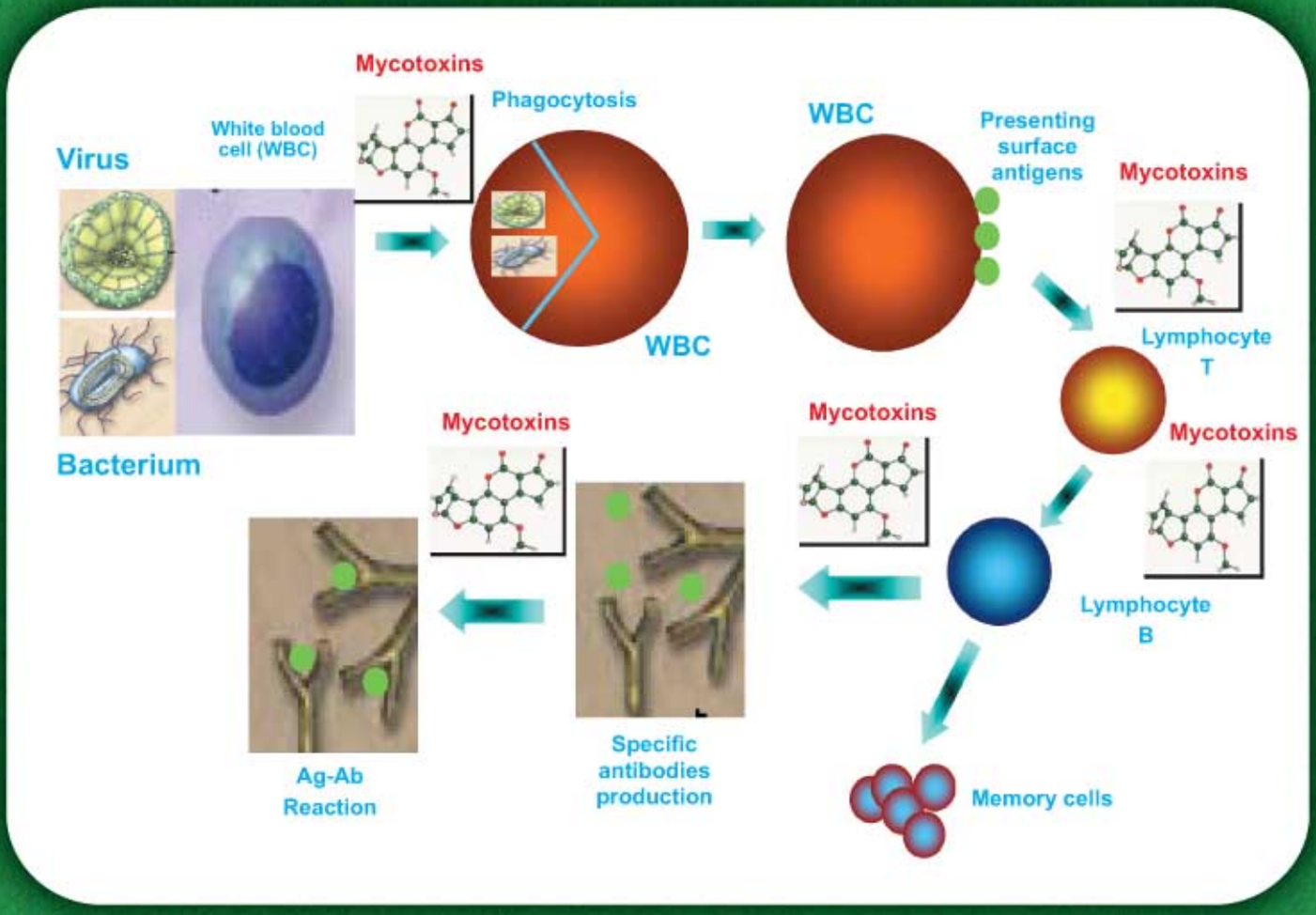
MYCOAD A-Z®



GENERAL TECHNICAL MANUAL

SPECIAL NUTRIENTS, INC.
The mycotoxins specialist

TABLE OF CONTENTS

Page

- Introduction 3

- Mycoad AZ characteristics and quality control 5

- *In vitro* evaluation 7

- Questions and answers 9

- Attachments

  - Swine
  - Poultry
  - Cattle (Mycoad AZ and Mycoad DF)

