18
19 201 safe, clean handling practices of mixed feed. It should be emphasised that at the end of the
20
21 202 storage period grains can not be less contaminated with mycotoxins than at the beginning.
22
23 203 The entrance of mycotoxins in the nutritional chain can be avoided by storing only high
24
25 204 quality products in silos, and by strictly observing good agricultural practices. Minimize
26
27 205 insect damage and fungal infection in the vicinity of the crop by proper use of registered
28
29 206 insecticides, fungicides and other appropriate practices within an integrated pest management
30
31 207 program. Control weeds in the crop by use of mechanical methods or by use of registered
32
33 208 herbicides or other safe and suitable weed eradication practices. Irrigation is a valuable
34
35 209 method of reducing plant stress in some growing situations. Excess precipitation during
36
37 210 anthesis (flowering) makes conditions favourable for dissemination and infection by
38
39 211 *Fusarium* sp.; thus irrigation during anthesis and during the ripening of the crops, specifically
40
41 212 wheat, barley, and rye, should be avoided.

42
43 213
44 214 Mycotoxins that were already present on the field can not be influenced either by a correct
45
46 215 method during storage or by using mould inhibitors, and the decontamination in later stages
47
48 216 of food production is difficult, thus it increases the cost of production and the results are not
49
50 217 always satisfactory. Because mycotoxin contamination often is unavoidable, developing and
51
52 218 implementing efficient detoxification methods are very important. According to FAO (Scott,
53
54 219 1998), the decontamination process must: 1. Destroy, inactivate or remove the mycotoxin 2.
55
56 220 Not produce or leave toxic, carcinogenic or mutagenic residues in final products or in food
57
58 221 products obtained from animals fed decontaminated feed; 3. Retain the nutritive value and
59
60 222 acceptability of the product; 4. Do not substantially alter important technological properties,
223
224 and 5. Destroy fungal spores and mycelia which could, under favourable conditions,
225
226 proliferate and form new toxins. In addition to these criteria, the process (es) should be
readily available, easily utilized, and inexpensive. These latter attributes facilitate
administration of a comprehensive decontamination program for affected feeds and foodstuffs

Detoxification of deoxynivalenol

227 and are more applicable in regions where less-stringent protocols exist. Detoxification efforts
228 have been mainly focused on the aflatoxins and many strategies to decontaminate aflatoxin
229 contaminated crops and products have been reported and reviewed. Therefore, this paper
230 gives an overview of methods used in industrial *Fusarium* toxin decontamination of food and
231 feed. It also addresses the possibilities of the use of adsorbents as farm animal feed additives.

232

Physical Methods of detoxification

234 There are several physical methods of decontamination of agricultural products known as the
235 removal of damaged grains or of a part of the contaminated crop, washing procedures,
236 radiation, ultrasound and extraction with organic solvents. The removal of damaged parts of a
237 crop (usually mould-contaminated) is possible when contamination is uneven or partial.

238

239 Physical methods have included sieving and dehulling (Trenholm et al., 1991), density
240 segregation of contaminated kernels from non contaminated kernels using water and saturated
241 sodium chloride solution (Babadoost et al., 1987) or water and 30% sucrose solution (Huff
242 and Hagler, 1982), and food processing practices such as milling (Lee et al., 1987; Sietz et al.,
243 1986; Scott et al., 1984; Young et al., 1984), cleaning or washing (Sietz et al., 1986; Scott et
244 al., 1983), and baking (Abbas et al., 1988; Sietz et al., 1986; Tanaka et al., 1986; Young et al.,
245 1984; El-Banna et al., 1983). Rinsing grain with water or sodium carbonate water solution
246 could lower the concentration of mycotoxin DON in wheat and maize (Voss, et al., 1996).
247 Density segregation decreases the level of contamination of DON in corn and wheat.
248 However, it should be noted that appearance and weight of a particular kernel do necessarily
249 indicate mycotoxin contamination. These methods are limited by the cost of seed drying, and
250 they are used only before wet milling and brewing. Milling was not effective in removing
251 DON from the naturally contaminated wheat (Lee et al. 1987). Cleaning or washing (Seitz et
252 al., 1986; Scott et al., 1983), and baking (Seitz et al., 1986; Young et al., 1984; Abbas et al.,
253 1988; Tanaka et al., 1986; El-Banna et al., 1986), whilst not a decontamination method per se,
254 dilution of contaminated grain with clean grain has also been used to reduce the toxicity of
255 contaminated feedstuffs. Density segregation, cleaning, and baking have been largely
256 unsuccessful in completely removing the DON from flour fractions or from whole wheat
257 (Tanaka et al., 1986). Most mycotoxins are heat resistant, and high temperatures are not used
258 in the decontamination of cereals and other agricultural products. However, autoclave
259 treatment of DON-contaminated maize and wheat with sodium bisulphite was shown to be

