

The present invention relates to a feed additive for inactivation of mycotoxins in animal feed or in the digestive system of animals, wherein the feed contains an enzyme preparation being produced of organisms selected from the groups of plants, bacterias, yeasts or protozoons in an amount of 0,1 to 3 kg/1000 kg of the feed. It relates further to the use of a feed additive for inactivation of mycotoxins in animal feed or in the digestive system of animals and to a process for preparing a feed containing the feed additive.

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From EP-A 0 257 996 there has become known a feed premix and a method for producing the same, which feed premix contains added, besides a physiologically acceptable carrier, for example grain flour, one or more enzymes. Also EP-A 0 286 056 shows a feed mixture and a method for treating feed raw materials, which feed mixture contains an added enzyme preparation. Both of the known feed mixtures contain essentially added amylases, because such grain-decomposing enzymes lack to young animals, in particular young piglets.

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Furthermore from AT-PS 397 756 there has for example become known a feed additive, which contains inorganic substances with great inner surface, such as silicates or zeolithes, and organic heterocyclic polymers for absorbing toxins.

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Mycotoxins, which are toxic metabolic products of moulds, are deemed unavoidable in the feed industry and in the livestock-breeding to a certain extent and are recognized to a high degree as cause of loss of performance in the bearing and breeding of domestic animals. Therefore, for a long time there were used several methods for avoiding the supply of mycotoxins to the animal body or for inactivating the mycotoxins supplied to the animal body to avoid the harmful action of the mycotoxins in the animal body. This was tried especially because of the fact that mycotoxins show a high chemical reactivity because of the functional groups being present in the molecule. This high chemical reactivity plays

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a central role in the toxicity behavior of such substances, because these groups interact with biologically important molecules, such as the nucleic acids, the enzymes but also the lipides of the cell membrane.

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For detoxifying the mycotoxins there were described up to now physical methods, such as the extraction, the heating, the irradiation and the adsorption of the mycotoxins, as well as the chemical inactivation of the same, such as a treatment
10 with acids, bases and different oxidizing agents. Among these different known processes the adsorption is the best usable in practice. This adsorption is normally accomplished by addition of larger amounts of adsorbents, such as clays, activated carbon or polymers. A complete inactivation or
15 fixation of all mycotoxins on the adsorbents is certainly not possible by such means, because of the mode of action of the adsorbents. The adsorption functions hereby essentially by the formation of a so called hydration cover on the adsorbent, on which polar molecules are accumulating because
20 of the van der Waals' forces. In this way it was possible up to now to inactivate polar mycotoxins such as aflatoxins satisfactorily. Mycotoxins, which have less strong polar functional groups, may be not sufficiently fixed on the adsorbents by such means and a sufficient inactivation of
25 such mycotoxins is by no means possible with the prior art adsorption processes.

Therefore the invention aims to provide a feed additive, in particular an enzyme preparation being based on organisms
30 selected from the groups of plants, bacterias, yeasts or protozoons, for inactivation of mycotoxins in animal feed or in the digestive system of animals with which mycotoxins, which are usually present in feed, are adsorbed as well as at least partially degraded und therefore the adversary effects,
35 which may be caused by the mycotoxins in the feed, are extensively removed.

The invention aims further at the use of a feed additive, which is able to inactivate the mycotoxins present in the feed or in the digestive system of animals, for avoiding a loss of performance in the bearing of domestic animals.

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For solving this problem, in the inventive feed additive for inactivation of mycotoxins in animal feed or in the digestive system of animals, wherein the feed contains an enzyme preparation being produced of organisms selected from the groups of plants, bacterias, yeasts or protozoons in an amount of 0,1 to 3 kg/1000 kg of the feed, the organisms producing enzymes are selected from the groups, which are able to produce epoxidases and/or lactonases. Because of the fact that the organisms producing enzymes are selected from the groups, which are able to produce epoxidases and/or lactonases, it is possible to degrade the mycotoxins having a high chemical reactivity, being therefore extremely toxic and being present in a feed to a large extent by the enzyme preparation contained in the feed additive and/or to chemically convert in less toxic or non toxic compounds, whereby the toxicity may be strongly reduced.