Fungal contamination of agricultural products is often unavoidable and of worldwide concern because these products frequently contain toxic metabolites known as mycotoxins. Mycotoxins contamination can occur in the crop, during harvest, at storage, or even after feed manufacturing. Mycotoxins are fairly stable compounds that cause a wide variety of deleterious effects in livestock, depending on the nature and concentration of toxins in the diet, animal species, age, and nutritional and health status at the time of exposure to contaminated feed. Several factors have an influence on the development of molds, including the moisture content of the grain or feed, environmental humidity and temperature, oxygen concentration, pH, and storage length.

Mycotoxins cause toxic, teratogenic, mutagenic, and carcinogenic effects, and/or depression of the immune system. Clinical immunosuppressive conditions can be confused with those caused by other pathogens. This is why it is frequently difficult to establish a precise differential diagnosis. The fact that a great variety of mycotoxins affect different organs in the urinary, digestive, and reproductive tracts, or the nervous and immune systems, among others, makes recognizing a mycotoxicosis condition even harder.

For many years, mycotoxins were studied individually in each species, disregarding that under field conditions contamination with only one mycotoxin normally does not occur. Only recently, the combination effect of several mycotoxins started to be evaluated. The scientific community is concerned about the fact that mycotoxin levels that used to be considered as safe in the past, have recently shown their ability to cause problems when combined with "low" levels of other mycotoxins. This potentiation of mycotoxin effects is due to the synergy that occurs when several mycotoxins are combined in the feed. One example of combined contamination in grains and feeds is the aflatoxin-fumonisin combination. Another example is vomitoxin and zearalenone, which are naturally found combined in the same grain or oil seeds/meals.

It has been consistently shown that the immune system is an important target for mycotoxins, causing adverse effects on the normal immune response, resulting in suppression of one or more immune functions. These immune failures predispose animals to severe vaccine reactions, low humoral/local antibody levels, and the presentation of diseases that are typically controlled with normal vaccination programs. In many occasions these effects cannot be seen, so that only lack of uniformity and poor productive parameters are reported.

For the last 20 years, hydrated sodium and calcium aluminosilicates (HSCAS) have been used for the control of the deleterious effects of mycotoxins. During 1987 Phillips and several collaborators analyzed the adsorption capacity of several adsorbents, demonstrating their aflatoxin-adsorbing effectiveness. Numerous myths have risen throughout the years about the use of clays for the control of mycotoxins. In some regions, people believe that all clays are polar, so that they only absorb aflatoxin. Some producers consider that most or all clays absorb nutrients, and they also believe that high inclusion levels (5 - 20 kg per metric ton) are needed for these clays to be effective. Regarding the idea that all clays are similar, and that they only adsorb aflatoxin, a scientific mineralogy literature review will show the existence of several types of bipolar clays, which can adsorb more than one mycotoxin. Regarding nutrient absorption, we can argue that many poultry studies demonstrated that several clays that adsorbed mycotoxins did not affect nutrient absorption whatsoever.

The terms clay and HSCAS are synonyms. They represent a great variety of minerals, including many types of clays with a wide variety of physicochemical traits. To show this wide diversity, we should state that no two equal clays exist in the world, since in any single mine, clay characteristics can change, and even minor variations that do not seem to affect the end product can vary its mycotoxin adsorption ability, as well as the types of mycotoxins adsorbed. Each clay type has a function in animal agriculture. Some of them are used to absorb water, and they are excellent for pelleting purposes, while others absorb ammonia in the GI tract of ruminants, or in the litter of poultry or pets. However, those types of clays have a limited action as mycotoxin adsorbents since –if any– they adsorb only aflatoxin.

