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# Safety of improved Milbond-TX mycotoxin binder when fed to broiler breeders above recommended levels

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## ABSTRACT

An increasing concern in poultry nutrition is the effects of mycotoxins in contaminated grain. Several new products have come onto the market that chemically bind these toxins preventing mycotoxicosis. However, many of these products have not been tested for safety if accidentally overfed to broiler breeders. In order to simulate a feed mixing error at a feed mill, Improved Milbond-TX<sup>®</sup> was overfed to broiler breeders to see if this would cause any negative effects on bird performance. A typical corn-soybean based diet supplemented with Milbond-TX mycotoxin binder at three different levels of inclusion (0%, 0.5%, and 1%) was fed to 300 broiler breeder hens. Data were collected on egg production, egg weights, hatchability, fertility, and chick weights from 24 to 35 weeks of age. Eggs per hen housed were not significantly different between the three treatments. The differences in egg weights, hatchability, fertility, and chick weights were also insignificant among the three treatments. We were able to conclude that overfeeding Improved Milbond-TX had no negative effect on bird performance and is safe to feed at a level of up to 1%.

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\* Michael Schlumbohm is a senior with a poultry science major.

† Judy England is the project/program manager for Dr. Coon.

§ Rueben Kriseldi is a May 2014 graduate with a major in poultry science.

‡ Dr. Coon is the mentor and a professor of Poultry Nutrition in the Department of Poultry Science Center of Excellence for Poultry Science.

## MEET THE STUDENT-AUTHORS



*Michael Schlumbohm*

I am the son of Aron and Marie Schlumbohm of Leipsic, Ohio and I am a senior Poultry Science major. I have a background with exhibition poultry and I continue to work with our extension service in judging poultry shows and organizing the state fair poultry show. However, my other interest is with commercial poultry production and particularly with poultry nutrition. I am a member of the Poultry Science Club and I currently work for Dr. Coon at the poultry research farm.

Last summer I had the opportunity to study abroad in Nampula, Mozambique where I worked with a broiler and layer farm for three weeks. After earning my B.S. degree I intend to go to graduate school and earn at least my master's degree in poultry nutrition. I would like to thank Dr. Coon for giving me this research trial to work on. I would also like to thank Judy England and Justina Caldas for giving me advice on writing and revising the paper, and helping me interpret the data.

My name is Ruben Kriseldi. I am a May 2014 graduate with a Poultry Science major. I am originally from Indonesia and I came to the U.S. for the first time in 2010 to get my bachelor degree. I chose University of Arkansas because of the poultry science program. The influence came from my father who has been involved in the industry for several years. In the past, he used to have several research projects in chicken and I often helped him. From there, I developed an interest in poultry science which led me to study here. My future plan is to get my Ph.D in poultry nutrition. I would like to work as a poultry nutritionist. After that, I hope that I can have my own company and wish to help feed the people in need.



*Ruben Kriseldi*

## INTRODUCTION

Mycotoxins are caused by fungi that grow on grain either in the field or during storage of complete feed (Fig. 1). Dangers of mycotoxin infections are especially high during drought years or if grain is improperly stored. These fungal infections are hard to avoid and exist in virtually all feed that livestock and poultry consume. Previous studies of mycotoxin levels in poultry feeds have revealed the presence of a number of different toxins. Many recent samples contain at least 10 contaminants (Croubels, 2013). The most prevalent mycotoxins include toxins from the genera of *Fusarium*, *Aspergillus*, and *Penicillium* (Croubels 2013). Most of the time there is no marked effect on animal performance. However, when exceptionally high levels occur in poultry feeds it can lead to mycotoxicosis. Mycotoxicosis can be a serious threat to poultry performance as it can cause lesions in the gastrointestinal tract, and oral cavity as well as erosion of the gizzard, inflammation of the proventriculus and epithelial mucosa of the intestinal wall (Fig. 2). These conditions can cause reduced uptake of nutrients and can leave the bird susceptible to further infection from other pathogens. Mycotoxicosis also results in reduced flock uniformity.

