

# Naturally innocuous

▶ TO ANIMALS



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## Introduction

Around the world, anti-mycotoxins additives have been used for decades to prevent the deleterious effects caused by them. Historically, one main concern is the possibility of these products negatively affecting the absorption of essential nutrients, such as minerals and vitamins from the gastrointestinal tract. Sporadically some farms have reported that some products are capable of harming the nutritional wellbeing of the animals. Taking into consideration all these factors, it is very important to review the results of *in vivo* trials, to determine if the product has the ability to absorb critical nutrients. It is important to point out that when we explain the materials and methods for the scientific experiments and field trials included in this brochure, we emphasize the part of the trial where the innocuity was evaluated. Therefore, we are not including many details on target organ protection and other measurements that also were evaluated in the trials reported here. If additional information on how the entire experiments were performed, by looking at the original publication, readers can see all the details on how they were performed. Among the works included here, we report an *in vitro* test showing the effect of MYCOAD AZ on vitamins and minerals. Other experimental trials with animals show how innocuous both products are. Regarding the effect on bone structure, bone composition is measured in broiler chickens and ducks, as well as the concentration of essential minerals in the blood of lactating cows. A golden indicator of problems with nutrients absorption is skin pigmentation in chickens which is measured in an experiment included here.

## *In vitro* test with MYCOAD AZ

The objective of this test was to demonstrate that MYCOAD AZ does not absorb vitamins or minerals. With this objective, 100 g of MYCOAD AZ was mixed separately with 100 ppm of vitamins D, E and B1 and 100 ppm of magnesium, zinc, iron, copper and selenium sulphates. Distilled water was used as diluent for water soluble vitamins and hexane for fat-soluble vitamins. Once the substances tested were mixed, they were kept shaking for 30 minutes and later filtered with filter paper. The solid residues obtained after filtration were separated and mixed again with similar aliquots of distilled water or hexane, depending on the element tested and kept for acid digestion for 30 minutes. After that step, the concentration of vitamins for each solution was measured using HPLC (High Performance Liquid Chromatography) with a standard variability of 5%. The concentration of minerals was determined with atomic absorption spectroscopy (AAS). In the final results, 6 of the 8 nutrients tested showed a difference of 0.3% to 1.2% between the initial and final concentrations. The nutrients showing a major difference were vitamin E (-2.5%) and Selenium (- 3.7%). Considering the normal variability expected in HPLC (+/- 5%) and AAS (+/- 2%), the results shown in this test for vitamin E are in the normal range. In the case of selenium, the results are slightly out of range. However, taking into consideration that commercial rations are not formulated using marginal levels of vitamins and minerals, specially vitamin E and selenium, we do not expect to observe any negative nutritional effect for the health and performance of animals where MYCOAD AZ is mixed in the ration.

*In vitro* evaluation of vitamins and minerals mixed with the equivalent of 2 kg of MYCOAD AZ/ MT of feed.

Source: Saarka Nutrición y Tecnología. México. April 2007. (Not published)

Nutrient	Initial ppm	Final ppm	Difference %
Vitamin D	100	99.1	0.9
Vitamin E	100	97.5	2.5
Vitamin B1	100	99.7	0.3
Magnesium	100	99.2	0.8
Zinc	100	98.8	1.2
Iron	100	99.4	0.6
Copper	100	99.3	0.7
Selenium	100	96.3	3.7

## Cows. MYCOAD AZ effect on critical minerals in blood

The objective of this experiment, performed in a commercial dairy farm in Puerto Chico in Mexico, was to measure the concentration of critical minerals in the blood of lactating dairy cows using the following parameters as a guide:

- Calcium (Ca): 9.0 to 12.4 mg/100 ml
- Phosphorous (P): 5.0 to 6.5 mg/100 ml
- Magnesium (Mg): 1.8 to 2.3 mg/100 ml

Three different inclusion rates of MYCOAD AZ were tested. A low dose of 10 gr/cow/day, equivalent to 0.5 kg/ MT of feed. A normal dose of 20 gr/cow/day, equivalent to 1.0 kg/ MT. A high dose of 50 gr/cow/day, equivalent to 2.5 kg/ MT. The results showed that MYCOAD AZ did not affect the absorption of the minerals measured in blood even when used at 5 times the recommended dose (50 g/cow per day).

