

MYCOAD AZ

Efficacy claims based on scientific *in vivo* trials with statistical significance on

Target  rgan Protection



 TECHNICAL MANUAL

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INTRODUCTION

Zearalenone (ZEA), fumonisin (FUM), aflatoxin, ochratoxin, deoxynivalenol (DON / vomitoxin), and other tricothecenes are very important mycotoxins affecting pig performance. ZEA affects reproduction in swine, primarily weaned and prepubertal gilts, causing vulvo vaginitis, hypertrophy of the mammary glands and uterus, with occasional prolapse of the rectum and vagina. On the other hand, FUM has been associated with pulmonary edema and immunosuppression, increasing the susceptibility to respiratory diseases such as APP (*Actinobacillus pleuropneumoniae*), PRRS (Porcine Reproductive and Respiratory Syndrome), and circovirus. Pigs are very sensitive to DON, which decreases appetite significantly with a reduction in liver size.

Currently, one of the most practical approaches used to decrease the toxic effects of mycotoxins in animals, consist of using adsorbents to reduce their absorption in the gastrointestinal tract. During the experimental evaluation of these products, the protection against damage to specific organs highly susceptible to some of these mycotoxins, what is commonly call a target organ, is critical. In addition, biochemical tests to detect alterations in the concentration of sphingolipids like sphinganine and sphingosine (biomarkers) indicate the inhibition of the enzyme responsible for this chemical transformation as a result of the intoxication caused by FUM in the liver.

The evaluation of the effect on target organs and the detection of biomarkers is of paramount importance when evaluating mycotoxin binders *in vivo* under experimental conditions. Unfortunately, some mycotoxin binders base their efficacy or effectiveness on a favorable effect on performance obtained because of the use of enzymes, beneficial bacteria, yeast and/or immuno-stimulants added to the product.

Target organs for several important mycotoxins in swine

Mycotoxin	Target Organ	Damage
Aflatoxin	Liver	Enlarged Fatty Friable
Ochratoxin	Kidney	Enlarged Urate deposits
ZEA	Female reproductive organs	Enlargement Vulvovaginitis
DON	Liver	Size reduction
FUM	Lungs Heart Liver	Enlargement

ZEARALENONE

Target Organ Protection: Reproductive Tract

Eighteen 20-day old recently weaned Yorkshire Cross gilts individually housed were randomly distributed into three dietary treatments with 6 replications each in an experimental farm at Trilogy Labs in Missouri, USA. The feed was experimentally contaminated with crystalline ZEA, determined to be over 99% pure. Performance was evaluated during the trial and at the end all pigs were euthanized and the internal reproductive organs weighed.

Performance after 30 days of treatment in 54 Day-old gilts

Treatment	BW gain kg	Total feed intake kg	FCR
Control	13.8 a	31.8 a	2.30 a
750 ppb ZEA	13.7 a	32.0 a	2.33 a
750 ppb ZEA + 1 kg / MT MYCOAD AZ	14.4 a	33.2 a	2.30 a

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

Relative internal reproductive organs (% body weight) of 54 day-old gilts after 30 days of treatment.

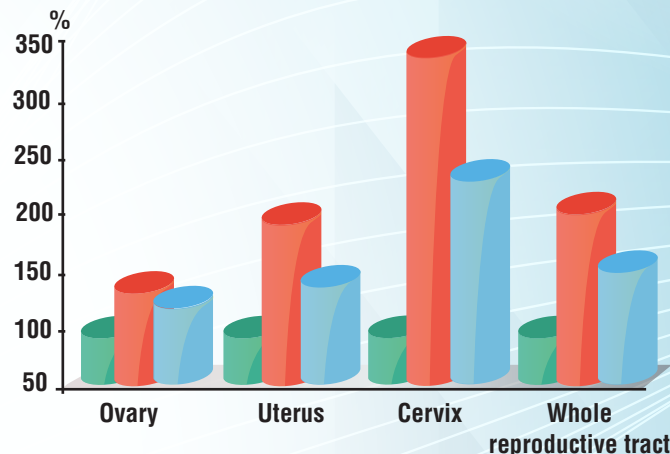
Treatment	Ovary	Uterus	Cervix	Total reproductive tract.
Control	3.0 a	28.9 a	3.0 a	37.1 a
750 ppb ZEA	4.2 b	55.9 b	10.8 b	73.5 b
750 ppb ZEA + 1 kg / MT MYCOAD AZ	3.7 ab	41.9 c	7.3 c	55.8 c

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

Note: It is important to emphasize that ZEA *per se* only affects the reproductive organs, not the productive parameters.

