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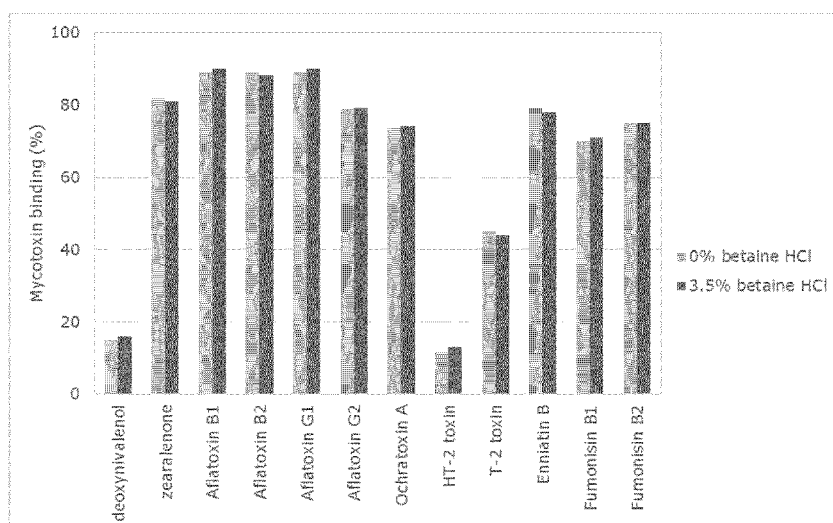
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(54) Title: COMPOSITION FOR USE AS MYCOTOXIN BINDER IN ANIMALS

Fig. 1



(57) Abstract: The current invention concerns a composition for binding and inactivation of mycotoxins, comprising a yeast component and one or more mineral clays, characterized in that said composition further comprises betaine or a betaine derivative.



COMPOSITION FOR USE AS MYCOTOXIN BINDER IN ANIMALS

TECHNICAL FIELD

- 5 The invention is directed to compositions and their use as feed additives to reduce mycotoxin contamination, preferably in animal feed.

BACKGROUND

- 10 Mycotoxins are metabolites produced by fungi that are toxic to humans and/or animals. These fungi occur on natural materials such as grains and plants, where the mycotoxins are then formed. These mycotoxins are fairly resistant to all kinds of treatments and are, in addition, difficult to detect. As a result, fungi infested plant material acts as carrier for mycotoxins, allowing them to enter the animal food chain through the feed
15 produced thereof, where they can cause discomfort and disease. The physical effects of mycotoxins differ from reduced food intake and poor food conversion to animal disease. The observed symptoms may also vary from animal to animal. Acute mycotoxicosis is often recognizable, but chronic mycotoxicosis takes its toll as it has an impact on animal performance and a reduction in immunity with all the known economic consequences.
20 Liver damage and deterioration is one of the main characteristics of both acute and chronic mycotoxicosis, further compromising the animals' health.

- Known state of the art solutions include the addition of (myco)toxin binders to the feed, in order to inactivate the mycotoxins before being taken in by the animal, or in the
25 animal itself. EP 1 079 696 for instance discloses a composition comprising a clay mineral and a yeast component capable of binding mycotoxins into the feed. However, once the animal has ingested the toxins, there is also a need for a composition which helps to restrain the damage caused by the toxins, limiting their toxicity, thus assisting the animal to cope with the infection. WO 2 014 014 334 A1 describes a food supplement
30 for animals comprising a clay mineral, a yeast component and trace minerals. This food supplement, however, does not deal with the issues of mycotoxicity. RU 2 391 975 describes the use of high doses of betaine in feed containing mycotoxins to prevent mycotoxicosis in farm animals.

- 35 The invention aims to provide a composition that is, on the one hand, functional as a toxin binder in feed while, on the other hand, protecting liver function in the animal, thereby having a double positive influence on the well-being of the animal and its performance.

SUMMARY OF THE INVENTION

The present invention provides a composition as stated in claim 1 for binding and
5 inactivation of mycotoxins in animals, while protecting liver function. More specifically, the composition comprises (myco)toxin binders combined with betaine, thereby having a double positive influence on the well-being of the animal and its performance.