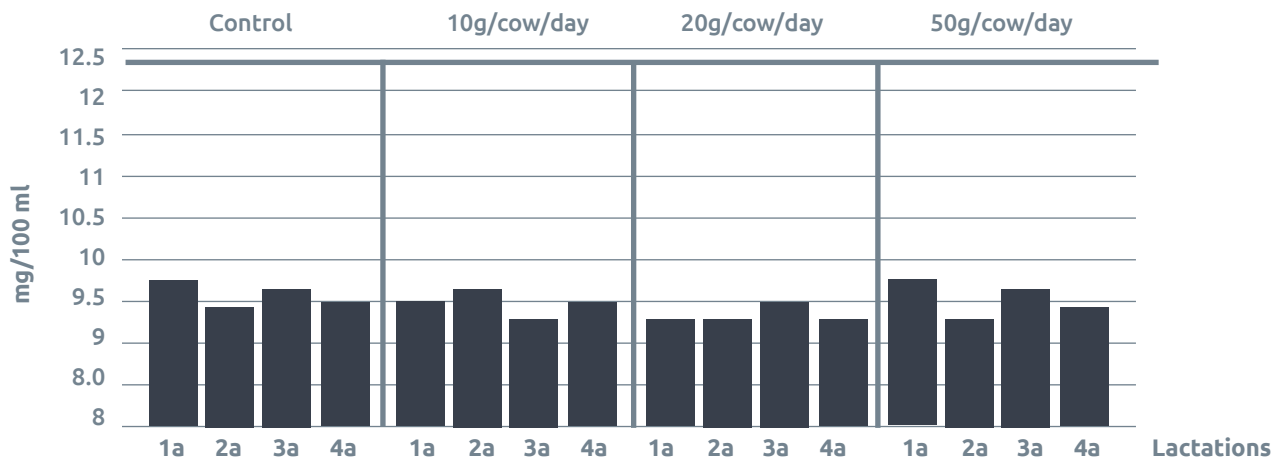
Effect of three inclusion rates of MYCOAD AZ in the concentration of critical minerals in the blood of commercial lactating cows.

Lactations #	Date	Calcium (mg/ 100ml)			Phosphorus (mg/ 100ml)			Magnesium (mg/ 100ml)		
		Jan 30/02	Feb/11/02	Feb/14/02	Jan/30/02	Feb/11/02	Feb/14/02	Jan/30/02	Feb/11/02	Feb/14/02
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 10g	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 20g	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 50g	1	8.7	10.4	9.7	5.1	6.2	5.1	1.9	2.2	2.1
	2	9.1	10.6	9.4	5.0	5.8	5.7	1.9	1.9	1.9
	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

\* = Not measured

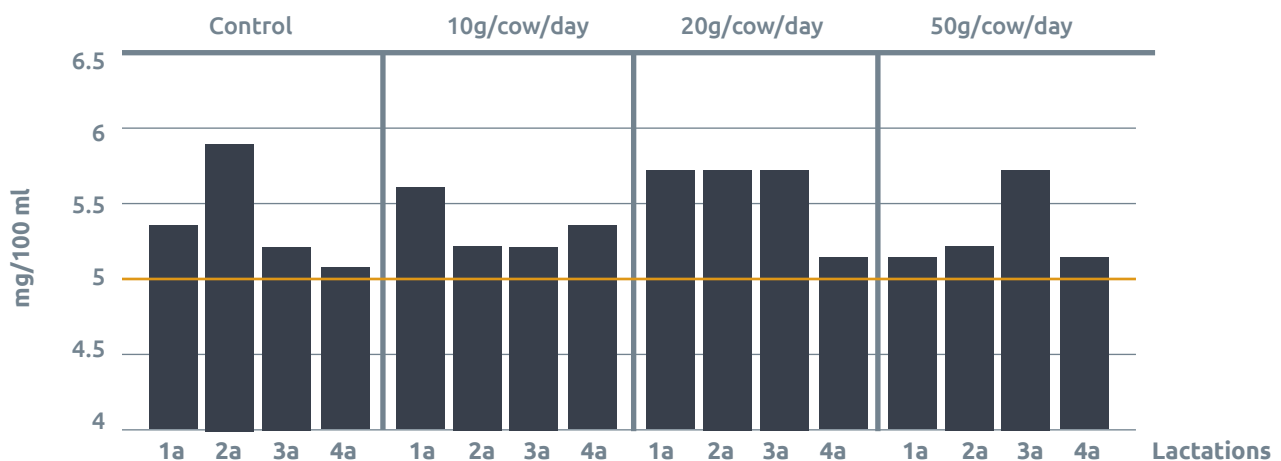
## Ca concentration in blood with different treatments