Detoxification of deoxynivalenol

260 useful in reducing the DON concentration (Young et al., 1986; Young, 1986). Danicke et al.
261 (2005) found that the hydrothermal treatment of deoxynivalenol (DON)-contaminated wheat
262 in the presence of sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) is an effective tool to reduce DON toxic
263 effects. The effect of oxidizing agents has been also studied. Bleaching of flour with chlorine
264 in a commercial mill resulted in a 10% reduction of DON content and treatment with ozone
265 caused a significant reduction (Young, 1986).

266
267 Various wavelengths have been tested on their detoxification capacities. In wheat, γ -radiation
268 successfully reduces the concentration of DON (Scott, 1998). Unfortunately, radiation
269 produces metabolites or results in even more toxic by-products. It should be noted that
270 radiation is effective in decontamination only when applied to a thin layer of grain.

271

Chemical methods of detoxification

272
273 A wide variety of chemicals has been found to be effective against several mycotoxins in
274 different commodities. These include calcium hydroxide monomethylamine, sodium bisulfite,
275 moist and dry ozone, chlorine gas, hydrogen peroxide, ascorbic acid, hydrochloric acid, sulfur
276 dioxide gas (moistened), formaldehyde, ammonia, and ammonium hydroxide. Chemical
277 detoxification does not meet the FAO requirements, because some compounds leave behind
278 their toxic metabolites and others reduce the nutritional value of treated food and feed.
279 Sodium bisulphite is a common food additive that can significantly reduce DON in maize–
280 based pig feed (Hagler, 1991). Another effective method of mycotoxin degradation is based
281 on reaction with ozone (O_3) gas. Several studies indicate that ozone degrades DON (Young,
282 1986; Young et al., 1986)

283

284 In certain instances, physical and chemical treatments have been used in combination and
285 resulted in a more effective decontamination than either treatment alone. All mentioned
286 physical and chemical methods need special equipment and additional time leading to an
287 increase in costs. The efficiency of these methods highly depends on the level of
288 contamination and the distribution of the mycotoxins throughout the grain. Subsequently, the
289 results obtained are uncertain and often connected with high product losses. Moreover, some
290 of these treatments are relatively cost intensive and may remove or destroy essential nutrients
291 in the feed. In addition, the handling of the chemicals poses a potential risk to the workers.
292 Mostly the nutritive value and the palatability of the feed are changed. The feed quality in

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293 general is negatively influenced by those treatments and even toxic by-products cannot be
294 excluded. Thus, there is no practical application of these methods.

295
296 For a decontamination method to be commercially feasible it needs to be effective without
297 creating new toxins, introducing new toxic compounds, or altering the nutritional value or
298 other desirable parameters of food or feed; it should be simple and inexpensive and use
299 existing technology (Young, 1986).

300
301 ***Efficacy of different adsorbents for the binding of the Fusarium mycotoxin***
302 ***deoxynivalenol***

303 Costs and limitations of physical and chemical treatments of feed prompted the search for
304 other solutions concerning the mycotoxin hazard. Consequently, techniques were investigated
305 based on deactivation of mycotoxins directly in the gastrointestinal tract of animals (in vivo).