Relative internal reproductive organs weight of 54 day-old gilts after treatment for 30 days

● Control ● 750 ppb ZEA ● 750 ppb ZEA + 1 kg **MYCOAD AZ**



Effect of ZEA on the reproductive organs size



CONCLUSIONS

The addition of **MYCOAD AZ** to contaminated diets resulted in gilts with a statistically significant reduction in the weight of the reproductive tract as compared to those fed 750 ppb of ZEA. Even though the addition of 1.0 kg/ MT of **MYCOAD AZ** to diets contaminated with 2 to 3 times the level of ZEA producing problems under commercial conditions did reduce the abnormal growth of the reproductive organs, they were still heavier than those from gilts fed the control diet. These results indicated that **MYCOAD AZ** at 1.0 kg/ MT was effective in reducing the estrogenic effects of ZEA.

Reference: Journal of Animal Science. Vol. 85 Suppl. 1 p 67 2007.

ZEARELENONE + DON

Target Organ Protection: Reproductive Tract and Liver




Eighteen female Yorkshire Cross of 19 days of age with an average BW of 5.84 kg. were exposed to diets naturally contaminated with ZEA and DON. Gilts were distributed using a randomized design of 3 treatments with 6 replicates of 1 pig each; individually housed with access to its own feeder and automatic water system in a experimental farm at Trilogy Labs in Missouri, USA. Performance, the weight of liver, reproductive tract, and the height and width of the vulvae were measured at the end of the experiment.

Effect on performance after 21 days of treatment.

Treatment	Initial BW kg	Final BW kg	Weigh gain kg	Feed intake kg	Feed intake + waste kg	FCR (intake + waste)
Control	5.45	8.39	2.94 a	6.76 a	14.06 a	4.77 a
1.2 ppm ZEA + 6 ppm DON	6.20	7.24	1.04 b	2.39 b	13.33 a	12.82 b
1.2 ppm ZEA + 6 ppm DON + 1 kg/ MT MYCOAD AZ	5.95	8.17	2.22 a	5.11 a	12.25 a	5.53 a

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

DON effect on relative liver weight (g/100 g BW) and size of 40 day-old gilts exposed to test diets for 21 days

Control	1.2 ppm ZEA + 6 ppm DON	1.2 ppm ZEA + 6 ppm DON + 1 kg/ MT MYCOAD AZ
3.76 g ^a	2.89 g ^b	3.12 g ^c
		

a, b, c Values with different letters are significantly different (P< 0.05).
BW= Body weight

Effect of ZEA on vulva measurements

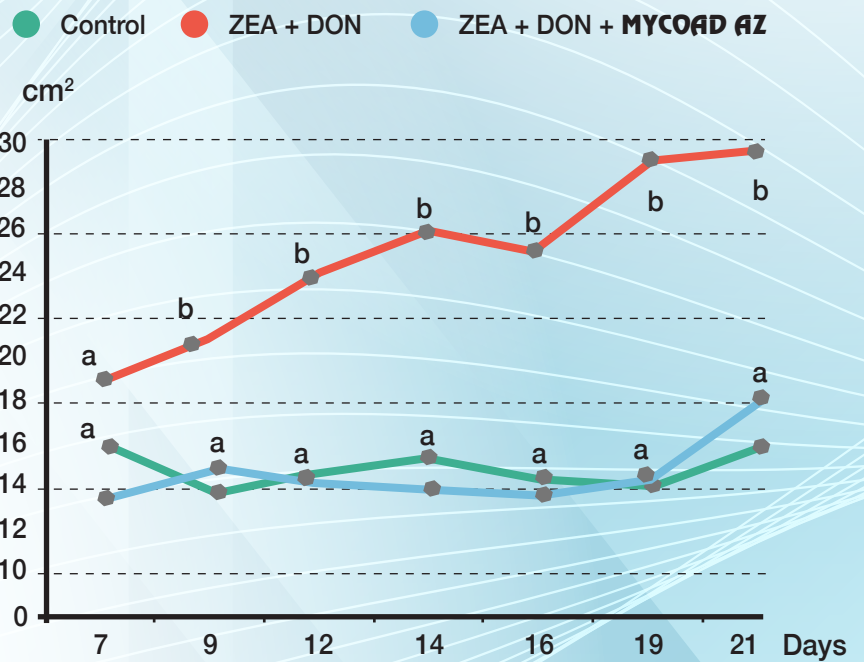


Height



Width




BW= Body weight



Treatment	Day 7 cm ²	Day 9 cm ²	Day 12 cm ²	Day 14 cm ²	Day 16 cm ²	Day 19 cm ²	Day 21 cm ²
Control	16.6 a	14.8 a	15.3 a	15.8 a	15.1 a	15.0 a	16.5 a
1.2 ppm ZEA + 6 ppm DON	19.4 a	21.2 b	24.0 b	25.8 b	25.1 b	28.5 b	29.1 b
1.2 ppm ZEA + 6 ppm DON + 1 kg / MT MYCOAD AZ	14.8 a	15.3 a	15.2 a	15.1 a	14.9 a	15.3 a	18.6 a