Normal range: 9.0 a 12.4 mg/100 ml



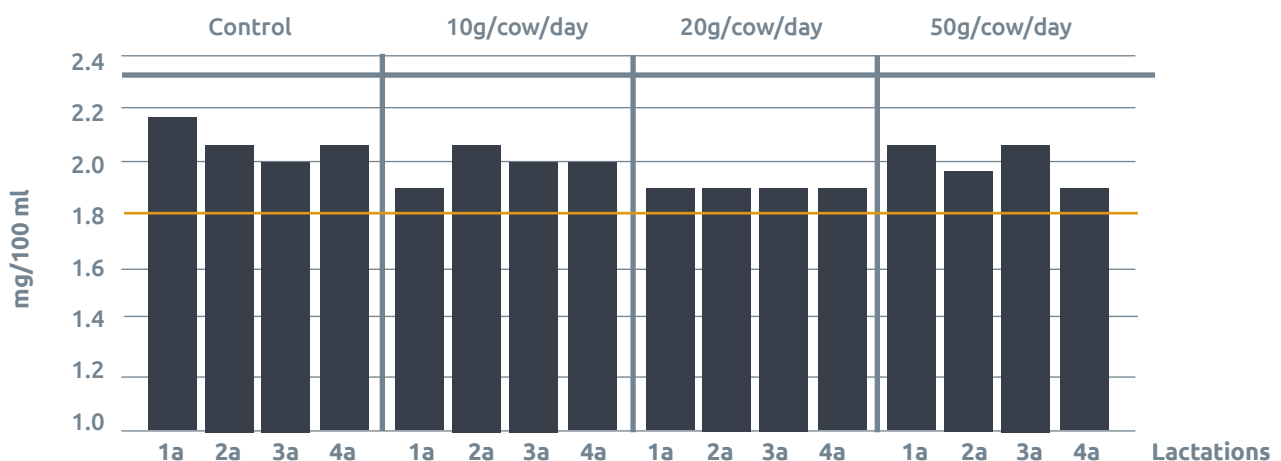
## P concentration in blood with different treatments

Normal range: 5.0 a 6.5 mg/100 ml



## Mg concentration in blood with different treatments

Normal range: 1.8 a 2.3 mg/100 ml



## Broiler chickens. MYCOAD AZ effect on bone structure

One of the objectives of the trial was to determine the effect of MYCOAD AZ on day-old commercial male broiler chicks fed experimental diets for 33 days. This experiment was conducted at an experimental facility located in Querétaro, Mexico. Bone ash, bone calcium and bone phosphorus were measured in all broilers from treatments 1 and 2.

Effects of MYCOAD AZ and T2 toxin on bone composition and performance of 38 day-old broilers exposed to test diets for 33 days.