In particular, hereby an enzyme preparation is used which was produced advantageously by producing a pure culture or pure cultures of the organisms which are able to produce epoxidases and/or lactonases in a manner known per se dependent on its optimal growth conditions. Thereafter the cells of these multiplied pure cultures are inactivated, also in a manner known per se, for obtaining the enzymes, which are not altered and therefore remain undamaged. As enzyme preparation it is thereafter possible to directly admix these inactivated cells or to admix a pure or crude extract of these cells to the feed. According to an especially preferred embodiment epoxidases and/or lactonases are used. Enzymes or enzyme preparations have the advantage that they degrade or chemically alter mycotoxins, which are contained in very different classes of chemical substances. The reason for this

is, because many of the mycotoxins are cyclic hydrocarbons with an oxygen as a heteroatom, wherein the linkage of the oxygen in these cycles is often in form of an epoxide or lactone. The epoxidases preferably used, which fall into the group of hydrolases, cleave the epoxide ring of the mycotoxin by the formation of two hydroxyl groups, wherein the hydroxyl groups may be then easily degraded by the animal body. The lactonases, which are preferably used, act in an analogous manner.

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For achieving this effect, it is possible to effect a partial degradation of the mycotoxins still before the use as fodder by mixing the feed, the inventive feed additive and water, which is adjusted to a slightly acid or neutral pH value, and using this mixture as fodder. It is too be taken care that the feed mash is not made to acid for not adversely affecting the feed customs of the animals or causing health problems. In this connection, it is especially favourable is that the degradation of the mycotoxins functions without providing additional energy and may be obtained only by homogenous admixture of the enzyme preparation to the feed in the environment of the digestive system of the animal. If necessary, this can already be achieved by admixing with water which is adjusted to an adequate pH value.

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According to the present invention there is preferably used an extract of *Saccharomyces*-strains, in particular *Saccharomyces telluris*. Strains of *Saccharomyces* are a special class of yeasts, which are one of the most important groups for obtaining the enzymes. The yeasts may produce different hydrolytic enzymes in extremely high amounts and it has been shown that the *Saccharomyces*-strains, which were used in the feed industry for totally different purposes, may also be used for an especially fast and complete degradation of mycotoxins, which may comprise an epoxy group as well as a lactone group. *Saccharomyces telluris* is preferably used, with which the degradation of trichothecenes, in particular

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of T-2 toxin, H-T-2 toxin and deoxynivalenole, may be achieved under natural conditions being present in the gastrointestinal tract of animals. By the addition of *Saccharomyces telluris*, in particular it was possible to
5 cleave the epoxide ring of the above mentioned mycotoxins and the dihydroxy compound being formed is metabolized without any toxic effect.

For receiving the greatest possible inactivation of feed the
10 inventive feed additive is preferably formed in that additionally a natural or synthetic porous mineral with great inner surface is contained in an amount of 0,5 to 8 kg/1000 kg, in particular 0,7 to 4 kg/1000 kg, of the feed. With a feed additive made of a mixture of an enzyme preparation and
15 of a natural or synthetic porous mineral with great inner surface it is assured that in an aqueous medium with a pH value corresponding to that in the gastrointestinal tract of animals all mycotoxins, which are present on the surface of the feed, in particular the polar mycotoxins, are either
20 adsorbed on the mineral or are degraded or chemically converted by the enzyme preparation. The presence of the natural or synthetic porous mineral with great inner surface has also the advantage that by adsorbing the mycotoxins on the surface of these minerals these mycotoxins are easier
25 decomposable by the enzyme preparation.