The current invention thereto aims to provide a composition comprising one or more
10 mineral clays and a yeast component as (myco)toxin binders, acting both in the feed and in the animal. Betaine or a betaine derivative is included in the composition, which is functional in the animal as a liver detoxifying agent due to its involvement in methylation reactions and detoxification of homocysteine.

15 In a second and third aspect, the present invention provides an animal feed comprising a composition as stated in claim 14 and a method as stated in claim 19.

The current invention thereto aims to provide an animal feed comprising the composition as stated above in combination with a preservative, and a method of providing this feed
20 to animals.

DESCRIPTION OF FIGURES

25 **Figure 1** illustrates the mycotoxin binding capacity of betaine hydrochloride in the gastric environment. The additive is added at 0.5% and comprises 0% or 3.5% betaine hydrochloride (betaine HCL). Mycotoxin binding is expressed relative to the negative controls.

30 **Figure 2** illustrates the mycotoxin binding capacity of a feed additive in the gastrointestinal track environment. The additive is added at 0.5% and comprises 0% or 3.5% betaine hydrochloride (HCl). Mycotoxin binding is expressed relative to the negative controls.

35 **Figure 3** illustrates the positive effect of a feed supplement containing betaine hydrochloride (HCl) on the health of pigs based on the daily measurement of the feed conversion ratio (FCR). The FCR was calculated for each experimental group as the proportion of the feed intake (g) to the daily weight gain (g).

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention concerns a composition to prevent the harmful effects of ingested mycotoxins present in animal feed.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill
10 in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

As used herein, the following terms have the following meanings:

15 "A", "an", and "the" as used herein refers to both singular and plural referents unless the context clearly dictates otherwise. By way of example, "a compartment" refers to one or more than one compartment.

"About" as used herein referring to a measurable value such as a parameter, an amount,
20 a temporal duration, and the like, is meant to encompass variations of +/-20% or less, preferably +/-10% or less, more preferably +/-5% or less, even more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, in so far such variations are appropriate to perform in the disclosed invention. However, it is to be understood that the value to which the modifier "about" refers is itself also
25 specifically disclosed.

"Comprise," "comprising," and "comprises" and "comprised of" as used herein are synonymous with "include", "including", "includes" or "contain", "containing", "contains" and are inclusive or open-ended terms that specifies the presence of what follows e.g.
30 component and do not exclude or preclude the presence of additional, non-recited components, features, element, members, steps, known in the art or disclosed therein.

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within that range, as well as the recited endpoints.

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The expression "% by weight" (weight percent), here and throughout the description unless otherwise defined, refers to the relative weight of the respective component based on the overall weight of the formulation.

For the purpose of the current invention, the term betaine is to be understood as referring to trimethylglycine. Trimethylglycine is an N-trimethylated amino acid. Betaine derivatives are to be understood as the products originated by conversion of betaine by strong acids to various salts. A betaine derivative can therefore be, but is not limited to, betaine hydrochloride which is yielded by conversion of betaine by hydrochloric acid.

The present invention provides a composition that can be used to reduce the negative effects of mycotoxins to the animals' health by, on the one hand, inactivating mycotoxins present in feed or in the animal itself and, on the other hand, by supporting the detoxification function of the liver in the animal.

In a first aspect, the invention provides a composition for binding and subsequent inactivation of mycotoxins while protecting liver function, comprising a yeast component, one or more mineral clays, characterized in that said composition further comprises betaine or a betaine derivative. Mineral clays are known as (myco)toxin binders, both in the feed as in the animal itself. The absorption of the toxins by the mineral clays prevents their uptake into the animals' blood stream. The combination of mineral clays with yeast cell wall extracts in animal feeds is known to further increase the mycotoxin binding effect. Binding of mycotoxins directly interferes with their toxicity mechanism.

The yeast component in the composition is preferably present in a concentration from 5% to 10% by weight and consists specifically of a yeast cell wall extract. In particular the cell wall is extracted from a yeast selected from the group of *Saccharomyces*, *Candida*, *Kluyveromyces*, *Torulaspora* or a combination thereof. More in particular, the yeast cell wall is extracted from a *Saccharomyces cerevisiae* yeast. More in particular, the yeast cell wall is hydrolysed prior to extraction and is, more specifically, composed of mannan-oligosaccharides.

