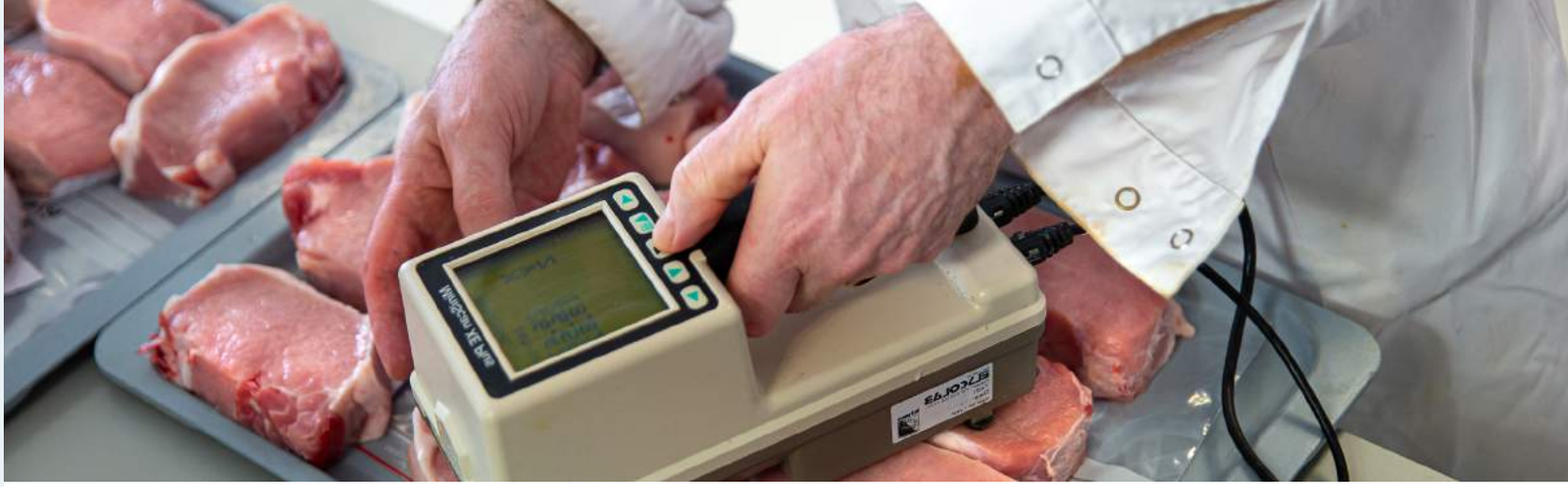
agrimprove
we farm ideas

ENDOTOXINS

IN ANIMALS

agrifirm

Table of content



What are endotoxins?

Gram-positive and Gram-negative bacteria are differentiated, among other things, by their type of cell envelope. Gram-negative bacteria are encircled by a thin peptidoglycan cell wall, that is also enclosed by an outer membrane containing lipopolysaccharides (LPS), one of the main components of the endotoxins responsible for the damage present in infected animals. Gram-positive bacteria do not have an outer membrane; therefore, endotoxins are not present in this group. Gram-negative bacteria have a cell envelope which contains three essential layers or membranes: cytoplasmic (inner), peptidoglycan or R-layer and the outer membrane. The latter contains phospholipids, proteins and LPS. On the other hand, LPSs consist of three elements: Lipid A, a hydrophobic component that serves as an anchor when a bacterium is invading a host's cell. The core, an oligosaccharide and the O antigen, which is a hydrophilic component. Lipid A is apparently responsible for most of the toxicity caused by endotoxins.