According to the present invention diatomaceous earthes, zeolites, 2- and 3-layer clay minerals or mixtures thereof with a cumulated pore volume of 0,05 to 4 cm³/g, in
30 particular 0,5 to 2,5 cm³/g are preferably used as natural or synthetic porous mineral with great inner surface, which have a pH value on the surface of 4,5 to 7,5. Diatomaceous earthes, zeolites and layer clay minerals or also mixtures thereof, which show a cumulated pore volume of 0,05 to 4
35 cm³/g, in particular 0,5 to 2,5 cm³/g, are able to adsorb mycotoxins very efficiently and therefore to inactivate them to a large extent. Such minerals preferably show a pore

diameter of 10 nm to 1 μ m and have a surface pH value of 4,5 to 7,5. Such minerals are prepared in a manner known per se, wherein preferably the minerals are calcinated without any fluxing agent at about 800 °C to 900 °C, whereby it is possible to achieve a uniformity of the surface pore structure. For example, it is possible to use mineral mixtures of 80% clinoptilolithe and 20 % muscovite, 80 % chabazite and 20 % muscovite, 75 % clinoptilolithe and 25 % kaolinite or 85 % chabazite and 15 % kaolinite.

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Finally the inventive feed additive for inactivation of mycotoxins in animal feed or in the digestive system of animals is generally characterized in that the feed additive consists of a mixture of 1 to 65 % per weight, in particular 5 to 50 % per weight, of the enzyme preparation and 99 to 35 % per weight, in particular 95 to 50 % per weight, of natural or synthetic, porous minerals with great inner surface. By using relatively large amounts, i.e. 1 to 65 % per weight, in particular 5 to 50 % per weight, of the enzyme preparation it is secured that all mycotoxins in the feed being degradable are degraded to a large extent. On the other side the present amount of natural or synthetic, porous minerals with great inner surface secures that those mycotoxins, which may not be degraded chemically, are bound on the surface of these minerals and are therefore also not able to metabolize and may not show their action being damaging for the animals. It has been shown to be favourable to adjust the activity of the enzyme preparation for the epoxidase to 470 - 600 UI/g and for the lactonase to 280 - 400 UI/g by admixing the natural or synthetic porous materials.

Feed additives according to the present invention, with or without the addition of natural or synthetic, porous minerals with great inner surface may especially be used for inactivation of aflatoxins, T-2 toxin, deoxynivalenole, nivalenole, zearalenones, monoacetoxyscirpenole, H-T-2 toxin, verrucarine, roridine, satratoxin, baccharinole, trichoder-

mole, diacetoxyscirpenole, fumonisine, ochratoxin, moniliformine, fusarenone X and crocine in a feed or in the digestive system of an animal.

5 Because of the great bandwidth of mycotoxins, which may be degraded with the enzyme preparations according to the present invention, the use of the feed additive according to the present invention is a very favourable and effective method for removing toxic substances from feed. By the use of
10 the feed additive it is secured that feed, which is highly contaminated with mycotoxins, may not show its action being damaging to domestic cattle. Furthermore it is possible that feed, especially natural crops, which are only submitted to a crushing, drying or other treatment enhancing durability,
15 after admixing with the feed additive according to the present invention may be fed to domestic cattle either directly or after conditioning with water, which is adjusted to a slightly acid pH value, without hesitation and without further examination by scientific institutes.

20 Furthermore the present invention is directed to a process for the preparation of a feed, wherein optionally crushed and/or dried crops are mixed with an inventive feed additive and water, which is adjusted to a pH value of 1 to 4,5
25 suitable for reaction by addition of an acid, are stirred for 3 to 30 minutes at 15 to 40 °C and are dried by the use of a stream of warm air after an adjustment to a pH value suitable for feed and after a draw-off of the liquid ingredients. The adjustment of the pH value or the acidification may be
30 achieved with pharmaceutically acceptable acids, especially hydrochloric acid, acetic acid etc., wherein in particular the pH value is adjusted between 1 to 4,5 for achieving a fast inactivation of the mycotoxins contained in the feed. According to the present invention the temperature range is
35 adjusted between 15 °C to 40 °C, e.g. from room temperature to body temperature, for a time range of 3 to 30 minutes, for achieving a nearly complete inactivation of the mycotoxins.