306
307 Several adsorbent products have been tested for their effectiveness to detoxify DON
308 contaminated feed, but the results have been unsatisfactory. Activated charcoal in aqueous
309 solution can adsorb most of the mycotoxins efficiently, whereas differently activated
310 charcoals have less and even no effects against mycotoxicosis. This might be due to the fact
311 that activated charcoal is a relatively unspecific adsorbent and hence, essential nutrients are
312 also adsorbed particularly if their concentrations in the feed are much higher compared to
313 those of the mycotoxin. Most studies related to the alleviation of mycotoxicosis by the use of
314 adsorbents are focused on aluminosilicates, mainly zeolites and hydrated sodium calcium
315 aluminosilicates (HSCAS), and aluminosilicate-containing clays, all consisting of aluminates,
316 silicates and some interchangeable ions, mainly alkali metal and alkaline earth metal ions
317 (Barrer, 1989; Mumpton, 1999).

318
319
320 In several studies, hydrated sodium calcium aluminosilicates (HSCAS) have proven to be the
321 most promising adsorbents. However, while good and scientifically explained results were
322 obtained for counteracting aflatoxins (Ramos and Hernandez 1996; Scott 1998), adsorption of
323 other mycotoxins was limited (e.g. zearalenone, ochratoxin A, fumonisin) or even failed (e.g.
324 trichothecenes, like deoxynivalenol) under field conditions (Friend et al. 1984; Kubena et al.
325 1990; Huff et al. 1992 Kubena et al. 1993; Ramos et al. 1996).

Detoxification of deoxynivalenol

1
2
3 326
4
5 327 Some types of activated carbons have been shown to adsorb DON from aqueous solutions of
6
7 328 the toxin with a saturation limit of ca. 2 µg DON/mg activated carbon (Galvano et al., 1998).
8
9 329 In addition, Avantaggiato et al. (2004) found that the dietary inclusion of activated carbon
10
11 330 produced a significant reduction in the intestinal mycotoxin (DON) absorption.

12 331
13
14 332 Although the different methods used at present for detoxification of *Fusarium* mycotoxins are
15
16 333 to some extent successful, they have big disadvantages with limited efficacy and possible
17
18 334 losses of important nutrients (Chung et al., 1990; Kubena et al., 1993) and are normally
19
20 335 expensive. Most of the commercially available mycotoxin-binders failed in sequestering
21
22 336 *Fusarium* mycotoxin in vitro. Only a small number of adsorbent materials possessed the
23
24 337 ability to bind more than one mycotoxin. Cholestyramine was proven to be an effective binder
25
26 338 for fumonisins and zearalenone in vitro, which was confirmed for zearalenone in experiments
27
28 339 using a dynamic gastrointestinal model and for fumonisins by conducting in vivo
29
30 340 experiments. No adsorbent materials, with the exception of activated carbon, showed relevant
31
32 341 ability in binding deoxynivalenol and nivalenol. Many scientists in the field are of the opinion
33
34 342 that the best solution for decontamination should be detoxification by biodegradation, giving
35
36 343 a possibility for removal of mycotoxins under mild conditions without using harmful
37
38 344 chemicals and without substantial losses in nutritive value and palatability of the
39
40 345 decontaminated food or feed.

346

347 ***Microbial Biodegradation of Fusarium mycotoxin deoxynivalenol***

42 348 The elimination of trichothecenes from contaminated feedstuffs is an unsolved problem and,
44
45 349 once contamination has occurred in grains, few strategies can be adopted for limiting adverse
46
47 350 effects in livestock (WHO, 2001). Investigation of the biodegradability of mycotoxins has
48
49 351 become an area of great interest. Efforts have been done to screen microorganisms from
50
51 352 different origins, which could detoxify trichothecene mycotoxins in vitro and in vivo. This
52
53 353 approach is defined as biodegradation or biotransformation that leads to less toxic products.
54
55 354 The use of microorganism in the in the field of mycotoxin detoxification is not new research
56
57 355 approach (Schatzmayr et. al. 2006). Some microorganisms with mycotoxin degradation
58
59 356 activity were isolated; the first was *Flavobacterium aurantiacum* with the ability to detoxify
60
357 aflatoxins (Ciegler et al., 1996). Wegst and Lingens (1983) provided evidence of the
358
degradation of ochratoxin A by the aerobic bacterium *Phenylobacterium* immobile.