Figure 1. Differences between Gram negative and Gram positive bacteria

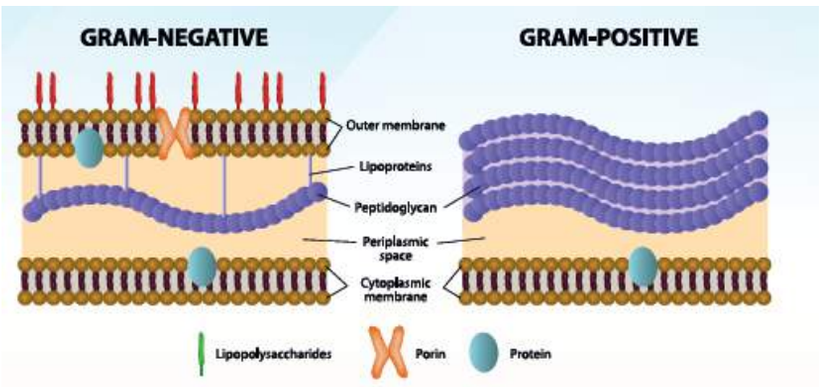
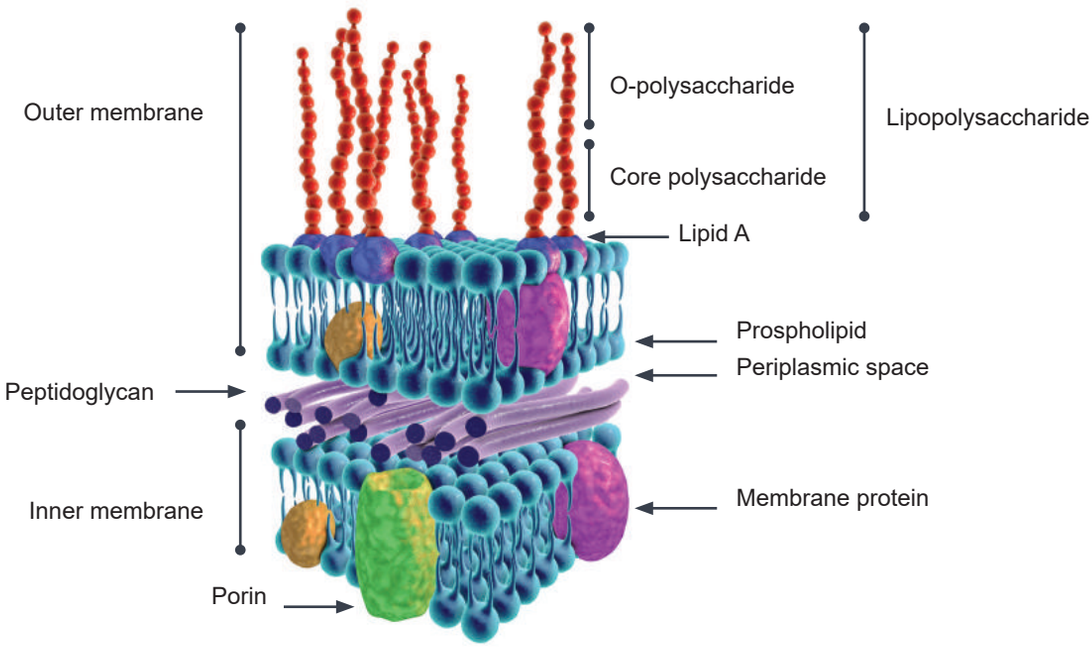


Figure 2. Important structures of a Gram negative bacteria



Endotoxins are common in the gastrointestinal tract (GIT) of animals, particularly in the large intestine. The most important bacteria that contain endotoxins in its structure belong to a very common group of bacteria called enterobacterias (typically present in the intestines). They are also present in the bioaerosols that produce inflammatory reactions particularly in the respiratory tract. It has been detected that bioaerosols, coming from feed, litter and feces have the highest concentration of bacterial endotoxins. When endotoxins are present in the GIT and excreted together with feces, these endotoxins will be attached to the dust particles present in the farm and end up in the air, which cause the inhalation of particles by the animals. As a result, we can observe respiratory or gastrointestinal diseases affecting the animals that inhaled them.

Under normal commercial production conditions, a small number of endotoxins will be transferred from the GIT to the blood stream. According to scientific papers, it represents concentrations lower than 10 pg/ml (picograms per milliliter) and these levels are capable of stimulating the immune system. Under stress, as during labor, the level of free endotoxin in the GIT will be higher. All this stress can produce a dysbiosis or dysbacteriosis, that is, microbial imbalance in the intestines, and the production of more endotoxins in the system. It is critical to emphasize the importance of keeping the correct balance in the intestines because diseases as Salmonellosis or Colibacillosis are intrinsically associated with the unrestricted growth of pathogenic bacteria that displace the intestinal favorable microflora (Lactobacillus to mention one example).

When bacteria are eliminated as a result of the administration of antimicrobials or because of the work of the immune system, bacterial cell will be destroyed and the final consequence is the liberation of endotoxins that will harm the animal. Endotoxins are released from the bacterial cell wall during the growth and division phases of the microbe. Also, they are liberated when getting in contact with water. Once endotoxins are released inside the body of the animal, an immune reaction is started that depends on the type of LPS present. Other factors important in the effect are the duration of exposure, concentration, genetic makeup of the animal affected and the presence of other bacterial or viral infections.