Because of the fact that a feed with a pH value in the above mentioned highly acid region can not be directly fed to animals it is possible that a neutralisation, for example with sodium hydroxide, sodium hydrogen carbonate, sodium carbonate etc., to a slightly acid or neutral pH value can be achieved after inactivation. Thereafter after a stripping of the liquid components the feed is dried in a stream of warm air for obtaining a feed being free from mycotoxins and being storable for a long period. It is therefore especially preferred that the feed mash is pressed to pellets before drying in a manner known per se. With these pellets it is possible to have an reduced oxidation of the nutrients of the feed by providing a favourable storage and transportability at the same time.

The invention is further explained by the following example.

Example

Study concerning the clinical effect of an inventive feed additive to mycotoxins

Feed additive: 60 % per weight of a yeast preparation containing epoxidases and lactonases

10 % per weight kaolinite
30 % per weight clinoptilolithe

48 young pigs of both sexes with an average starting weight of 8,16 kg, a variance coefficient of 20,83 %, an average age of 28,6 days were selected for the test. Comparable young pigs were selected for each test stage. During the whole test the temperature and the humidity was controlled in the pigsty.

A diet with three different contents of mycotoxins and two different types of mycotoxins was fed to the animals. Four test groups were administered the inventive feed additive

too. One test group was administered neither mycotoxins nor the inventive feed additive.

In principle the feed contained soybean, millet and milk-
5 powder and was given 4 to 5 times a day, water was given ad
libitum. The mycotoxins were dissolved in ethanol and then
admixed with a small amount of the diet for a following
administration into the feed. The groups are shown in table
1, wherein the young pigs of both groups with identical
10 mycotoxin content show the same age and the same origin.

Table 1: Test groups with different mycotoxin content with
and without supplement of the feed additive.

15		T-2 toxin	feed additive
		+ aflatoxin	
	1	0,3 ppm + 0,1 ppm	0 kg/t
	2	0,5 ppm + 0,1 ppm	0 kg/t
	3	1,0 ppm + 0,1 ppm	0 kg/t
20	4	0,3 ppm + 0,1 ppm	2 kg/t
	5	0,5 ppm + 0,1 ppm	2 kg/t
	6	1,0 ppm + 0,1 ppm	2 kg/t
	7	0,0 ppm + 0,0 ppm	2 kg/t
	8	0,0 ppm + 0,0 ppm	0 kg/t

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The examined parameters were the body weight, the feed
uptake, the average daily augmentation of weight and the
feed-transformation rate.

30 The results of the performance parameters are shown in table
2.

Table 2: Average feed uptake, average daily augmentation of weight and feed-transformation rate of the eight test groups.

	group	average feed uptake	daily augmenta- tion of weight	feed- transformation rate
5	1	904	543	1,99
	2	909	455	1,99
	3	796	471	1,69
10	4	916	530	1,73
	5	910	487	1,86
	6	818	492	1,66
	7	922	520*	1,77
	8	899	509	1,77

15 * corrected by elimination of a stunted animal

Clinical evaluation:

20 - group 1 (0,3 ppm T-2 toxin + 0,1 ppm aflatoxin B₁ and 0,0 kg feed additive):
mild conjunctivitis, body condition and skin were normal, a slightly reduced feed uptake was detected.

25 - group 2 (0,5 ppm T-2 toxin + 0,1 ppm aflatoxin B₁ and 0,0 kg feed additive):
an undoubtable and severe irritation of the eyes, snout and muzzle were detected, body condition was normal, a reduced appetite was detected.

30 - group 3 (1,0 ppm T-2 toxin + 0,1 ppm aflatoxin B₁ and 0,0 kg feed additive):
all young pigs showed an irritation of the eyes, snout and muzzle and of the lateral parts of the neck and the thorax. All young pigs showed abdominal wind and a serious reduction
35 of appetite beginning in the second week. One young pig vomited on the 12th day.