Treatment	Bone ash	Calcium in bones (%)	Phosphorus (%)	ADG (g)	ADI (g)	FCR
1. Control	45.95a	23.87a	8.71a	54.8a	105.3a	1.92a
2. Control + 0.25% MYCOAD AZ	44.20a	25.11a	8.66a	51.3a	103.0a	2.01a
3. Control + 1.25 PPM T-2	N/M	N/M	N/M	N/M	N/M	N/M
4. Control + 0.25 % MYCOAD AZ	N/M	N/M	N/M	53.5a	101.4a	1.90a

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

ADG= average daily gain | ADI= average daily intake | FCR= feed conversion ratio | N/M= Not measured

A. Casarin et al. Evaluation of the efficacy of a commercial purified phylosilicate to reduce the toxicity of T-2 toxin in broiler chicks. 2006 International Poultry Scientific Forum. Atlanta, GA, USA.

## Broiler chickens. MYCOAD AZ effect on skin pigmentation

The effect on skin pigmentation in the carcass before refrigeration and performance was evaluated in commercial broilers. The first 14 days, no pigment was added to the diet and from 14-49 days 60 ppm of total xanthophyll was added. All rations included 60 ppm of Salinomycin. The color in the skin was measured at 49 days of age with the Minolta method CR 300. The results indicated that no significant difference was detected in skin coloration when the group treated with MYCOAD AZ was compared to the treatment including only xanthophyll.

Effect of 1.0 kg of MYCOAD AZ on performance and skin pigmentation of broilers at 49 days of age.

Treatment	Weight gain (g)	Feed intake (g)	FCR
Control	2987a	6056a	2.03a
Control + xanthophyll	2987a	6032a	2.02a
Control + xanthophyll + 1 kg MYCOAD AZ	2982a	6027a	2.06a

a Values within one column with different letters are significantly different ( $P < 0.05$ ).

Effect of 1.0 kg of MYCOAD AZ on skin pigmentation of 49 day-old broilers.

Treatment	Clarity l*	Red a*	Yellow b*	Croma (orange) c*
Control	74.2a	5.1a	19.9a	20.6a
Control + xanthophyll	70.0b	3.0ab	41.6b	41.9b
Control + xanthophyll + 1 kg MYCOAD AZ	71.4b	1.4b	41.1b	41.2b

a, b Values within one column with different letters are significantly different ( $P \leq 0.05$ )

Miguel Forat, M. et al. Effect of Mycoad AZ on performance and skin pigmentation of broilers. Instituto Internacional de Investigación Animal, Querétaro, México Octubre 2011 (unpublished).

## Ducks. MYCOAD effect on bone structure, organs weight & performance

Commercial one day-old meat-type ducks (Cherry Valley) were fed a diet naturally contaminated with 120 ppb of AFL at the Khon Kaen University's Poultry Research farm in Thailand. The objective was to demonstrate the innocuity of MYCOAD in ducks based on the effect on performance, bone strength, liver characteristics and target organ protection. When MYCOAD (2.5 kg/ ton) was added to the diet, without the inclusion of AFLA (treatment 2) a better performance and bone structure was obtained than in the group where no mycotoxin nor MYCOAD was administered (treatment 1). When MYCOAD (2.5 kg/ mt) plus 120 ppb AFLA (treatment 5) was evaluated, it showed a significantly improved performance, target organ protection and bone strength than the ducks that only received AFLA in the diet. As far as the effect of MYCOAD *per se* in the liver, the treatment including only MYCOAD showed the same results as the negative control (T1). This is a proof that in a very sensitive species such as ducks, MYCOAD not only does not adsorb nutrients but it helps to improve adsorption.

Effect of MYCOAD and Aftatoxin on performance and bone strength in 4-week-old ducks.

Treatment	Feed intake (g)	BW gain (g)	FCR (g/g)	Livability (%)	Tibial bone porosity
Control	2777a	1630a	1.70a	97.5a	1.08b
Control + 2.5 kg MYCOAD	2776a	1660a	1.67a	100a	1.03a
120 ppb AFLA	2595c	1406c	1.85b	87.5b	1.70e
120 ppb AFLA + 1.5 kg MYCOAD	2680b	1533b	1.75ab	100a	1.25d
120 ppb AFLA + 2.5 kg MYCOAD	2826a	1629a	1.74ab	97.5a	1.17c
120 ppb AFLA + 3.5 kg MYCOAD	2845a	1644a	1.73ab	97.5a	1.14c

a, b, c, d Means within a column without a common superscript differ significantly ( $P < 0.05$ ).