30

The clay minerals in the composition are preferably present in a concentration from 40% to 80% by weight. In particular, one or more mineral clays are selected from the group consisting of zeolites, bentonites, aluminosilicates, magnesium aluminum silicates or mixtures thereof. In a preferred embodiment of the invention the mineral clay consists of a clinoptilolite.

35

The composition also aims at supporting the animals' endogenous defense mechanisms once mycotoxins have been ingested. After mycotoxin ingestion, the liver is burdened

with the inactivation and degradation of the mycotoxins. Accordingly, liver damage and deterioration is one of the main characteristics of both acute and chronic mycotoxicosis.

The composition thereto comprises betaine or a betaine derivative, which is involved in
5 methylation reactions and in the detoxification of homocysteine. Betaine and betaine derivatives are used in this invention as detoxifying agents, supporting the detoxification functions of the liver, eventually contributing to the improvement of the animals' health.

Betaine is used in the liver as a methyl group donor. The liver is the major organ within
10 which methylation reactions occur. Dietary intake of methyl donors like betaine could influence the methylation of DNA and histones, thereby altering the epigenetic regulation of gene expression. Many of the hepatic genes involved in pathways for the development of fatty liver, hepatic fibrosis, and hepatocarcinomas are epigenetically regulated. In addition, methylation reactions are also important for several metabolic
15 processes such as the synthesis of creatine and carnitine.

The composition comprising a yeast component, one or more mineral clays, and betaine or a betaine derivative has a positive effect on animals' health which is larger than the effect expected of the individual components on the animals' health. Betaine or a betaine
20 derivative is preferably present in the composition in a concentration from 0.001% to 5% by weight of the composition. When betaine or a betaine derivative is present in the composition in a concentration of at least 0.001% by weight of the composition, the synergetic effect of the composition is more pronounced. Above a betaine or a betaine derivative concentration of more than 5% by weight of the composition, the synergism
25 between the different components of the composition was no longer observed. In a particularly preferred embodiment of the invention, the betaine derivative included in the composition is betaine hydrochloride.

The inventors of the present invention have found that a composition as described above
30 is exceptionally suited to counteract the harmful effects of a broad range of toxins. The binding and inactivation of in particular Ochra toxin, zearalenones, deoxynivalenol (DON), fumonisins, aflatoxins, HT-2 toxins, T-2 toxins or enniatins contributes to the protection of the animals' health.

35 In a preferred embodiment, the composition described in the present invention or a feed supplement comprising the composition described in the present invention comprises a preservative composed of a calcium, sodium or ammonium salt of propionate. This preservative prevents bacterial and fungal growth in the composition or in the animal

feed or drinking water upon addition of the composition. More in particular, the preservative is present in a concentration from between 5% to 10% by weight of the composition.

5 In a second aspect, the composition of the present invention as described above has a remarkable positive effect on the animal when taken in and is thus well suited as a feed supplement or as addition to the animals' feed. Addition of the composition to feed directly or as (part of) a feed supplement therefore results in healthier animals. In one embodiment, the composition or supplement is a powder. In a different embodiment,
10 the composition or supplement is a liquid. In yet another embodiment, the composition or additive is added to the animals' drinking water. In a preferred embodiment of the invention, the effective amount of the composition comprises from 0.5% to 1% by weight of the feed.

15

In particular the composition according to the present invention is suited for administration to animals selected from the group consisting of avian, bovine, porcine, equine, ovine, and caprine species

20 In a third aspect, the present invention also describes a method of feeding animals with a feed supplemented with a feed supplement according to the present invention, wherein the animal obtains the feed additive in a content of between 5 g/kg and 10 g/kg bodyweight / day. In a specific embodiment of the invention, the composition is admixed with the animal feed prior to feeding. In an alternative embodiment, the composition is
25 admixed with the animal's drinking water before it is provided to the animal.