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

Effect of ZEA on the reproductive organs size (100 g BW x 1000)

Control	1.2 ppm ZEA + 6 ppm DON	1.2 ppm ZEA + 6 ppm DON + 1 Kg/ MT MYCOAD AZ
53.9 g ^a	104.5 g ^b	89.5 g ^c
		

a, b, c Values with different letters are significantly different (P< 0.05).
BW= Body weight

CONCLUSIONS

The deleterious effects of ZEA + DON in gilts were completely independent; hyperestrogenism due to ZEA and poor performance with reduced liver size due to DON. The addition of 1 kg of **MYCOAD AZ** / MT was very effective in preventing both the toxic effects of DON and the estrogenic effects of ZEA in pre-pubertal gilts.

Reference: Journal of Animal Science. Vol. 87 E-Suppl. 2 p 440. 2009.

FUMONISIN

Target Organ Protection: Lungs / Liver / Heart

Two experiments of different lengths of time (28 and 56 days) were conducted using 12 male pigs with an average initial BW of 58.5 kg in each experiment. Pigs were individually housed with feed and water provided *ad libitum* and randomly distributed into 3 treatments with 4 replications each.

FUM was obtained from a culture material containing 72% FB1 and 38% FB2, produced at LAMIC, Santa Maria, Brazil. Performance data were recorded every 7 days for 28 and 56 days. Relative organ weight (lungs, liver, and heart) was measured at 27 days. Blood serum (plasma proteins and SA:SO ratio) was evaluated at 56 days.

Effect on performance after 28 days of treatment

Treatment	Initial BW kg	Final BW kg	Average daily/ gain g	Daily feed intake g	FCR
Control	58.5 a	86.1 a	985 a	2505 ab	2.56 a
25 ppm FUM	58.0 a	78.3 b	722 b	2315 b	3.08 b
25 ppm FUM + 4 kg / MT MYCOAD AZ	59.3 a	83.4 ab	861 ab	2615 a	2.70 a

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

Effect on performance after 56 days of treatment

Treatment	Average daily / gain g	Daily feed intake g	FCR
Control	1076 a	2979 a	3.23 a
25 ppm FUM	996 b	2810 b	3.46 b
25 ppm FUM + 4 kg / MT MYCOAD AZ	1084 a	2948 a	3.21 a



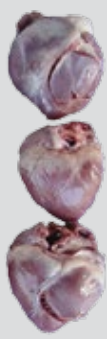
a, b Values within one column with different letters are significantly different (P< 0.05).

Serum sphinganine: sphingosine ratio and total plasma proteins after 56 days of treatment




Treatment	Serum SA:SO Ratio	Total plasma proteins g / dl
Control	0.38 a	8.82 a
25 ppm FUM	0.78 b	7.67 b
25 ppm FUM + 4 kg / MT MYCOAD AZ	0.49 a	8.63 a

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

Relative heart and liver weight (g / kg body weight) after 28 days of treatment




Control 3.75 a	FUM 4.87 b	FUM + AZ 3.75 a
		

a, b Values with different letters are significantly different (P < 0.05).

Control 18.4 a	FUM 20.9 b	FUM + AZ 18.6 a
		

a, b Values with different letters are significantly different (P < 0.05).

Relative weight of lungs (g/ kg body weight) after 28 days of treatment

Control 6.17 a	FUM 9.69 b	FUM + AZ 6.68 a
		

a, b Values with different letters are significantly different (P < 0.05).

CONCLUSIONS

The addition of **MYCOAD AZ** was very effective in preventing all the toxic effects of FUM on performance, relative organs weight, and blood serum parameters of finishing pigs.

Reference: Journal of Animal Science. Vol. 87 Suppl. 2 p 250. 2009.

ZEA+AFLA+T-2 TOXIN

Field trial

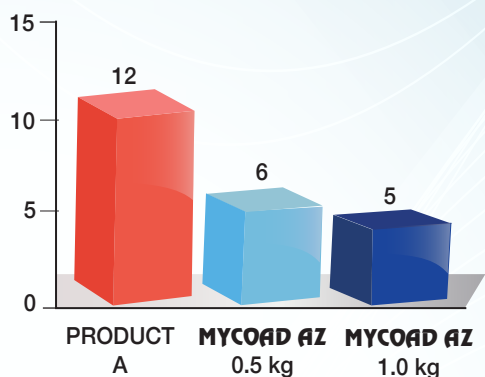
The presence of natural contamination with 3 different mycotoxins was detected in a commercial farm in Thailand (130 ppb ZEA, 44 ppb Aflatoxin and 15 ppb of T-2 toxin). Ninety nine gilts of approximately 40 kg presenting vulvovaginitis were selected and divided in 3 treatments of 33 gilts each to verify the effectiveness of two mycotoxins binders. **MYCOAD AZ** at 0.5 and 1.0 kg/ MT and another product identified as Product A at 1.0 kg/ MT were used for 15 days to study the effect on the regression of swollen vulvae and performance.