How to prevent their damage

1. Vaccination with a portion of the endotoxins. Lipid A has been tested in vaccinations to protect against the deleterious effects caused by endotoxins. The main problem of this approach is the high cost of producing these types of vaccines.

2. Use of mycotoxin absorbents (modified clays) in the feed. Not all binders are capable of adsorbing substances that are liposoluble (soluble in fat) inside the gastrointestinal content. Since Lipid A is a lipophilic toxin, it is necessary to use a binder that has that capacity.

In vitro trials

This certificate shows the *in vitro* adsorption results of MYCOAD AZ against an endotoxin using HPLC (High Precision Liquid Chromatography). A commercial endotoxin, E-Toxate, from Sigma-Aldrich Corporation, USA, was used in the test. This endotoxin is produced from Escherichia coli O55:B5 LPS and is standardized against USP (U.S. Pharmacopeia) reference standard endotoxin. The test was performed using a pH of 5.0 for adsorption and 6.5 for desorption with the objective of mimicking the conditions present in the GIT of animals. The results of this *in vitro* test have been corroborated in several occasions.

Certificate of Analysis

TRILOGY
Analytical Laboratory

Special Nutrients
7764 Douglas Road
Miami, FL 33133

Date Received: 4/27/2016
Date Reported: 5/4/2016
Certificate No: 2473875
Trilogy ID: S1610511126

Sample Description: MYCO-AD A-Z 1 APRIL 2016 16D01PZX

% Desorption	0.0	0.0
	0.0	0.0
	0.0	0.0
% Desorption Average	0.0	0.0
% Efficiency	99.1	98.6
Inclusion Rate:	1 kg/ton	500g/ton

Toxin Concentration: 45.2 EU/mL
Adsorption pH: 5.0
Desorption pH: 6.5

Reference: Trilogy Analytical Lab Internal SOP 12.110 with Extension adaption

Results Approved By:

Julio Knuckhorst Laboratory Representative

The results of these tests are performed under ambient temperature and humidity. These results relate only to the samples tested. This report shall not be reproduced except in full, without written approval of the laboratory. Samples retained for 30 days after receipt. All analytical results inherently contain a degree of uncertainty. Our methods have been validated and specific uncertainties determined for each method. Uncertainty measurements are available upon request.

Page 1 of 1 Trilogy Analytical Laboratory, Inc. 870 Woodbine Drive Washington, MO 63090 Phone: 636.239.1521 Fax: 636.239.1531

MYCOAD AZ vs. endotoxins in blood

In this evaluation performed in blood, the endotoxin concentrations were obtained using an Elisa kit originally developed for the detection of endotoxins in humans and manufactured by Biochek in Germany. This evaluation was conducted at the University of Leipzig and the sows were located in commercial farms. Sows were bled at the beginning of the experiment before adding MYCOAD AZ and bled again after 21 days of treatment with MYCOAD AZ.

Table 1. Effect of feeding MYCOAD AZ (1 kg/ton) to commercial sows for 15 consecutive days on the concentration of natural endotoxins in the blood of commercial sows.

Sow Identification #	Endotoxin (ml/L) before adding MYCOAD AZ	Endotoxin (ml/L) in blood after adding MYCOAD AZ
123	4.55	<0.05
125	30.86	<0.05
126	<0.05	<0.05
127	>50	<0.05
128	22.88	<0.05
129	2.85	<0.05
130	47.33	<0.05

Conclusion

Under the conditions of this field evaluation, MYCOAD AZ was able to reduce the concentration of endotoxins detected in blood.

MYCOAD AZ effect vs. endotoxins in feces

Objective

Determine the direct influence of MYCOAD AZ on the level of free endotoxins in feces suspensions of commercial pigs tested.