Characteristics

Mycoad AZ, a purified HSCAS produced in Texas under a proprietary purification process, is a combination of dipolar phylosilicate clays that form stable and irreversible covalent bonds in the gastrointestinal tract, with the most important toxins affecting pigs, cows and poultry. The purification process allows the extraction of essentially all the non-clay fractions that interfere with the adsorption of mycotoxins, increasing the adsorption capacity of dipolar mycotoxins and/or of structurally difficult to capture mycotoxins such as zearalenone, a lipophilic mycotoxin. These properties facilitate the production of stable and irreversible complexes with the mycotoxins that are present in the lumen of the gastrointestinal tract. The process of activation also widens the spectrum and produces a mycotoxins binder with a higher potency.

Quality Control

During the production process, the clay is tested continuously to obtain a product which properties are consistently the same. Characteristics as level of moisture, particle size and concentration of surfactants in the final product are measured in each batch. If the standard levels are not accomplished the product is eliminated and used in other industries (oil fields). Also Mycoad AZ is tested for the presence of dioxin by Xenobiotic Detection Systems in Raleigh, North Carolina, USA, and each shipment sent to countries regulating the concentration of dioxin in the product, is tested before shipment to comply with the regulation from the European Union. To reassure that every batch produced is effective in mycotoxins control, each 18,000 kg shipment is tested *in vitro* using high performance liquid chromatography (HPLC) by a totally independent laboratory (Trilogy Analytical Labs, Missouri, USA) using 1 kg of Mycoad AZ tested against 3,000 ppb of zearalenone, fumonisin and ochratoxin, respectively (see Figure 5). Currently, this is the only mycotoxin binder supported by that type of testing, which represents the highest quality control standard in the industry. Besides the *in vitro* results, many trials have been performed *in vivo* demonstrating the efficacy against the most important mycotoxins affecting the swine, poultry and dairy industries worldwide. The *in vitro* results show an adsorption capacity of over 90% against the most important mycotoxins affecting these species. In the case of zearalenone a minimum efficacy of 95% is certified and for fumonisin and ochratoxin the efficacy is 85% and 90% respectively.