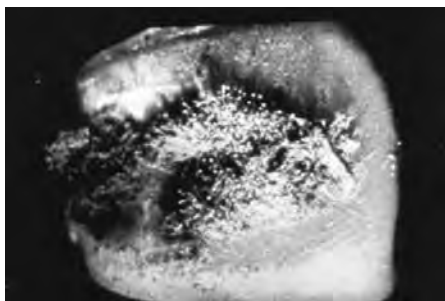
Because of the ill-effects of mycotoxicosis, there have been several methods employed to reduce the amount of mycotoxins ingested by animals. Among these is the use of genetically resistant crops (Wu et al., 2006; Kabak et al., 2006). Proper crop rotation and management, use of biological and chemical agents, and irradiation are also ways to prevent mycotoxin growth in grains (Kabak et al., 2006). Recently there has been research into the use of clay-based adsorbents. Improved Milbond-TX<sup>®</sup> is an inert montmoril-

lonite clay-based hydrated sodium calcium aluminosilicate (HSCAS) adsorbent that originates from natural clay deposits (Miles and Henry, 2007a). Though there has been research testing the safety of Improved Milbond-TX in broiler and layer diets, there has not been as much research done with broiler breeders. The basic principle of clay-based adsorbents is similar to a chemical reaction and therefore, the release of free energy is the driving force of every adsorption (Huwig et al., 2001). Physical structure of the adsorbent such as total charge and charge distribution and surface area are important features as well. Along with this the properties of the adsorbate molecules, the mycotoxins, such as polarity, solubility, size, shape and—in case of ionized compounds—charge distribution and dissociation constants play a significant role. Because of the variability of these properties, it is important to investigate the effectiveness of each product when it interacts with the mycotoxins (Huwig et al., 2001).

However, it was not certain how higher than recommended levels of Improved Milbond-TX would affect egg production, egg weight, hatchability, fertility, or chick weights of broiler breeders. The goal of this study was to determine if or how bad the effect of higher than recommended levels of this product would affect bird performance. Based on previous studies done with this product, it was hypothesized that the effect would be slight if at all.

## MATERIALS AND METHODS

The birds raised for this experiment were Cobb 500 females and MX males (Cobb-Vantress, Inc., Siloam Springs, Ark.). All of the feed that was fed from 0 to 21 weeks of age also was supplied by Cobb-Vantress. The birds were fed on



**Fig. 1.** Corn kernel infected by *Aspergillus flavus*. Infection by this mold can lead to mycotoxicosis. Photo courtesy of Dr. William Huff, USDA.



**Fig. 2.** Aflatoxin affected liver (left) vs healthy liver (right). Photo courtesy of Dr. William Huff, USDA.

a skip-a-day schedule and reared to the body weight growth curve recommended by the primary breeder.

At 21 weeks, the birds were divided between 12 pens. There were 4 pens per treatment. In each treatment there were a total of 100 hens making for a total of 300 hens used. The hens were housed in floor pens with 25 hens and two cocks per pen.

Also at 21 weeks, the birds were switched over from the grower diet to the breeder diets that included the Milbond-TX mycotoxin binder; it was also at this age that they were light stimulated. There were three treatments, each with a different level of inclusion of Improved Milbond TX. Treatment one had an inclusion of 0%, treatment two, 0.5%, and treatment three, 1.00% (Table 1). In all three treatments, birds were fed the same amount of feed daily (g/bird/day). The amount of feed was adjusted for pens that had mortality. The males were also fed according to the recommendations of the primary breeder.

All diets were formulated to ideal protein profile, 15.5% crude protein (CP) and to contain 2915 apparent metabolizable energy (AME) kcal/kg (Table 2). Hens were fed according to the primary breeder's recommendations for feeding hens into production. At peak feed consumption, hens were receiving 154 g feed/bird/day. At peak consumption, hens were consuming 450 kcal/bird/day and 23.9 g CP/bird/day. Feed was withdrawn post-peak egg production. Samples of the feed were submitted to the Central Analytical Laboratory (University of Arkansas Poultry Science Center, Fayetteville, Ark.) for analysis (Table 3).