Materials and methods

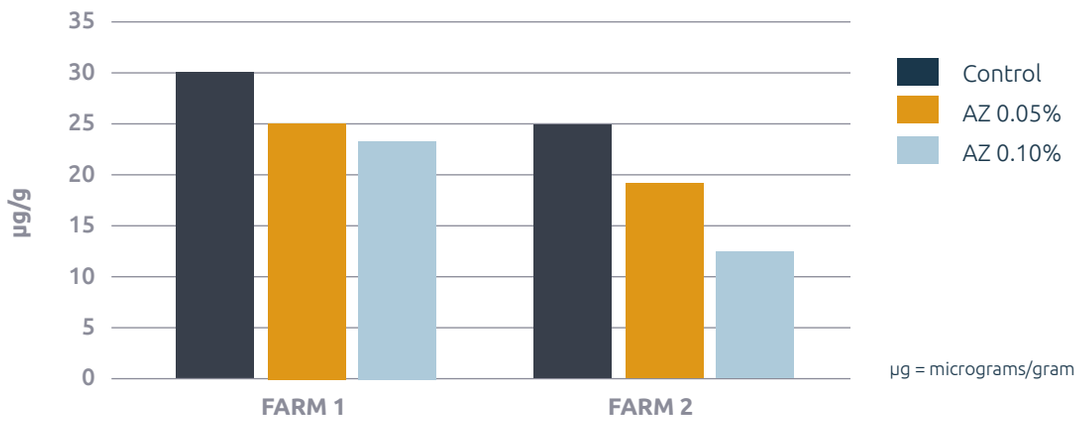
Several commercial farms with a history of bacterial endotoxins detected in the blood of sows, piglets, weanling and fattening pigs were tested in this experiment. Five pooled fecal samples coming from different farms were evaluated. Fecal samples from 10 randomly selected animals from the 5 herds were collected. The ages of the animals tested were the following: post-partum sows, 10 and 20-day-old piglets, weanling and fattening pigs. The concentrations of MYCOAD AZ (0.05% and 0.10%) tested were performed in duplicates. Sterile saline solution (PBS) was used as medium to mix the feces taken from the animals and diluted 1:10 with the medium and placed for one hour at 37 C in a shaker. After centrifugation, the supernatant was used to measure the concentration of free endotoxins. An Elisa kit developed by Biochek in Germany for the identification of endotoxins in human was used in this experiment. This evaluation was conducted at the University of Leipzig in Germany.

Table 2. Free endotoxin (mg/g) in pooled pig feces of different age groups and farms after adding Mycoad AZ at two different concentrations.

Farm	Age	Control	MYCOAD AZ 0.05% (0.5 kg/T)	MYCOAD AZ 0.10% (1 kg/T)
1	Sow post-partum	19.70	11.40	16.70
	10 d	2.30	2.90	3.00
	20.d	4.70	2.90	2.30
	fattening	3.20	2.20	3.30
2	Sow post-partum	3.40	4.20	5.00
	20.d	146.30	54.70	31.80
	weaning	1.00	1.90	1.20
	fattening	28.00	18.20	4.80
3	Sow post-partum	33.80	12.10	13.70
	10.d	31.50	31.10	24.00
	20.d	19.70	25.20	20.60
	weaning	22.80	22.80	18.20
4	Sow post-partum	33.80	12.10	13.70
	20.d	30.40	25.70	12.50
	weaning	2.30	4.20	3.30
	fattening	4.60	9.40	5.00
5	Sow post-partum	22.30	16.10	8.50
	10.d	12.10	12.00	8.90
	weaning	14.80	10.60	8.50
	fattening	3.20	2.20	3.30

n= 10 in each group

Graph 1. Free entotoxin µg\g in sow feces of two farms 1-2 days post-partum.



Conclusion

Table 2 shows the effect of adding MYCOAD AZ on free endotoxin concentration in feces in 5 different farms at different stages of production. There is no clear effect when the endotoxin levels are low. On the other hand, when the endotoxin concentration is high, the use of MYCOAD AZ normally results in a decrease of endotoxins. This effect can be observed clearly in the results of two farms shown in Graph 1. There, the addition of MYCOAD AZ at two different dosages resulted in a decrease of free endotoxin in feces of sows 1-2 days post partum. This preliminary study indicates some degree of reduction in the concentration of endotoxins, in particular when endotoxin levels are high.

MYCOAD AZ vs. effectiveness on performance

Three commercial experiments were performed in Holland from 2007 until 2011 to evaluate the effectiveness of MYCOAD AZ in the performance of piglets not exposed to significant levels of mycotoxins. Animals were kept in an experimental farm under an excellent husbandry. In the 3 trials reported here, the average nutritional values of the weaner and starter diets for all treatments are listed below.

Table 3. Average nutritional values for all the experimental diets tested from 2007 through 2011.