Detoxification of deoxynivalenol

1
2
3 359 *Gliocladium roseum* detoxified zearalenone by opening the ring structure with subsequent
4
5 360 decarboxylation in yields ranging between 80 and 90% (El-Sharkawy and Abul-Hajj 1988). It
6
7 361 is known, that the 12, 13- epoxide ring is responsible for trichothecenes toxic activity and
8
9 362 removal of this epoxide group entails a significant loss of toxicity.

10 363 Of the microorganisms screened for their ability to degrade DON, some have been found to
11
12 364 be able to convert DON to the far less toxic compound (Table 1).
13

14 365

366 *Environmental microorganism*

17 367 DON is the most common mycotoxin produced on cereal crops and is chemically stable but
18
19 368 does not appear to accumulate in agriculture soils, implying the possibility of biological
20
21 369 mineralization of DON in nature (Völkl et al., 2004). Among 1285 microbial cultures
22
23 370 obtained from farmland soils, cereal grains, insects and other sources, one mixed culture was
24
25 371 able to transform DON into 3-keto- DON (Völkl et al., 2004). The culture was also capable of
26
27 372 transforming 3ADON, 15ADON and FUS. However, the microorganisms responsible for the
28
29 373 biotransformation have not been identified. In addition, mixed soil microorganisms have been
30
31 374 reported as transforming DON and 3ADON into five unidentified metabolites that showed
32
33 375 toxicity reduction in the bioassay with *Saccharomyces cerevisiae* (Binder et al., 1998). With
34
35 376 an enrichment strategy that used DON as the sole carbon source in a medium containing
36
37 377 mineral salts. *Agrobacterium-Rhizobium* strain E3-39 was isolated from soil samples (Shima
38
39 378 et al., 1997). The strain transformed DON into 3-keto DON under aerobic conditions. The
40
41 379 transformation activity of the strain was considerably persistent and the enzyme(s)
42
43 380 responsible for the microbial transformation was found to be in cells-culture, cell-free filtrate,
44
45 381 but not in the cell extract (Shima et al., 1997). Six agricultural soils capable of transforming
46
47 382 DON into several products were obtained after enhancement using *F. graminearum* infected
48
49 383 mouldy maize. These enhanced soils transformed DON by more than 87%, and two of them
50
51 384 completely removed DON from culture media after incubation at 28 °C for 3 days (Zhou,
52
53 385 2008). A bacterium strain Barpee was isolated from one of the soil samples and showed
54
55 386 strong DON transforming activity. Under aerobic conditions, the bacterial strain transformed
56
57 387 DON into at least two products: the major one was identified as a stereo-isomer of DON, and
58
59 388 the minor one as 3-keto-DON.

56 389

58 390 *Ruminal fluid microorganisms*

59 391 The studies on the biodegradation of DON by microorganisms in rumen fluid from different
60
392 ruminant species (Table 1). Microorganisms from sheep rumen fluid were found to have a

Detoxification of deoxynivalenol

1
2
3 393 deacetylation function (Kiessling et al., 1984). However, DON was not transformed
4
5 394 (Kiessling et al., 1984). In contrast, the microorganisms in the rumen fluid from a dairy cow
6
7 395 transformed DON into 3a, 7a, 15-trihydroxytrichothec-9, 12-dien-8-one (de-epoxy DON),
8
9 396 which has been named DOM-1 (Yoshizawa et al., 1983). The deepoxidation of DON by
10
11 397 rumen fluid was also reported by Cote et al. (1986) and He et al. (1992). It appeared that
12
13 398 microorganisms from rumen fluid could have both deacetylation and deepoxidation functions,
14
15 399 transforming 3ADON to DON and DOM-1 (King et al., 1984). These transformations by
16
17 400 rumen fluid were also observed when animal feed containing DON and 3ADON was used in
18
19 401 a test (Binder et al., 1998).