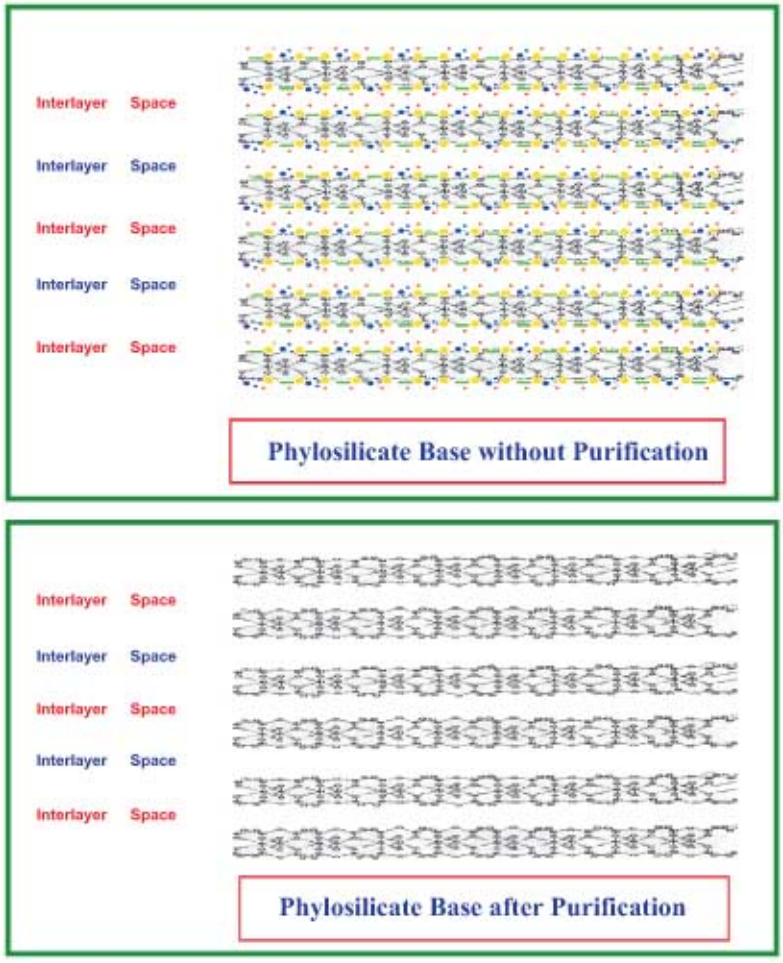


Figure1. Structural appearance of a phylosilicate (clay) before and after purification.

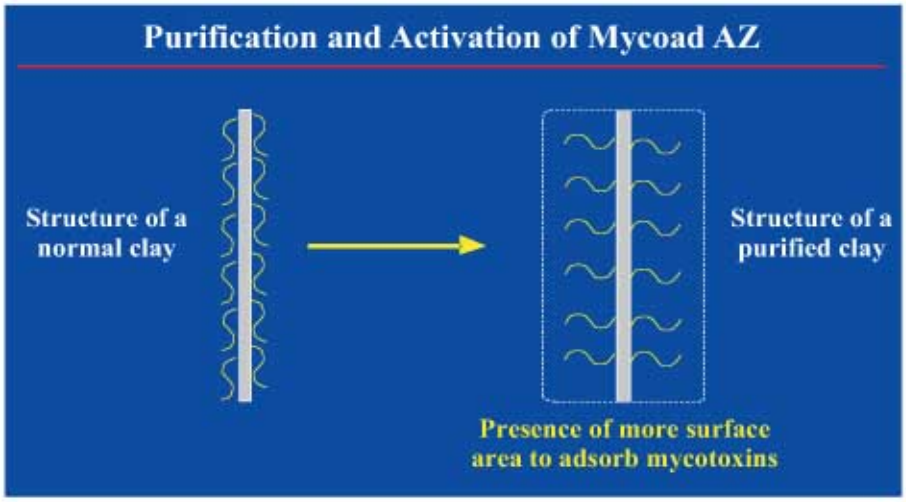
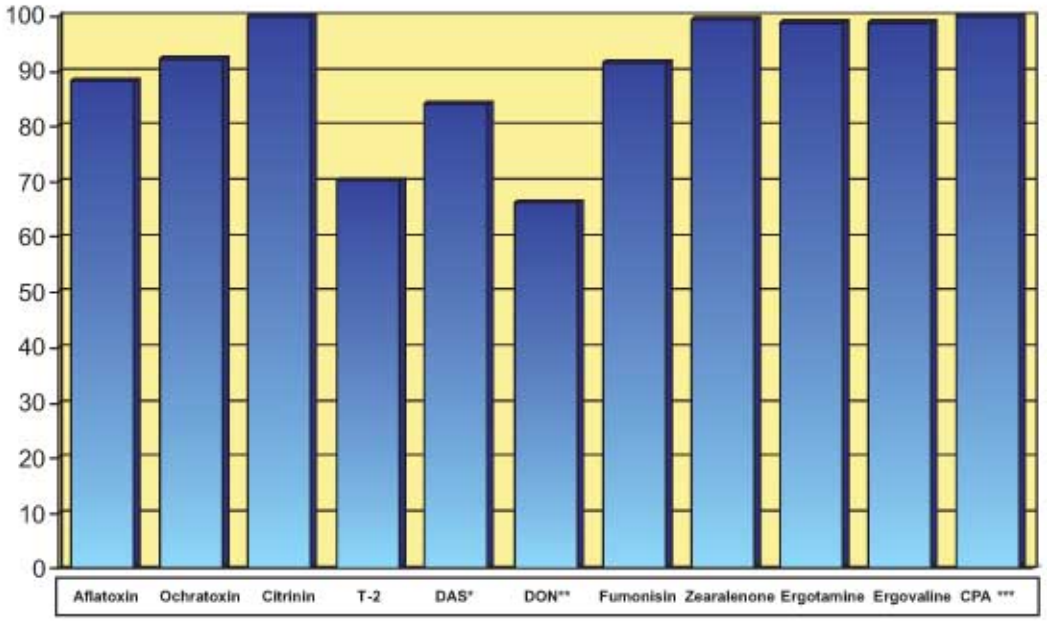