Effect of different treatments in the relative weight, degree of paleness and fatty composition of livers in 4 week-old ducks.

Treatment	Liver % BW	Liver paleness	Liver fat %
Control	2.31a	1.10a	16.0a
Control + 2.5 kg MYCOAD	2.26a	1.09a	15.8a
120 ppb AFLA	2.85c	1.87d	22.4c
120 ppb AFLA + 1.5 kg MYCOAD	2.60ab	1.36c	17.6b
120 ppb AFLA + 2.5 kg MYCOAD	2.43a	1.29bc	16.8ab
120 ppb AFLA + 3.5 kg MYCOAD	2.40a	1.19ab	16.5a

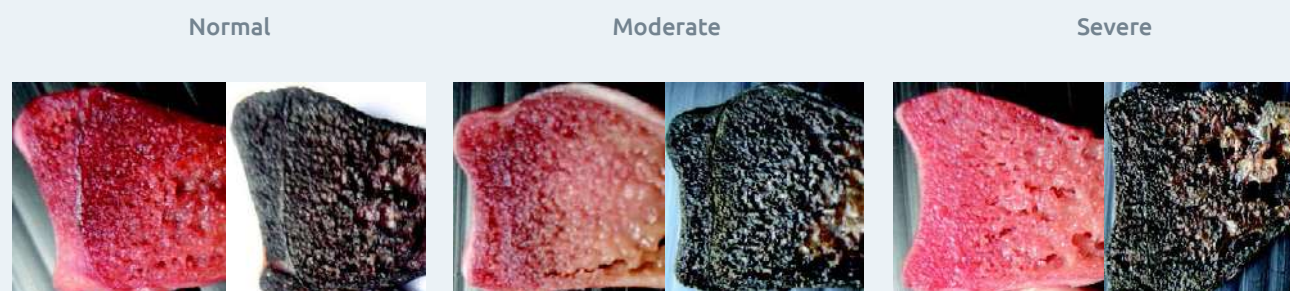
a, b, c, d Means within a column without a common superscript differ significantly (P<0.05).

Effect of MYCOAD and aflatoxin in the organs weight of 4-week-old ducks.

Treatment	Heart % BW	Pancreas % BW	Kidneys % BW	Spleen % BW
Control	0.57a	0.39a	0.90a	0.11a
Control + 2.5 kg MYCOAD	0.57a	0.38a	0.89a	0.10a
120 ppb AFLA	0.71b	0.49b	1.52b	0.21b
120 ppb AFLA + 1.5 kg MYCOAD	0.63ab	0.44ab	1.10ab	0.13a
120 ppb AFLA + 2.5 kg MYCOAD	0.59a	0.42ab	0.96ab	0.12a
120 ppb AFLA + 3.5 kg MYCOAD	0.58a	0.41ab	0.92ab	0.12a

a, b Means within a column without a common superscript differ significantly (P<0.05).

Tibial bone porosity score on fresh bone and on drip in silver nitrate 5%.



Wongtangintharn, S, et al. Efficacy of Mycoad to ameliorate the toxic effect of aflatoxin in ducks. XXV World's Poultry Congress. Beijing, China. September 2016.



## MYCOAD effect on tilapias

Tilapia of the Nile (*Oreochromis niloticus*) were placed in plastic cages at the experimental facilities of Samitec in Santa Maria, Brazil. 5,000 ppb of Aflatoxin were added to some of the treatments. Performance and microscopic lesions in the liver were evaluated.