20
21 402
22 403 *Eubacterium* strain BBSH 797 is by far the most intensively studied bacterial isolate that is
23
24 404 capable of transforming trichothecenes. The strain was isolated from the bovine rumen fluid
25
26 405 and its primary function was to transform DON into DOM-1 in vitro and in vivo (Binder et
27
28 406 al., 1998; Schatzmayr et al., 2006). A BBSH 797 based commercial feed additive product is
29
30 407 available on the market.

31
32 408
33 409 Bacterial mixtures originating from the rumen of a fistulated dairy cow showed both
34
35 410 deacetylation and deepoxidation functions (Swanson et al., 1987a, b). Microbial
36
37 411 transformations were obtained after the tested mycotoxins were incubated within the bacterial
38
39 412 suspensions at 4.9×10^7 per ml anaerobically at 38 °C for 24 or 48 hours. DON was
40
41 413 transformed into DOM-1

Chicken digesta microorganisms

42
43 415
44 416 A very effective deepoxidation activity was achieved when DON was mixed with the digesta
45
46 417 of the large intestines of chickens; DON was completely transformed to DOM-1 (He et al.,
47
48 418 1992). It was shown that the deepoxidation function of the intestinal microorganisms of
49
50 419 chickens may vary greatly among the samples collected from different chicken varieties,
51
52 420 individuals and intestinal regions. The variation was also noticed in DON deepoxidation
53
54 421 activity by microorganisms from swine intestine, either no activity (He et al., 1992) or with
55
56 422 activity (Kollarczik et al., 1994).

57 423
58 424 In general, several authors described the de-epoxidation reaction of ruminal or intestinal flora
59
60 425 (Kollarczik et al., 1994; He et al., 1992; Yoshizawa et al., 1983, 1994). However, Binder et al.
426 (2000a) were the first to isolate a pure bacterial strain, which was able to biotransform the

Detoxification of deoxynivalenol

1
2
3 427 epoxide group of trichothecenes into a diene (Fuchs et al., 2002). Deoxynivalenol, the best
4
5 428 known trichothecene is enzymatically reduced by an epoxidase of *Eubacterium* DSM 11798
6
7 429 to the less toxic metabolite deepoxy- deoxynivalenol (DOM-1, Figure 1). The degradation of
8
9 430 the epoxide group is carried out by reductive cleavage of the toxic 12, 13 epoxy ring. This
10
11 431 strain was isolated out of bovine rumen fluid; its mode of action was proven in vitro and also
12
13 432 in vivo. The detoxifying strain *Eubacterium* BBSH 797 was the first microbe used in a
14
15 433 mycotoxin deactivating feed additive (Binder et al. 2000 b). Awad et al. (2004, 2006)
16
17 434 indicated that the *Eubacterium sp.* DSM 11798 was capable of completely compensating for
18
19 435 the adverse effect of the trichothecene deoxynivalenol in poultry.
20
21 436

21 437 Please inset Figure 1 about here

22 438
23
24 439 The rumen and intestinal microflora detoxify DON by reductive deepoxidation under strictly
25
26 440 anaerobic conditions (King et al. 1984; Kollarczik et al. 1994). Biological degradation of
27
28 441 DON under aerobic conditions appears to be much more difficult to achieve (Karlovsky
29
30 442 1999).
31
32 443

33 444 The investigations in this field (Line and Bracket 1995a, b; Line et al., 1994) concentrated on
34
35 445 possible mechanisms of aflatoxin degradation by *Flavobacterium Aurantiacum*. The first
36
37 446 important question which must be answered is: whether the bacteria actually degrade the
38
39 447 toxin or whether the disappearance of the toxin resulted from adsorption to the cells. Without
40
41 448 this knowledge, no real advantage can be taken of this microbial method of removing the
42
43 449 toxin (Figure 2).
44

45 450 Different strains of lactic acid bacteria (LAB) are able to bind the main *Fusarium* mycotoxins
46
47 451 (DON), but biodegradation did not appear to be the mode of action, as no toxin derivatives
48
49 452 were observed and removal was not impaired in nonviable bacteria. The binding ability of
50
51 453 selected strains could be used to decrease the bioavailability of toxins in contaminated silages
52
53 454 (Niderkorn et al., 2006).
54