Figure 2. Graphic representation of the purification effect on mycotoxins adsorption.

In vitro evaluation is the first step that must be taken for the identification of a mycotoxin binder. This is so important that many researchers consider that if the product does not work *in vitro*, it will hardly work when used in animals (*in vivo*). Generally it is considered that a product with an 80% absorption capacity *in vitro* can potentially do a good job *in vivo*. It is important that *in vitro* results represent the net absorption of the product, meaning that both the adsorption and desorption processes have occurred, through a pH change, mimicking what occurs in the gastrointestinal tract (GIT) of animals. Adsorption results from tests performed only at a low pH do not assure that the product can retain the mycotoxins when the pH rises.



* DAS = diacetoxyscirpenol.

** DON = Deoxynivalenol. Mycoad AZ was tested at an inclusion rate of 2.5 kg/MT.

*** CPA = Cyclopiazonic acid.

Figure 3. Average net adsorption capacity of Mycoad AZ using HPLC test with 3 ppm (3,000 ppb) of all the mycotoxins tested and the equivalent of 1.0 kg of product per MT.

Universiteit Utrecht

Deoxynivalenol and *Zearalenone*
adsorption by two products obtained from
Jadis Additiva

RESULTS

Figure 4. *In vitro* test results of Mycoad AZ tested against zearalenone and vomitoxin at an independent laboratory in the European Union.

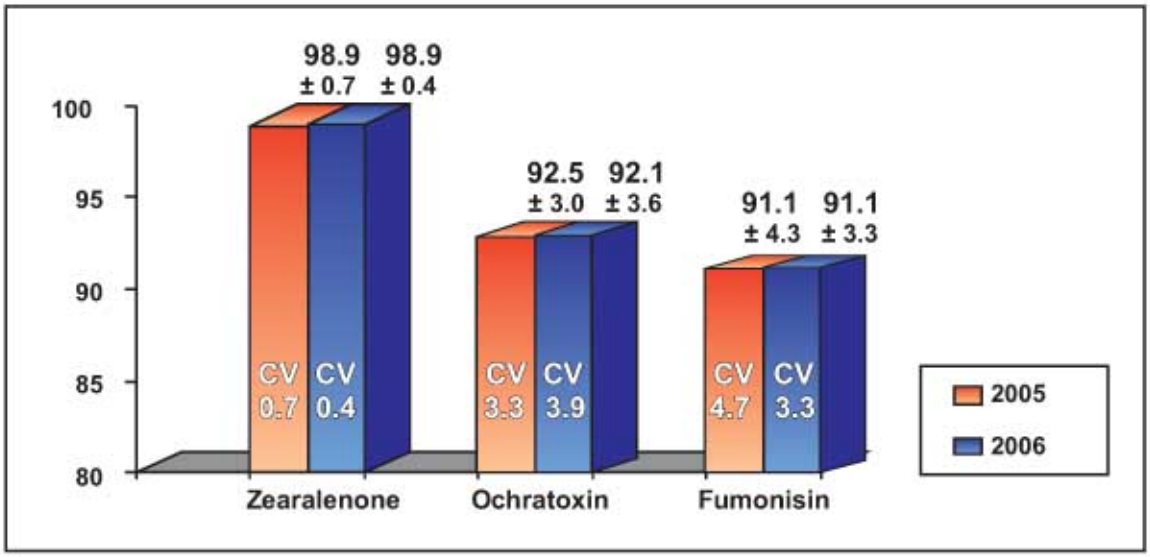


Figure 5. Average *in vitro* net adsorption results of more than 200 batches of Mycoad AZ tested in 2005 and 2006 showing its consistent effectiveness. *