- groups 4 & 5 (0,3 ppm or 0,5 ppm T-2 toxin + 0,1 ppm aflatoxin B₁ and 2 kg feed additive):

mild symptoms of conjunctivitis and skin irritation were detected, the feed uptake and the body condition were
5 considered as normal.

- group 6 (1,0 ppm T-2 toxin + 0,1 ppm aflatoxin B₁ and 2 kg feed additive):

2 young pigs (of 6) showed only a mild conjunctivitis, all
10 other observed values were in the normal region.

- group 7 (0,0 ppm T-2 toxin + 0,0 ppm aflatoxin B₁ and 2 kg feed additive): and

- group 8 (0,0 ppm T-2 toxin + 0,0 ppm aflatoxin B₁ and 0,0
15 kg feed additive) (comparison):

The young pigs were healthy.

From the above evaluation a positive influence of the
inventive feed additive concerning the feed uptake, the daily
20 augmentation of weight and the feed-transformation rate can
be clearly seen in each of the three dosages. The untreated
groups showed significantly more serious clinical symptoms of
mycotoxicosis compared with the groups, which were treated
with the feed additive. Concerning the performance parameters
25 as well as also from the clinical point of view the
beneficial effect of the inventive feed additive - namely the
effective protection against T-2 toxin and aflatoxin
contamination - is obvious.

C l a i m s



1. Feed additive for inactivation of mycotoxins in animal feed or in the digestive system of animals, wherein the feed
5 contains an enzyme preparation being produced of organisms selected from the groups of plants, bacterias, yeasts or protozoons in an amount of 0,1 to 3 kg/1000 kg of the feed, wherein the organisms producing enzymes are selected from the groups, which are able to produce epoxidases and/or
10 lactonases.
2. Feed additive according to claim 1, wherein the enzyme preparation is formed by a crude extract of an inactivated pure culture of organisms producing enzymes.
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3. Feed additive according to claim 1 or 2, wherein an extract of Saccharomyces-strains, in particular Saccharomyces telluris, is used as enzyme preparation.
- 20 4. Feed additive according to claim 1, 2 or 3, wherein additionally a natural or synthetic porous mineral with great inner surface is contained in an amount of 0,5 to 8 kg/1000 kg, in particular 0,7 to 4 kg/1000 kg, of the feed.
- 25 5. Feed additive according to claim 4, wherein diatomaceous earthes, zeolites, 2- and 3-layer clay minerals or mixtures thereof with a cumulated pore volume of 0,05 to 4 cm³/g, in particular 0,5 to 2,5 cm³/g, are used as natural or synthetic porous mineral with great inner surface, which have a pH
30 value of 4,5 to 7,5 on the surface.
6. Feed additive according to any of the claims 1 to 5, wherein the feed additive consists of a mixture of 1 to 65 % per weight, in particular 5 to 50 % per weight, of the enzyme
35 preparation and 99 to 35 % per weight, in particular 95 to 50 % per weight, of natural or synthetic, porous minerals with great inner surface.



7. Use of a feed additive according to any of the claims 1 to 6 for inactivation of aflatoxins, T-2 toxin, deoxynivalenole, nivalenole, zearalenones, monoacetoxyscirpenole, H-T-2 toxin, verrucarine, roridine, satratoxin, baccharinole, trichoderma, diacetoxyscirpenole, fumonisine, ochratoxin, moniliformine, fusarenone X and crotochine in a feed or in the digestive system of an animal.
8. Process for the preparation of a feed, wherein optionally crushed and/or dried crops are mixed with a feed additive according to any of the claims 1 to 6 and water, which is adjusted to a pH value of 1 to 4,5 suitable for reaction by addition of an acid, are stirred for 3 to 30 minutes at 15 to 40 °C and are dried by the use of a stream of warm air after an adjustment to a pH value suitable for feed and after a draw-off of the liquid ingredients.
9. Process according to claim 8, wherein the feed mash is pressed to pellets before drying in a manner known per se.