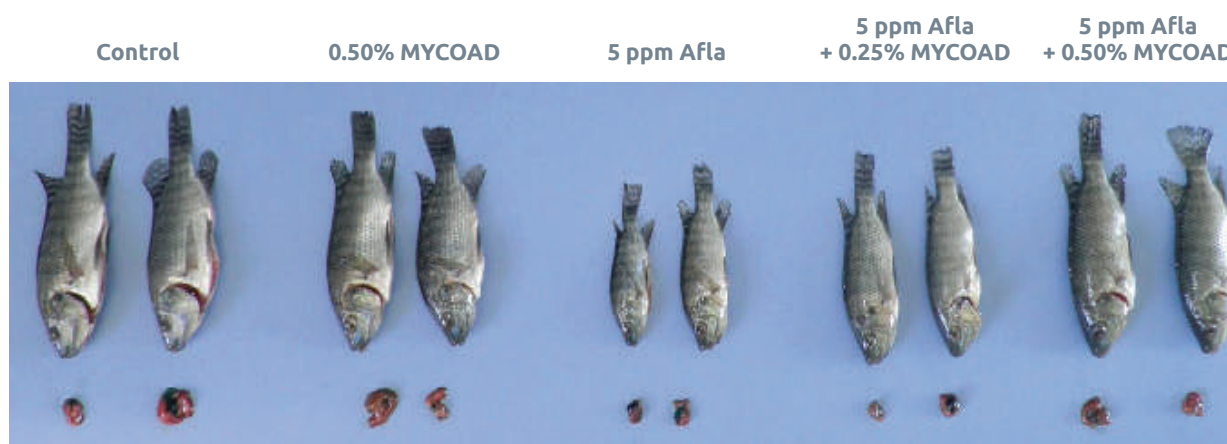
No statistically significant negative effect was reported in the treatment where only MYCOAD was added at an inclusion rate five times higher than the one recommended under commercial conditions. In the treatment where AFLA was added, the deleterious effect caused by this mycotoxin were clearly demonstrated. MYCOAD was able to significantly prevent the damage caused by Afla.

Average body weight and total size of fish after 21 days of treatments.

Treatment	Day 0		Day 7		Day 14		Day 21	
	ABW g	SIZE cm	ABW g	SIZE cm	ABW g	SIZE cm	ABW g	SIZE cm
Control	2.74a	3.89a	5.02a	6.19a	7.61a	7.27a	11.17a	8.19a
0.50% MYCOAD	2.73a	3.89a	4.85a	6.19a	7.55a	7.30a	10.66ab	8.19a
5 ppm Aflatoxin	2.65a	3.86a	4.45a	6.04a	6.02b	6.77b	8.24d	7.27b
5 ppm Afla + 0.25% MYCOAD	2.48a	3.82a	4.45a	5.95a	6.18b	6.86b	8.40cd	7.49b
5 ppm Afla + 0.50% MYCOAD	2.58a	3.81a	4.59a	6.11a	6.76ab	7.05ab	9.52bc	7.87a

a, b, c, d Means in the same column with different superscripts differ significantly ( $P \leq 0.05$ ).

ABW = Average Body Weight



Mallmann, C. et al. Experimental trial for the approval of MYCOAD as an anti-mycotoxin additive in tilapias at Lamic in Brazil. January 2012.

Histopathological lesions caused by aflatoxin in the livers of 6 twenty one day-old fish per treatment.

Treatment	Necrosis of hepatocytes	Megalocytosis
Control	None	None
0.50% MYCOAD	None	None
5 ppm Aflatoxin	3 out of 6, + 2 out of 6, ++	3 out of 6, ++
5 ppm Afla + 0.25% MYCOAD	4 out of 6, + 1 out of 6, ++	5 out of 6, + 1 out of 6, ++
5 ppm Afla + 0.50% MYCOAD	4 out of 6, + 1 out of 6, ++	3 out of 6, +

Score: **Mild** (+), **moderate** (++) , **accentuated** (+++), **severe** (++++). Necrosis of the hepatocytes and megalocytosis are caused by aflatoxicosis

## MYCOAD AZ effect on gilts

Twenty commercial gilts with an average live weight of 11.07 kg were used in this experiment at the University of Santa Maria in Brazil. The Fumonisin used in the test was produce by Lamic in the same country. At the end of the trial all animals were euthanized and the target organs for this mycotoxin were evaluated. The addition of 5 times (5 kg/ MT) the recommended dose of MYCOAD AZ did not alter performance nor organs as can be established when no statistical difference is observed between the negative control and treatment 2.