* 1 kg of Mycoad AZ was tested in triplicate using 3,000 ppb of each mycotoxin.

What levels of mycotoxins can cause damage in different species?

The following tables include different mycotoxins concentrations that can cause damage in swine, poultry and cows. It should be taken into account that the levels regulated or recommended in the charts shown below are based in some cases on studies performed with only one synthetic mycotoxin, which are less toxic than natural mycotoxins. Therefore, the information included is only a guideline for the levels that can result in field problems. Another important limitation of these studies is that they do not consider the synergy of different mycotoxins, the interactions with other dietary noxious compounds, bacterial contamination, or the presence of livestock stressors such as poor ventilation, heat stress and poor management.

Mycotoxin	Maximum Concentrations Recommended
Aflatoxin	‹ 20 ppb (recommended by FDA, USA)
Zearalenone	‹ 300 ppb
Vomitoxin (DON)	‹ 300 ppb
T-2 Toxin	‹ 1,000 ppb
Fumonisin	‹100 ppb
Ochratoxin	‹ 200 ppb

Table1. Mycotoxins concentrations capable of causing deleterious effect in swine under experimental and commercial conditions.

Mycotoxin	Regulation or Recommendation USA & EU	Levels that Can Result in Immunosuppression
Aflatoxin	‹ 20 ppb	‹ 5 ppb
T-2 Toxin	‹ 500 ppb	‹ 100 ppb
Ochratoxin	‹ 20 ppb	‹ 5 ppb

Table 2. Mycotoxins concentrations capable of causing deleterious effect in poultry under experimental and commercial conditions.

Mycotoxin	Regulations or Recommendation USA & EU
Aflatoxin in feed	‹ 20 ppb (recommended by FDA, USA)
AFM1 in milk (Metabolite of aflatoxin)	EU ‹ 0.05 ppb (50 ppt) USA ‹ 0.5 ppb (500 ppt)
Zearalenone	‹ 250 ppb
Vomitoxin (DON)	‹ 300 ppb
T-2 Toxin	‹ 100 ppb
Ochratoxin	‹ 250 ppb
Fumonisin	‹ 3,000 ppb

Table 3. Mycotoxins concentrations capable of causing deleterious effect in dairy cattle under experimental and commercial conditions.

Where should samples be obtained from in order to detect mycotoxins contamination?

Establishing a sound feedstuff quality control program is crucial. Both, raw materials and finished feeds should be sampled. Obtaining representative samples for mycotoxin analysis is not an easy task, since mycotoxins are found in some areas inside the bins. They are not evenly spread in the feed ingredients or in the ration. In order to reduce errors, samples should be obtained from moving ground grain. Finished feed samples should be obtained directly from the farm feeders, so that they represent what the animals are actually eating. Even if ideal sampling recommendations are observed, in many cases it is not possible to detect high mycotoxin levels. This is why many practitioners use laboratory analyses such as histopathology, to reconfirm the diagnosis of mycotoxicosis observed in the field.

Is the concentration of mycotoxins related to the number of fungal spores detected in a gram of feed?

No. There is not a direct relationship.

Can molds or mycotoxins be eliminated with high temperatures?

Mycotoxins are not destroyed by high temperatures (extrusion or pelletization). During the cooling process of extrusion or pelleting molds are growing back in the feed

Do mycotoxins affect only feed conversion and mortality rates?