	Weaner rations (fed for the first 13 days of age)	Starter rations (fed from 13 to the end of the trial)
Crude protein (%)	18.5	18.0
Crude fat (%)	5.9	4.6
Crude fiber (%)	4.0	4.3
Ash (%)	5.5	5.4
Net energy (kcal/MJ)	2361/9.9	2306/9.7
Lysine (g/kg)	11.5	11.2

Trial 1

The goal of this trial, performed in 2007 was to evaluate the effect of 0,5 kg/ton MYCOAD AZ in the performance of weaned piglets which did not show any clinical symptoms of mycotoxicosis. Two treatments were evaluated, one was a control group and the second was administered 0.5 kg/ton MYCOAD AZ in the weaner and starter diets feeds for a total of 32 days. 386 piglets (Topigs 30 x Tempo) were equally divided over 60 pens, (30 pens per treatment with 6-7 piglets per pen), based on genetics, sex and weaning weight. The animals consumed a weaner diet from day one till day 13. From day 13 until the end the trial, they were fed a starter diet. Weight gain and feed intake were recorded per pen.

Results

A feed intake and weight gain 2% higher in the treatment that included MYCOAD AZ was detected. No difference was reported in feed conversion. This trial showed that supplementing diets of weaned piglets with MYCOAD AZ has a positive effect on the performance of piglets until 32 days after weaning.

Table 4. Performance from weaning (26 days of age) until the end of the trial (59 days of age).

	Control	0.5 kg/T MYCOAD AZ	Difference
Weaning weight	8.1	8.1	0%
Weight day 59	23.8	24.2	+2%
Daily weight gain	492	502	+2%
Daily feed intake	733	751	+2%
Feed conversion	1.49	1.50	0%

Trial 2

After getting the results reported in trial # 1, a second test was organized in 2010 adding a higher dose of MYCOAD AZ (1.0 kg/ton instead of 0.5 kg. /ton). Again, two groups were evaluated, a negative control and a group which received diets containing 1.0 kg/ton MYCOAD AZ. 178 piglets (Topigs 30 x Tempo) were equally divided amongst 28 pens (14 pens per treatment with 6-7 piglets per pen), based on genetic, sex and weaning weight. From weaning until 13 days after weaning a weaner diet was fed, from 13 days after weaning until the end of the trial a starter diet was fed. The diets were also analyzed for mycotoxin detection and all the values present were below tolerable levels (Table 5). Weight gain and feed intake were recorded per pen.

Table 5. Analysis of mycotoxins in the rations used in the experiment.

Mycotoxin analyses (µqlkg)				
Aflatoxin B1, B2, G1, G2	<1	<1	<1	<1
Deoxynivalenol*	<100	<100	<100	<100
Fumonisin B1, B2*	<5	<5	<5	<5
T-2 Toxin*	<50	<50	<50	<50
Zearalenone*	15.8	13.9	15.8	13.9

* Analysis of these mycotoxins in average sample of control diets and diets with MYCOAD AZ

Results

Table 6 shows that in the group treated with MYCOAD AZ a feed intake 6% higher and a weight gain 6% higher than the treatment where no product was added. Regarding feed conversion results, in the weaner period FC was higher in the MYCOAD AZ group, while this group had a lower feed conversion ratio in the starter period. Overall feed conversion ratio is slightly better for the piglet fed diets with MYCOAD AZ.

Table 6. Results from weaning (25 days of age) until end of trial (59 days of age).

	Control	1 kg/T MYCOAD AZ	Difference
Weight			
Weaning weight	8.1	8.1	0%
Weight day 38	12.1	12.2	+1%
Weight day 59	23.4	24.3	+4%
Daily weight gain			
Weaner period	303	309	+2%
Starter period	564	608	+8%
Overall	461	490	+6%
Daily feed intake			
Weaner period	337	368	+9%
Starter period	905	943	+4%
Overall	681	717	+5%
Feed conversion ratio			
Weaner period	1.12	1.19	+6%
Starter period	1.61	1.56	-3%
Overall	1.48	1.47	-1%



In conclusion, the inclusion of 1.0 kg/ton MYCOAD AZ in the absence of significant levels of mycotoxins, had a positive effect on feed intake and weight gain of the piglets evaluated in this trial. Final weights were almost 1 kg higher at 59 days of age. When comparing these results to the ones obtained in the previous trial where 0.5 kg/ton of MYCOAD AZ was added, the improvement in performance (feed intake and weight gain) is about two times higher and therefore dose-dependent.