Eggs were gathered three times daily from the start of production until peak production. After the hens reached peak, eggs were picked up twice daily. At each egg gathering, the number of eggs was recorded. All eggs were saved for hatching; a marker was used to write the pen number on each egg in order to track the hatchability and fertility by pen.

**Table 1. Experimental Breeder Diets.**

<b>Ingredient<sup>†</sup>, %</b>	<b>Treatment 1 (0%)</b>	<b>Treatment 2 (0.5%)</b>	<b>Treatment 3 (1.00%)</b>
Corn 9.5% CP	64.30	63.70	62.10
Soybean meal 47.1% CP	17.10	17.20	17.60
Wheat Shorts	5.00	5.00	5.00
Poultry Fat	3.12	3.30	3.82
Limestone	7.51	7.51	7.51
Dicalcium Phosphate	1.51	1.51	1.52
Salt	0.33	0.33	0.33
Sodium Bicarbonate	0.20	0.20	0.20
Methionine 98.5%	0.30	0.30	0.30
Lysine	0.20	0.20	0.20
Choline Chloride 60%	0.12	0.12	0.12
Vitamin Premix	0.20	0.20	0.20
Trace Mineral	0.08	0.08	0.08
Selenium Premix 0.6%	0.02	0.02	0.02
Ethoxoquin	0.02	0.02	0.02
Milbond-TX	0.00	0.50	1.00

<sup>†</sup> These were the formulas used to provide the necessary nutrients for optimum bird performance based on primary breeder recommendations, plus the addition of the Milbond-TX mycotoxin binder. CP = crude protein.

**Table 2. Calculated nutrient content.**

<b>Calculated Nutrient<sup>†</sup></b>	<b>Diets</b>		
	<b>0%</b>	<b>0.5%</b>	<b>1.00%</b>
AME, Kcal/Kg	2,915.00	2,915.00	2,915.00
CP, %	15.50	15.50	15.50
Calcium, %	3.25	3.25	3.25
Available Phosphorous, %	0.41	0.41	0.41
Digestible Lysine, %	0.75	0.75	0.75
Digestible TSAA, %	0.68	0.68	0.68
Digestible Threonine, %	0.42	0.42	0.42

<sup>†</sup> Nutrient content of each diet based on mathematic calculation.

AME = Apparent metabolizable energy, CP = crude protein,

TSAA = Total sulfur amino acids.

**Table 3. Actual nutrient analysis.**

Analyzed Nutrient <sup>†</sup>	Diets		
	0% (treatment 1)	0.5% (treatment 2)	1.00% (treatment 3)
DM, %	89.80	89.40	89.90
CP, %	15.30	16.00	15.30
Ash, %	11.39	11.56	12.42
Fat, %	5.45	5.48	6.27
Calcium, %	3.27	3.37	3.38
Total Phosphorous, %	0.60	0.64	0.60
Calories/g	3,788.00	3,787.00	3,772.00

<sup>†</sup> Nutrient content of the diets as determined by laboratory analysis.

DM = dry matter, CP = crude protein.

Egg weights were recorded two days of each week. The weight was recorded by gathering all the eggs in a pen and weighing all the eggs on a digital scale and then dividing by the number of eggs gathered.

Starting the week after the hens came into production, the eggs were set in an incubator weekly for 12 weeks total. At transfer on the 18th day, the eggs were candled for infertile, contaminated, and early dead embryos. These eggs were then broken open to determine if it was infertile, contaminated, or died early during incubation. When the hatch was pulled each chick was weighed individually using a digital scale. The remaining unhatched eggs were broken open to determine when the embryo died. At the end of the trial, the percent hatch and fertility were compared between the treatments. The number of eggs, egg weights, and chick weights were also analyzed and compared.

Data were analyzed using JMP Pro 10 (SAS Institute, Inc., Cary, N.C.) using standard least square analysis reduced maximum likelihood (REML) method. Pen was considered as a random effect. When significant differences were found, means were separated using Tukey's honestly significant difference (HSD),  $\alpha = 0.05$ .