Mycotoxins first affect the immune system. This results in secondary problems that are typically misdiagnosed/confused with other pathologic conditions. The effect on the immune system results in stronger respiratory live vaccine reactions. Antibiotic use must be increased in order to control associated bacterial infections. In the case of enteric problems, increased susceptibility to salmonellosis and colibacillosis occurs.

When a mycotoxin binder is used, should a mold inhibitor be added as well?

It is important to remember that mold inhibitors cannot destroy mycotoxins. They inhibit the growth of the mycotoxin-producing molds. This means that if mycotoxins are already present in the feed, the effect of the mold inhibitor will have only limited value. Ideally, both mold inhibitors and mycotoxin binders should be used. But if one must be chosen, use only the mycotoxin binder, since the effect of mycotoxins on animals is much worse than that of molds.

Do mycotoxin binders have the ability to absorb essential nutrients for animal development?

This depends on the type of binder. Some of them can affect the absorption of certain nutrients in the GI tract, mainly those with a high cationic exchange capacity and that are also expansible. They retain water together with certain water-soluble nutrients. This group includes several bentonites and zeolites. This is why it is important to review the results of *in vivo* trials, in order to determine if the product has the ability to absorb some nutrient types. For many years many mycotoxin binders have been tested to determine if they are capable of absorbing nutrients in chickens, pigs and cows. The following tables show the results obtained in some of these evaluations in dairy cows and broilers where it is clear that the product used in these cases did not have a negative effect on the animals treated.

	Lactations #	Calcium (mg/100ml)			Phosphorus (mg/100ml)			Magnesium (mg/100ml)		
		30/01/02	11/02/02	14/02/02	30/01/02	11/02/02	14/02/02	30/01/02	11/02/02	14/02/02
CONTROL	1	9.4	*	9.8	6.5	*	5.3	2.3	*	2.2
	2	9.0	*	9.3	6.1	*	5.9	2.1	*	2.1
	3	8.7	*	9.7	6.3	*	5.2	2.0	*	2.0
	4	9.1	*	9.5	6.5	*	5.1	2.1	*	2.1
AZ 10 g	1	9.1	9.3	9.5	5.0	5.5	5.6	1.9	2.0	1.9
	2	9.1	10.2	9.7	5.0	5.6	5.0	2.0	1.9	2.1
	3	9.2	11.7	9.4	5.2	6.1	5.0	1.9	2.1	2.0
	4	9.0	9.7	9.5	5.4	5.6	5.3	1.9	2.0	2.0
AZ 20 g	1	9.3	10.6	9.4	5.0	5.8	5.7	1.9	1.9	1.9
	2	9.3	10.6	9.4	5.0	5.8	5.7	1.9	1.9	1.9
	3	9.2	10.6	9.5	5.5	5.9	5.7	1.9	1.9	1.9
	4	9.2	11.7	9.4	6.3	6.2	5.0	2.0	1.9	1.9
AZ 50 g	1	8.7	10.4	9.7	5.1	6.2	5.1	1.9	2.2	2.1
	2	9.1	10.6	9.3	5.9	5.5	5.0	2.0	2.3	2.0
	3	9.0	10.0	9.6	5.9	5.0	5.7	2.1	1.9	2.1
	4	9.1	10.4	9.3	5.6	5.5	5.1	2.0	1.9	1.9

Table 4. Effect of Mycoad AZ on the concentration of critical minerals in the blood of commercial cows during lactation after using 10, 20 and 50 g/cow/day.

* No test performed

TREATMENT	Bone Ash %	Bone Calcium %	Bone Phosphorus %
Control	45.95[a]	23.87[a]	8.71[a]
Control + Mycoad AZ	44.20[a]	25.11[a]	8.66[a]

Table 5. Effects of Mycoad AZ on bone mineralization of 38-day-old broilers exposed to test diets for 33 days.

[a] Means within columns with no common superscripts differ significantly ($P \leq 0.05$).