The present invention may be described by the following embodiments:

1. A composition for binding and inactivation of mycotoxins, comprising a yeast component and one or more mineral clays, characterized in that said composition
30 further comprises betaine or a betaine derivative.
2. The composition of embodiment 1, wherein the yeast component comprises a yeast cell wall extract.
3. The composition of any of the embodiments 1 to 2, wherein the yeast component comprises a yeast cell wall extract from a yeast selected from the group
35 consisting of *Saccharomyces*, *Candida*, *Kluyveromyces*, *Torulaspora* or a combination thereof.
4. The composition of any of the embodiments 1 to 3, wherein the yeast component comprises a yeast cell wall extract from a *Saccharomyces cerevisiae* yeast.

5. The composition of any of the embodiments 1 to 4, wherein the yeast component comprises a yeast cell wall extract composed of mannan-oligosaccharides.
6. The composition of embodiment 1, wherein said one or more mineral clays are selected from the group consisting of zeolites, bentonites, aluminosilicates, magnesium aluminum silicates or mixtures thereof.
7. The composition of any of embodiments 1 to 6, wherein said mineral clay is clinoptilolite.
8. The composition of embodiment 1, wherein said betaine derivative is betaine hydrochloride.
9. The composition of embodiment 1, wherein said composition has a total concentration of mineral clay of between 40% and 80% by weight of the composition.
10. The composition of embodiment 1, wherein said composition comprises between 5% and 10% yeast component by weight of the composition.
11. The composition of embodiment 1, wherein said composition comprises between 0.001% to 5% betaine or betaine derivative by weight of the composition.
12. A composition for use as toxin binder in animals and animal feed, said composition is a composition according to any of the embodiments 1 to 11.
13. The composition of embodiment 12, wherein said toxin is selected from the group consisting of OchrA toxin, zearalenones, deoxynivalenol (DON), fumonisins, aflatoxins, HT-2 toxins, T-2 toxins and enniatins.
14. An animal feed comprising a composition according to any of the embodiments 1 to 11.
15. The animal feed of embodiment 14, wherein the effective amount of the composition comprises from 0.5% to 1% by weight of the feed.
16. The animal feed of embodiment 14, wherein the animal is selected from the group consisting of avian, bovine, porcine, equine, ovine, and caprine species.
17. The animal feed of any of embodiments 14 or 16, wherein said composition comprises a preservative that is composed of a calcium, sodium or ammonium salt of propionate.
18. A method for feeding animals, where the animals consume a feed comprising a composition as stated in any of embodiments 1 to 11 or an animal feed as stated in any of embodiments 14 to 18, wherein the effective amount of the composition comprises from 5g/kg to 10g/kg bodyweight/day.
19. The method of embodiment 19, wherein the composition is admixed with the animal feed prior to feeding.

The invention is further described by the following non-limiting examples which further illustrate the invention, and are not intended to, nor should they be interpreted to, limit the scope of the invention.

- 5 The present invention will be now described in more details, referring to examples that are not limitative.

It is supposed that the present invention is not restricted to any form of realization described previously and that some modifications can be added to the presented
10 example of fabrication without reappraisal of the appended claims.

EXAMPLES

Example 1: (simulation of the gastric environment)

15 The following experiment demonstrates betaine hydrochloride does not have mycotoxin binding activity in the gastric environment.

Sample combinations were prepared in citrate buffer pH 3 (cf. gastric pH). After a 1
20 hour incubation at 37°C while shaking, the mycotoxins were extracted according to a solvent-switch method and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Internal standards were therefore included in each sample. Mycotoxin binding is expressed relative to the negative controls in % and is shown in Figure 1.

25

Sample combinations consisted of:

- negative control 1: mycotoxin standard mixture containing deoxynivalenol, zearalenone, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A, HT-2 toxin, T-2 toxin, Enniatin B, Fumonisin B1 and Fumonisin B2.
- 30 - negative control 2: 0.5% feed additive (Table 1)
- mycotoxin inhibitor: 0.5% feed additive (Table 1) + mycotoxin standard mixture containing deoxynivalenol, zearalenone, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A, HT-2 toxin, T-2 toxin, Enniatin B, Fumonisin B1 and Fumonisin B2.