Performance of sows after 15 days of treatment

Treatment	Average daily feed intake g	Average daily gain g	Conversion rate
Product A 1.0 kg/ MT	870	800	2.19
MYCOAD AZ 0.5 kg/ MT	960	866	2.23
MYCOAD AZ 1.0 kg/ MT	930	872	2.08

Number of gilts (out of 33) remaining with swollen vulva after 15 days of treatment with two mycotoxin binders

Number of swollen vulvae



Swollen vulva before treatment



Vulva regression



CONCLUSIONS

MYCOAD AZ at 0.5 and 1.0 kg/ MT was very effective on reversing in 15 days the swollen vulvae of gilts affected by ZEA. Feeding 1.0 kg of **MYCOAD AZ** for 15 days improved performance in addition to the regression of vulvae in gilts fed naturally contaminated diet.

Reference: Personal Communication. Dr. Sakda Thepprechasakul. Innovet. Thailand. 2005.

Quality Control

Two methods of evaluation are followed. The first is the certificate of analysis (COA) which ensures that the same clay is always used, based on physical-chemical analysis and x-ray diffraction. The second control consists of a certificate of efficacy (COE) from an external independent laboratory where the *in vitro* net adsorption is measured for each batch produced. This certificate accompanies each lot sold, showing efficacy against the mycotoxins listed below.

In vitro net absorption of 1 kg / MT of MYCOAD AZ from all batches produced from year 2009 to 2015

Year	Zearalenone 3000 ppb	Ochratoxin 3000 ppb	Fumonisin 3000 ppb
2009	97.5 ± 0.8	95.1 ± 1.6	90.1 ± 2.7
2010	97.7 ± 0.6	94.6 ± 1.3	90.1 ± 2.5
2011	97.5 ± 0.5	95.5 ± 1.9	93.3 ± 2.5
2012	97.4 ± 1.1	94.5 ± 2.5	94.2 ± 2.0
2013	97.6 ± 1.1	94.6 ± 2.1	92.7 ± 1.8
2014	97.6 ± 0.9	94.9 ± 1.6	90.9 ± 3.2
2015	97.6 ± 0.7	93.1 ± 2.5	92.4 ± 1.9
Average	97.6 ± 0.8	94.5 ± 2.0	92.1 ± 2.4

The certificate of efficacy is the fingerprint of the clay



Characteristics

During its production, two industrial processes are carried out: purification and activation. The purification process removes impurities and heavy fractions from the clay, allowing access to chemical structures with greater possibilities of binding mycotoxins. The activation is obtained by adding a surfactant that produces structural changes in the clay, increasing its surface and creating specific conditions to adsorb mycotoxins of very weak polarity. Through these processes, higher adsorption capacity and speed is achieved.

In vitro adsorption rate and speed of 1 kg / MT of MYCOAD AZ against ZEA and FUM

3000 ppb Zearalenone

3000 ppb Fumonisin

Absorption %	Desorption %	Efficacy %	Time in minutes	Absorption %	Desorption %	Efficacy %
98.6	0.3	98.3	1	83.5	0.8	82.7
98.4	0.4	98.0	5	83.3	1.0	82.3
98.5	0.4	98.1	10	87.0	1.1	85.9
100	0	100	15	92.7	2.9	89.8
100	0	100	30	93.9	1.8	92.1
100	0	100	60	92.6	2.1	90.5
100	0	100	90	93.9	1.9	92.0

Does your anti-mycotoxin additive meet the basic TOP and FACTS?

Target 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
Recommended commercial dosage		2.5 kg / MT	1 kg / MT
The clay is always obtained 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
ENDOTOXIN adsorption		N/A	YES
Efficacy approved by LAMIC and other institutions against the following number of mycotoxins		4	4
Efficacy approved by LAMIC and other institutions in different types of animals		6	5
Nutrient absorption		NO	NO
<i>In vitro</i> efficacy test every:		100 MT	18 MT

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* Test performed with 4 Kg / MT with 30,000 ppb of fumonisin
N/A= not applicable

MYCOAD = Cobind, Toxfree Standard
MYCOAD AZ = Cobind AZ, Toxfree