Reference: Casarin, A. M. Forat, E. Soto, and D. Zaviezo. Evaluation of the efficacy of a commercial purified phylosilicate to reduce the toxicity of T-2 toxin in broiler chicks. International Poultry Scientific Forum. Atlanta, GA, USA, 2006.

What is the advantage of using a low inclusion level mycotoxin binder in the feed?

Traditionally, nutritionists refuse to use high inclusion level mycotoxin binders since these products use valuable space within the feed formulation without contributing with any nutritional value. This is more critical in broiler diets, where high energy/amino acid density should exist in order to fulfill the requirements of genetically rapid growing broiler strains. One additional advantage is that using low inclusion rate mycotoxin binders reduces their possibilities to absorb essential nutrients such as vitamins and minerals. Also, the cost of the product inclusion into the feed is decreased when compared with other similarly-priced binders.

Numerous technical manuals recommend a lower commercial dose rate than the significantly effective dose shown in the scientific studies that appear in the same manual. Why?

This is done to show that the addition of the binder has little incidence in the final cost of the feed. In the poultry industry, the cost of the mycotoxin binder inclusion is very important, since the production cost of feed is consistently questioned. Feed typically represents in excess of 2/3 of the production cost of one pound of meat. If a low dose rate is recommended, inclusion will be less expensive, making it more attractive to be used in the feed. However, a reduced dose will not provide effective protection in the presence of mycotoxins.

Are the mycotoxin binders containing bacteria, yeasts, or enzymes of any value to degrade mycotoxins?

So far, no mycotoxin binders exist that do not contain clays, since clays are the only products *per se* that have shown efficacious, consistent results. Detoxification with enzymes, bacteria or yeasts should result in a fairly rapid mycotoxin biotransformation, prior absorption occurs in the small intestine. One additional concern about their mode of action is that biotransformation sometimes results in secondary metabolites that are more toxic than the original mycotoxin.

Enzymes. The main disadvantage of using enzymes is that their activity greatly depends on the conditions existing in the GI tract, especially acidity, and the presence of other dietary enzymes/ingredients. Most mycotoxins are denatured when subjected to pelleting/extrusion processes. Therefore, a simple analytical method should exist to determine enzyme levels prior to and after feed manufacturing.

Bacteria. One additional biotransformation mechanism, that occurs naturally, is through the action of anaerobic bacteria present in the distal GI tract. Successful colonization of the GI tract with foreign bacteria greatly depends on the microflora already established and on the conditions existing in the GI tract. When bacteria are used in the feed, it is important to avoid the use of antibiotics either as growth promoters or for therapeutic purposes. Antibiotics have shown potential to destroy beneficial microflora. In addition, high pelleting/extrusion temperatures are very likely to destroy the bacteria present in the feed.

MYCOAD A-Z®

DESCRIPTION

Mycoad AZ is an activated, broad spectrum, hydrated, sodium/calcium aluminosilicate (HSCAS), specially formulated to adsorb and retain all major mycotoxins affecting poultry, swine, and cattle health and productivity.

DOSE RATE

1 kg per metric ton of feed.

APPLICATION

Add **Mycoad AZ** to the mixer, together with all other feed ingredients. Mix homogeneously.

COMPATIBILITY

Mycoad AZ is compatible with all feed ingredients. **Mycoad AZ does not affect or adsorb** any of the feed components (amino acids, vitamins, minerals, antibiotics, and coccidiostats).

CHARACTERISTICS

Cream-colored, fine powder.

PACKAGING

25 kg bag. Four-ply bags (three paper plies and one inner plastic ply).

MYCOAD A-Z®

Each shipment is tested for efficacy (HPLC) using 3,000 ppb of the mycotoxins evaluated and 1 kg/MT of the product:

Zearalenone = > 95% efficacy

Fumonisin = > 85% efficacy

Ochatoxin = > 90% efficacy

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