Trial 3

After reviewing the results shown in experiment 1 and 2, a third trial was run in 2011 measuring the effect on performance of adding 1.0 kg/ton of MYCOAD AZ in weaned piglets, when the product was only added to the weaner diets. The nutritional formula for the starter rations were exactly the same for both groups. Again, two treatments were evaluated, a negative control without MYCOAD AZ and a treatment where a 1.0 kg/ton of MYCOAD AZ was included in the weaner diets. 186 piglets (Topigs 30 x Piétrain) were divided amongst 30 pens (15 pens per treatment with 6-7 piglets per pen), based on genetic, sex and weaning weight. From weaning until 13 days after weaning a weaner diet was fed, from 13 days after weaning until the end of the trial both groups received the same starter diet. The diets were also analyzed on mycotoxin content, all values were within tolerable limits (Table 7). Weight gain and feed intake were recorded per each pen.

Table 7. Mycotoxin levels detected in the diets evaluated in this trial.

	Weaner diets (weaning - 13 days after wenaing)		Weaner diets (weaning - 13 days after weaning - end trial)	
	Control	MYCOAD AZ	Control	MYCOAD AZ
Aflatoxin B1, B2, G1, G2	<1	<1	<1	<1
Deoxynivalenol*	<100	<100	<100	<100
Fumonisin B1, B2*	<5	<5	<5	<5
T-2 toxin*	<50	<50	<50	<50
Zearalenone*	15.8	13.9	15.8	13.9

Results

Although MYCOAD AZ was only supplied in the weaner phase, the positive effect on feed intake and weight gain is also observed in the starter phase. At the end of the trial the piglets were on average 0.8 kg heavier, when 1.0 kg/ton MYCOAD AZ was included in the weaner diet. No effect on feed conversion was detected.

Table 8. Results from weaning (25 days of age) until end of trial (59 days of age).

	Control	1 kg/T MYCOAD AZ	Difference
Weight			
Weaning weight	8.3	8.3	0%
Weight day 38	11.1	11.2	+1%
Weight day 59	23.1	23.9	+3%
Daily weight gain			
Weaner period	213	226	+6%
Starter period	580	611	+5%
Overall	439	463	+5%
Daily feed intake			
Weaner period	267	283	+6%
Starter period	841	881	+5%
Overall	620	650	+5%
Feed conversion ratio			
Weaner period	1.25	1.25	0%
Starter period	1.45	1.44	-1%
Overall	1.41	1.41	0%

The inclusion of 1.0 kg/ton MYCOAD AZ in the weaner diet only, resulted in 5% higher feed intake and 5% higher weight gain; both in the weaner and starter phases. This trial shows that the positive effect of MYCOAD AZ in low mycotoxin diets might be created in the weaner phase, but continues in the starter phase. The improvement in feed intake and weight gain are comparable with the results observed in previous trials. The results obtained in the 3 trials presented here show the positive effect on performance of adding MYCOAD AZ to the diet, even when mycotoxins are not detected in feed analyses. Based on the detection of endotoxins in the feed, we speculate that the improvement is related to the identification in endotoxins in feces and blood, as determined in the *in vitro* test performed at the University of Leipez in Germany. It is important to emphasize that the experimental farms used in these trials were extremely clean, as typically happens in the case of experimental farms.

Conclusion

MYCOAD AZ is an excellent option to control the deleterious effects caused by endotoxins in animal production.

Does your Anti-Mycotoxin Additive meet the basic TOP and FACTS?

Top Organ Protection

MYCOTOXIN	ORGAN	MYCOAD	MYCOAD AZ
Aflatoxin	Liver	YES	NO
Ochratoxin	Kidney	YES	NO
T-2 toxin	Oral lesion	YES	YES
Fumonisin	Heart/Lung/Liver	YES	YES*
Zearalenone	Reproductive	N/A	YES
DON	Liver	N/A	YES

FACTS	MYCOAD	MYCOAD AZ
In vivo dosage with TOP results	2.5 kg/MT	1 kg/MT*
Commercial recommended dosage	2.5 kg/MT	1 kg/MT
The clay component always from the same mine	YES	YES
Approved in Texas, USA, against Aflatoxin	YES	N/A
Approved in the European Union against Aflatoxin	YES	N/A
Number of mycotoxins tested by LAMIC and other institutions	4	4
Number of species tested and approved by LAMIC and other institutions	6	6
Nutrient Absorption	NO	NO
In vitro efficacy test every 18 MT or 100 MT	YES	YES
Endotoxin adsorption	N/A	YES

*Test performed with 4 kg with 30,000 ppb of Fumonisin

Claims associated with products may be different based on government requirements. Certain statements may also not be applicable in all regions.

More information
www.agrimprove.com
agrimprove@agrifirm.com

Better Together

agrifirm
SPECIAL NUTRIENTS
MEMBER OF THE ROYAL AGRIFIRM GROUP