35

Table 1: Feed additive composition

Component	Without betaine HCl	With betaine HCl
	(%)	(%)
Betaine hydrochloride	0	3.5
Mineral clays	80	76.5
Yeast component	10	10
Preservative	10	10

Addition of a feed additive comprising betaine hydrochloride (HCl) to the feed did not lead to increased mycotoxin binding in the gastric environment when compared to a feed additive without betaine HCl.

Example 2: (simulation of the gastro-intestinal track)

The following experiment demonstrates betaine hydrochloride does not have mycotoxin binding activity in the gastro-intestinal track environment.

Sample combinations were first prepared in citrate buffer pH 3 (cf. gastro-intestinal pH-range). After a 1 hour incubation at 37°C while shaking, the pH was brought to pH 7. Following a subsequent incubation of 3 hours at 37 °C while shaking, the mycotoxins were extracted according to a solvent-switch method and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Internal standards were therefore included in each sample. Mycotoxin binding is expressed relative to the negative controls in % and is shown in Figure 2.

Sample combinations consisted of:

- negative control 1: mycotoxin standard mixture containing deoxynivalenol, zearalenone, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A, HT-2 toxin, T-2 toxin, Enniatin B, Fumonisin B1 and Fumonisin B2.
- negative control 2: 0.5% feed additive (Table 1)
- mycotoxin inhibitor: 0.5% feed additive (Table 1) + mycotoxin standard mixture containing deoxynivalenol, zearalenone, Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin A, HT-2 toxin, T-2 toxin, Enniatin B, Fumonisin B1 and Fumonisin B2.

Addition of a feed additive comprising betaine hydrochloride (HCl) to the feed did not lead to increased mycotoxin binding in the gastro-intestinal track environment when compared to a feed additive without betaine HCl.

Example 3: Study of the clinical effect of mycotoxins in pigs

50 pigs of either sex with average initial weight of 8.2 kg and average age 29 days were
 5 selected for the study. The animals were divided in 12 experimental groups. Half of the
 groups were fed with a feed that was contaminated with mycotoxins while the other half
 was fed with mycotoxin-free feed. Additionally, the feed of each group further differed
 in the presence or absence of a 0.5% feed supplement comprising 0%, 2.5% or 5%
 betaine hydrochloride (HCl). The feed supplement (see Table 2 for feed supplement
 10 composition) was admixed with the animal feed prior to feeding. The feed was
 administered 4 times per day and water was available ad libitum. The feed intake and
 weight gain was measured daily for 30 days. The Feed Conversion Rate (FCR) was
 calculated for each experimental group as the proportion of the feed intake (g) to the
 daily weight gain (g) and is used as a measure for pig health: the lower the FCR, the
 15 healthier the pigs.

Table 2: Feed supplement compositions used in example 3.

Component	0% Betaine HCl	0.0125% Betaine HCl	0.025% Betaine HCl
Betaine hydrochloride	0%	2.5%	5%
Mineral clays	80%	77.5%	75%
Yeast component	10%	10%	10%
Preservative	10%	10%	10%

The results of the study are shown in Figure 3. A surprising effect was found when pigs
 20 were fed with mycotoxin contaminated feed that was provided with the feed supplement
 comprising betaine HCl. The FCR of pigs fed with the feed according to the current
 invention was much lower, indicating these pigs were much healthier due to the liver
 supporting function of betaine HCl. Notably, the positive effect on pig's health was larger
 than what could have been expected of the combination of feed supplement and betaine
 25 HCl based on the results of the feed supplement alone (0.5% supplement and 0%
 betaine HCl) or the betaine HCl alone (0% supplement and 0.0125% or 0.025% betaine
 HCl).