55 455
56 456 Please insert Figure 2 about here

57 457
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59 458 Further screening of microorganisms with the degrading or binding properties may lead to the
60
459 detection of more efficient and more applicable bacteria. First of all, the microorganisms

Detoxification of deoxynivalenol

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3 460 resistant to mycotoxins should be investigated. Investigations of several bacteria and yeasts
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5 461 have shown that differences may be found both in sensitivity and selectivity of these
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7 462 microorganisms against mycotoxins (Madhyastha et al., 1994), e.g. *Bacillus brevis* was
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9 463 sensitive to eight mycotoxins including zearalenone and ochratoxin-A, but had not affected by
10
11 464 high concentrations of trichothecene toxins. *Kluyveromyces marxianus* was sensitive to all
12
13 465 trichothecene toxins, but this yeast was not inhibited by other mycotoxins. The study of the
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15 466 mechanisms involved in resistance (e.g. selective uptake through membranes, decomposition
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17 467 by selective enzymes, blocking by complex formation etc.) may be helpful in finding
18
19 468 applicable microbes. The control of degradation products, the effects of detoxification on the
20
21 469 nutritive and organoleptic properties are, in every case, a decisive part of such research and
22
23 470 potential applications.

24
25 471
26 472 Schatzmayr et al. (2006) reported that for an effective use of mycotoxin-degrading
27
28 473 microorganisms as animal feed additives certain prerequisites have to be fulfilled, such as
29
30 474 rapid degradation of mycotoxins into non-toxic metabolites under different oxygen conditions
31
32 475 and in a complex environment, safe to use, and stable in the gastrointestinal tract at different
33
34 476 pH levels. In addition, the choice of the biodegradation approach depends on its effectiveness
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36 477 in practice and its economics. Anaerobic microorganisms isolated from animal guts are
37
38 478 generally suitable for developing feed additives (Zhou et al., 2008), which will act in the
39
40 479 intestines of the targeting animals. Survival and adaptation of the microorganisms in the
41
42 480 animal guts are key factors for successful detoxification. Additionally, Cheng et al. (2006)
43
44 481 reported that the MDE product (mycotoxin degrading enzyme) with esterase, epoxidase and
45
46 482 peptidase activity could block epoxide formation in DON metabolism and esterase
47
48 483 transformed ZON into a non-toxic metabolite in pigs. The feed additive was capable of
49
50 484 counteracting the adverse effects on animal performance

485

486

487 Conclusion

52 488 The scope for mycotoxin decontamination is still very limited therefore the elimination of
53
54 489 mycotoxins from foods is a big problem worldwide wide. *Fusarium* mycotoxin
55
56 490 deoxynivalenol contamination seems to be an important issue in animal nutrition especially in
57
58 491 Europe and North America. As a preventive measure, it is possible to use agents that can
59
60 492 inactivate mycotoxins, particularly substances with a high absorption capacity, and specific
493 enzymes, which can break down the chemical structure of the mycotoxins. The biological

Detoxification of deoxynivalenol

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3 494 methods described above may become the technology of choice, as enzymatic reactions offer
4
5 495 a specific, irreversible, efficient and environmentally friendly way of detoxification that
6
7 496 leaves neither toxic residues nor any undesired by-products. The isolation and
8
9 497 characterization of microorganisms that are able to biotransform mycotoxins is promising for
10
11 498 the practical application of biotechnology, in addition to the decontamination processes taking
12
13 499 place directly in the intestinal tract of animals. Finally, a technology must be developed that is
14
15 500 suitable for economically feasible treatment of cereals and legumes. Providing high quality
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17 501 and safe animal products is vital and the research priority must be to eliminate toxic residues
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19 502 produced by fungi.
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Table 1. Microbial Biodegradation of mycotoxin deoxynivalenol