Gilts average daily feed consumption (kg) after 28 days of consuming the experimental diets tested.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 1 - 4
Negative control	0.69a	1.10a	1.20a	1.36a	1.09a
5 kg MYCOAD AZ/MT	0.72a	1.19a	1.19a	1.29a	1.10a
30 ppm FUM	0.75a	1.09a	1.19a	1.34a	1.09a
30 ppm FUM + 2 kg MYCOAD AZ/MT	0.88a	1.18a	1.32a	1.45a	1.21a
30 ppm FUM + 5 kg MYCOAD AZ/MT	0.68a	1.11a	1.30a	1.41a	1.13a

a Means within a column without a common superscript differ significantly (P<0.05).

Gilts average body weight (kg) after 28 days of consuming the experimental diets tested.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Negative control	11.10a	13.50a	18.66a	23.43a	27.45a
5 kg MYCOAD AZ/MT	11.03a	13.56a	18.81a	23.56a	27.56a
30 ppm FUM	11.06a	14.25a	18.61a	23.86a	28.04a
30 ppm FUM + 2 kg MYCOAD AZ/MT	11.04a	14.18a	19.19a	23.78a	28.01a
30 ppm FUM + 5 kg MYCOAD AZ/MT	11.10a	13.51a	18.35a	23.84a	27.70a

a Means within a column without a common superscript differ significantly (P<0.05).

Gilts average feed conversion after 28 days of consuming the experimental diets tested.

<b>Treatment</b>	<b>Week 1</b>	<b>Week 2</b>	<b>Week 3</b>	<b>Week 4</b>	<b>Week 1 - 4</b>
Negative control	2.08a	1.50a	1.76a	2.07a	1.85a
5 kg MYCOAD AZ/MT	2.03a	1.59a	1.77a	1.93a	1.83a
30 ppm FUM	1.66a	1.81a	1.58a	1.95a	1.75a
30 ppm FUM + 2 kg MYCOAD AZ/MT	2.11a	1.67a	2.03a	2.12a	1.98a
30 ppm FUM + 5 kg MYCOAD AZ/MT	2.07a	1.64a	1.68a	2.27a	1.91a

a Means within a column without a common superscript differ significantly (P<0.05).

Gilts average daily weight gain (kg) after 28 days of consuming the experimental diets tested.

<b>Treatment</b>	<b>Week 1</b>	<b>Week 2</b>	<b>Week 3</b>	<b>Week 4</b>	<b>Week 1 - 4</b>
Negative control	0.343a	0.737a	0.681a	0.671a	0.608a
5 kg MYCOAD AZ/TM	0.363a	0.750a	1.679a	0.667a	0.614a
30 ppm FUM	0.455a	0.623a	1.750a	0.696a	0.631a
30 ppm FUM + 2 kg MYCOAD AZ/TM	0.448a	0.716a	0.655a	0.706a	0.631a
30 ppm FUM + 5 kg MYCOAD AZ/TM	0.345a	0.691a	1.784a	0.644a	0.616a

a Means within a column without a common superscript differ significantly (P<0.05).

Relative organ weight (g/100 g BW) of gilts fed the experimental diets tested for 28 days.

<b>Treatment</b>	<b>Liver</b>	<b>Heart</b>	<b>Lungs</b>
Negative control	2.32a	0.46a	0.79a
5 kg MYCOAD AZ/MT	2.24a	0.44a	0.89ab
30 ppm FUM	2.28a	0.44a	1.22b
30 ppm FUM + 2 kg MYCOAD AZ/MT	2.59a	0.50a	0.92ab
30 ppm FUM + 5 kg MYCOAD AZ/MT	2.31a	0.48a	0.87a

a, b Means within a column without a common superscript differ significantly (P<0.05)

BW = Body weight

Mallmann, C. et al. Efficacy of an anti-mycotoxin product for Fumonisin control in gilts. (Unpublished)

## 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
<i>In vivo</i> 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 Regulation #1831/ 2003 (1m 588)	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
<i>In vitro</i> 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.*