CLAIMS

1. A composition for binding and inactivation of mycotoxins, comprising a yeast component and one or more mineral clays, characterized in that said composition further comprises betaine or a betaine derivative.
- 5 2. The composition according to claim 1, wherein the yeast component comprises a yeast cell wall extract.
3. The composition according to any one of claims 1 to 2, wherein the yeast component comprises a yeast cell wall extract from a yeast selected from the group consisting of *Saccharomyces*, *Candida*, *Kluyveromyces*, *Torulaspora* or a combination thereof.
- 10 4. The composition according to any one of claims 1 to 3, wherein the yeast component comprises a yeast cell wall extract from a *Saccharomyces cerevisiae* yeast.
5. The composition according to any one of claims 1 to 4, wherein the yeast component comprises a yeast cell wall extract composed of mannan-oligosaccharides.
- 15 6. The composition according to any one of claims 1 to 5, wherein said one or more mineral clays are selected from the group consisting of zeolites, bentonites, aluminosilicates, magnesium aluminum silicates or mixtures thereof.
- 20 7. The composition according to any one of claims 1 to 6, wherein said mineral clay is clinoptilolite.
8. The composition according to any one of claims 1 to 7, wherein said betaine derivative is betaine hydrochloride.
9. The composition according to any one of claims 1 to 8, wherein said composition has a total concentration of mineral clay of between 40% and 80% by weight of the composition.
- 25 10. The composition according to any one of claims 1 to 9, wherein said composition comprises between 5% and 10% yeast component by weight of the composition.
11. The composition according to any one of claims 1 to 10, wherein said composition comprises between 0.001% to 5% betaine or betaine derivative by weight of the composition.
- 30 12. A composition for use as toxin binder in animals and animal feed, said composition is a composition according to any one of claims 1 to 11.
13. The composition for use according to claim 12, wherein said toxin is selected from the group consisting of OchrA toxin, zearalenones, deoxynivalenol (DON), fumonisins, aflatoxins, HT-2 toxins, T-2 toxins and enniatins.
- 35 14. An animal feed comprising a composition according to any one of claims 1 to 11.

15. The animal feed according to claim 14, wherein the effective amount of the composition comprises from 0.5% to 1% by weight of the feed.
16. The animal feed according to any one of claims 14 to 15, wherein the animal is selected from the group consisting of avian, bovine, porcine, equine, ovine, and caprine species.
- 5 17. The animal feed according to any one of claims 14 to 16, wherein said composition comprises a preservative that is composed of a calcium, sodium or ammonium salt of propionate.
- 10 18. A method for feeding animals, where the animals consume a feed comprising a composition according to any one of claims 1 to 11 or an animal feed according to any one of claims 14 to 17, wherein the effective amount of the composition comprises from 5 g/kg to 10 g/kg bodyweight/day.
19. The method according to claim 18, wherein the composition is admixed with the animal feed prior to feeding.