DON	Reaction	Product	Microorganism	Origin	Reference
DON	Oxidation	3-keto-DON	<i>Agrobacterium-Rhizobium</i> (strain E3-39)	Enriched soil	Shima <i>et al.</i> , 1997
DON	Oxidation	3-keto-DON	Mixed culture D107	Unknown	Völkl <i>et al.</i> , 2004
DON	Deepoxidation	DOM-1	Rumen Microorganisms	Cow	King <i>et al.</i> , 1984
DON	Deepoxidation	DOM-1	Rumen Microorganisms	Dairy cow	Swanson <i>et al.</i> , 1987a,b
DON	Deepoxidation	DOM-1	Chicken gut microorganisms	Chicken	He <i>et al.</i> , 1992
DON	Deepoxidation	DOM-1	Pig gut microorganisms	Pig	Kollarczik <i>et al.</i> , 1994
DON	Deepoxidation	DOM-1	Bacterial strain BBSH 797	Rumen fluid	Fuchs <i>et al.</i> , 2002
DON	Deepoxidation	DOM-1	Bacterial isolates LS100 & SS3	Chicken digesta	Young <i>et al.</i> , 2007
15ADON	Deepoxidation	de-epoxy 15ADON	Bacterial isolates LS100 & SS3	Chicken digesta	Young <i>et al.</i> , 2007
15ADON	Deacetylation	DOM-1	Bacterial isolates LS100 & SS3	Chicken digesta	Young <i>et al.</i> , 2007
15ADON	Deepoxidation	DOM-1	Bacterial isolates LS100 & SS3	Chicken digesta	Young <i>et al.</i> , 2007
3ADON	Deacetylation	DON	<i>Fusarium nivale</i> , <i>F. roseum</i> and <i>F. solani</i>	Fungus	Yoshizawa and Morooka, 1975
A3DON	Deacetylation	7,15DADON, 7ADON	<i>F. solani</i>	Fungus	Yoshizawa and Morooka, 1975

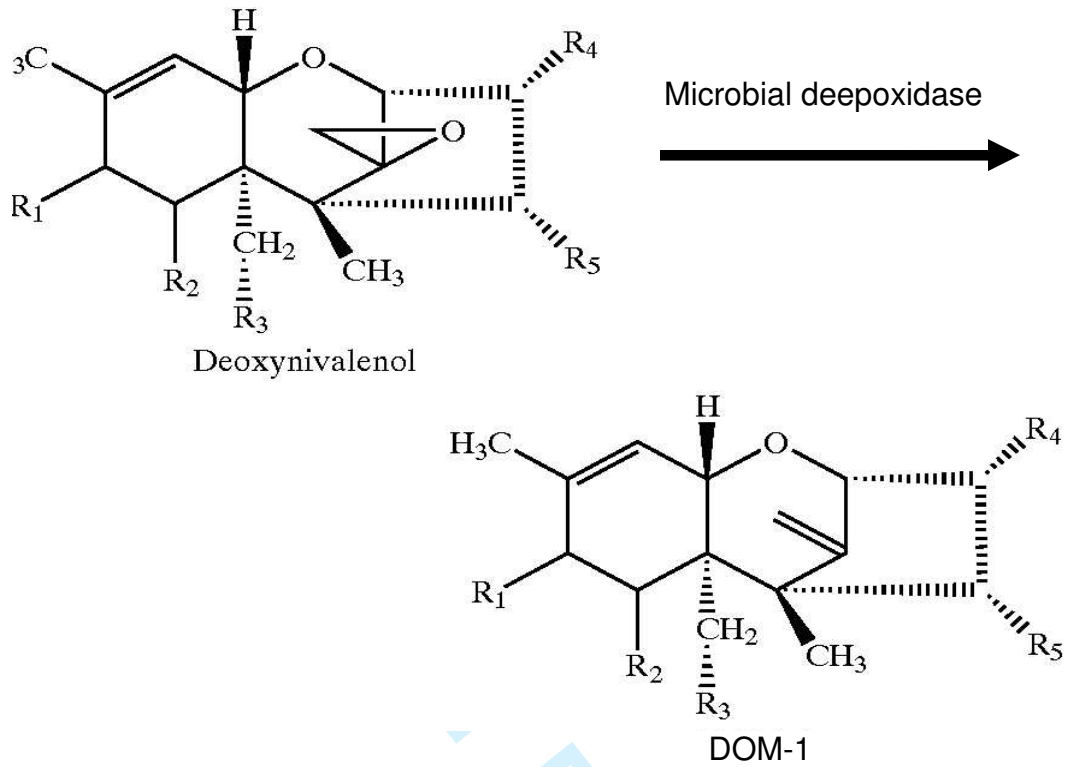


Figure 1. Microbial transformation (*Eubacterium sp.*) of deoxynivalenol to non-toxic metabolites