Fig. 1

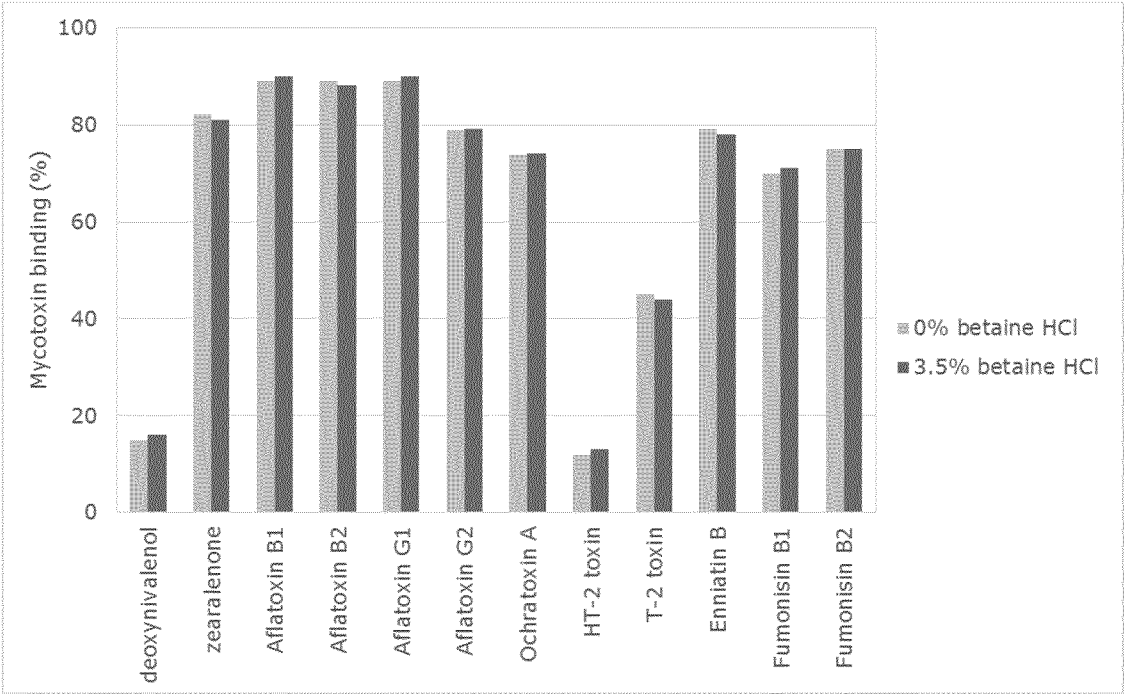


Fig. 2

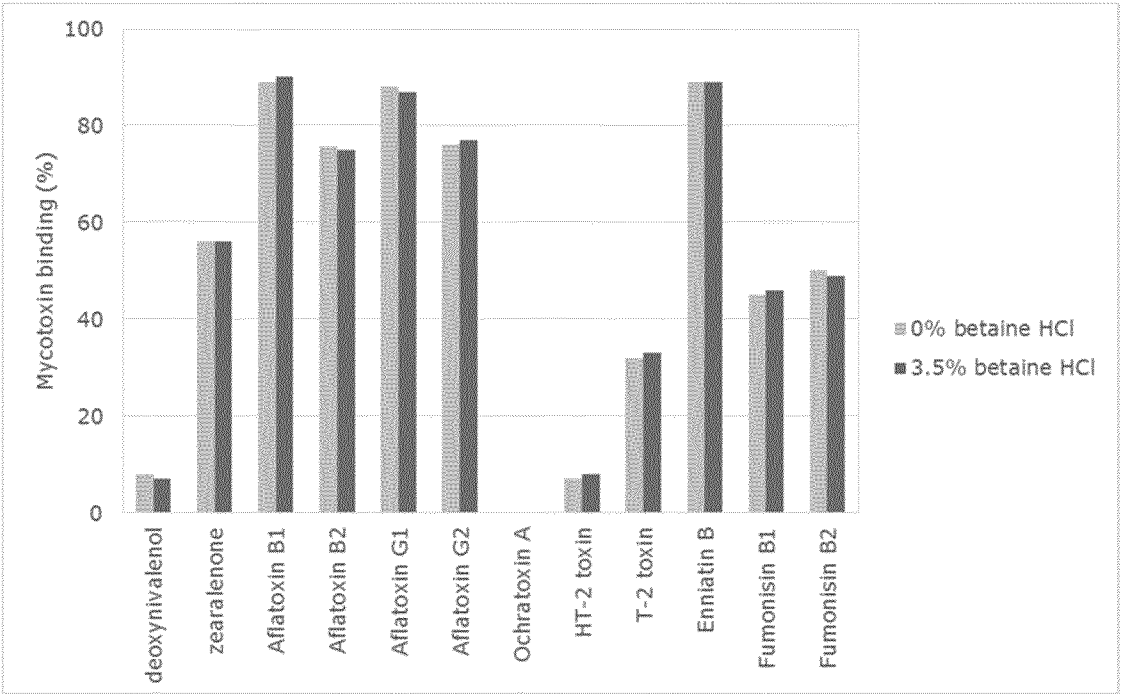
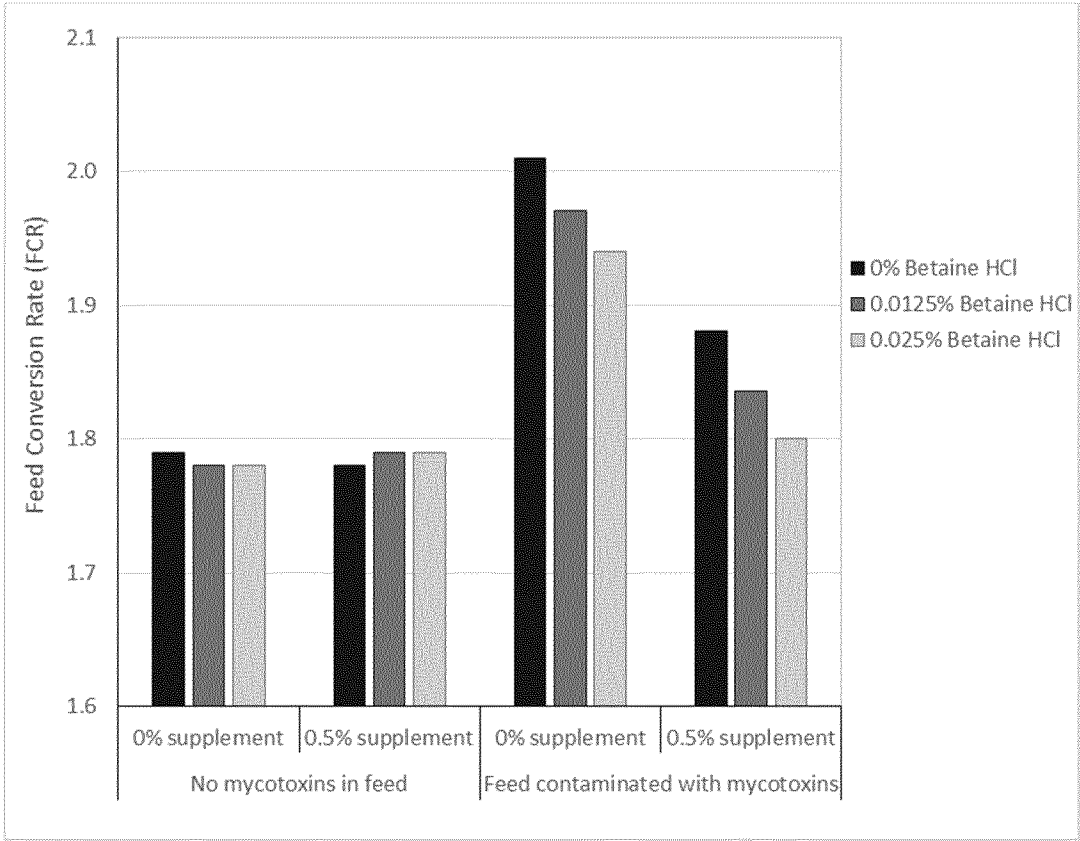


Fig. 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/078090

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K20/10 A23K20/153 A23K20/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K C11C A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 107 125 452 A (ZHENG QIQUAN) 5 September 2017 (2017-09-05) the whole document	1,6, 12-16
Y	----- WO 99/53772 A1 (ALLTECH INC [US]) 28 October 1999 (1999-10-28) cited in the application the whole document	1-19
Y	----- AU 2012 200 952 B2 (MANN STEPHEN; PARFITT DAVID) 23 October 2014 (2014-10-23) page 5, line 8 - page 13, line 12	1-19
Y	----- RU 2 391 975 C1 (G NAUCHNOE UCHREZHDENIE VSEKOS [RU]) 20 June 2010 (2010-06-20) cited in the application the whole document	1-19
	----- -/-	



Further documents are listed in the continuation of Box C.



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5 December 2018

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/078090

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/011825 A2 (TEXAS ENTEROSORBENT INC [US]; CARPENTER ROBERT H [US]; KEMP MAURICE CL) 25 January 2007 (2007-01-25) paragraphs [0004] - [0009]; claims 1-20; example 17	1-19
A	----- C Boudergue ET AL: "Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety", efsa, 1 January 2009 (2009-01-01), XP055383153, DOI: 10.2903/sp.efsa.2009.EN-22 Retrieved from the Internet: URL:https://www.efsa.europa.eu/en/supporting/pub/en-22 [retrieved on 2017-06-20] pages 19-71 -----	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2018/078090

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