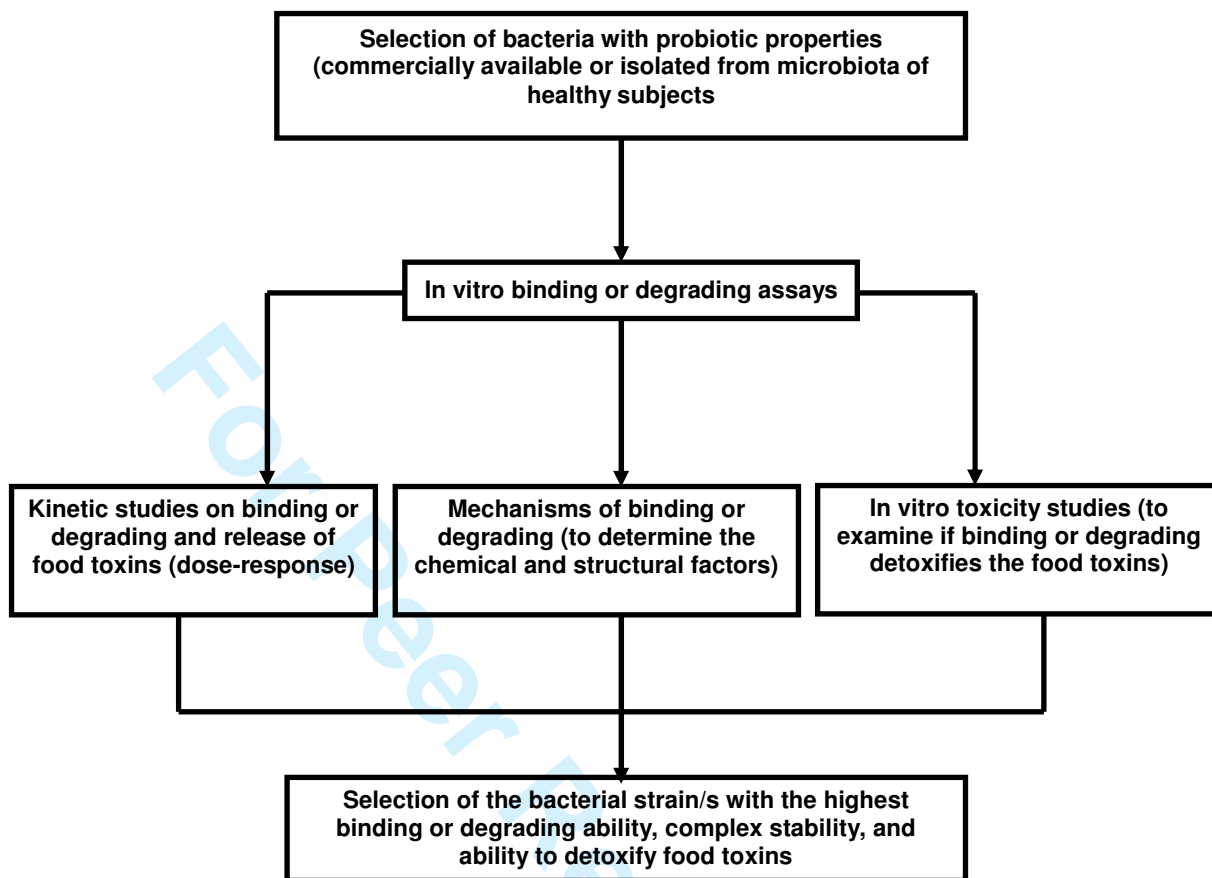


Figure 2. More in vitro studies are required to identify and explain the interaction between bacteria and mycotoxins (El Nazami et al., 2004)