## **RESULTS AND DISCUSSION**

Overall the birds used stayed in good health with total hen mortality at only 1.7%, not due to the inclusion of Improved Milbond-TX mycotoxin binder. Similarly, in trials done with broilers and commercial egg-type layers, mortality has been kept below 3% in one case and below 2% in the other. Mortality in these trials was not due to the inclusion of Improved Milbond-TX mycotoxin binder (Miles and Henry, 2007a,b).

**Egg Production.** In the current trial, the number of eggs per hen housed was not significantly different between the treatments. The numbers of eggs per hen housed were 55.5, 57.8, and 56.9 for treatments 1, 2, and 3, respectively ( $P = 0.4233$ , SEM = 1.2).

In an experiment done by Miles and Henry, 2007a with Leghorn hens fed Improved Milbond TX, there was a sig-

nificant difference between hens fed a diet with 0% and 1% inclusion. Hens fed diets with no added Improved Milbond TX had a higher average daily production from weeks 9-12. However, they were using hens from two different genetic lines, one selected for poor shell quality and one for good shell quality. When this was taken into account, there was no effect of the Improved Milbond TX on egg production (Miles and Henry, 2007a). Improved Milbond TX had no effect on feed intake and it appeared that no other nutrients relating to egg production were tied up by the binder.

**Egg Weights.** Egg weights were not significantly different among treatments (Table 4,  $P = 0.759$ , SEM = 0.689). The egg weights at 35 weeks of age were within 0.6 grams between treatment one and treatments two and three. Treatments two and three were exactly the same. It would seem that no nutrients were tied up by the binder that may affect egg weight or synthesis of protein.

Likewise, Miles and Henry, 2007a found no significant differences ( $P > 0.10$ ) between egg weights among treatments except for during two separate weeks of their trial. During weeks 16 and 20, they did find a significant difference between treatments which received 0%, 1%, and 2% inclusion of Improved Milbond-TX mycotoxin binder, with the weights of the treatment given 2% being the lowest ( $P > 0.05$ ; Miles and Henry 2007a)

**Hatchability.** Hatchability in this trial was calculated from total eggs set for the week. Overall hatchability was not significantly different among treatments ( $P = 0.3152$ ). Only in one week's hatch was there a significant difference. Between treatment 2 (0.5%) and treatments 1 (0%) and 3 (1%) there was a difference. Due to this occurring only one week of the whole trial, it is unlikely that this was because of feeding Improved Milbond TX. Some possibilities for this reduced hatchability could have been a result of a hatchery error that affected the trays containing eggs from this treatment or rough handling, cracked eggs etc. Besides this one week with significant difference ( $P = 0.0306$ ), hatchability was consistent throughout the trial and was not affected by feeding Improved Milbond TX (Table 5).

**Table 4. Egg weights (g) each week for each treatment. Average weight of eggs collected each week during the trial.**

Treatments	24	25	26	27	28	29	30	31	32	33	34	35
1	47.8	47.5	50.8	55.2	54.6	55.3	56.8	60.0	59.3	60.7	62.2	62.8
2	44.8	50.5	51.5	53.8	54.4	55.5	56.8	58.6	56.0	60.6	61.7	62.2
3	46.0	47.0	51.7	53.6	56.0	57.3	57.4	60.2	58.4	61.2	62.4	62.2
P-value	0.264	0.175	0.795	0.595	0.168	0.418	0.432	0.475	0.335	0.742	0.710	0.759
SEM	1.313	1.057	1.173	1.173	0.562	1.126	0.384	0.994	1.547	0.561	0.595	0.689

P-value less than 0.05 indicates significant differences. SEM is the standard error of the mean.

**Table 5. Hatchability of all eggs set (%). Percent of all eggs set that hatched.**

Treatments	26	27	28	29	30	31	32	33	34	35	Overall
1	85.0	88.0	90.1	88.9	90.2	90.2	90.0 <sup>††</sup>	89.7	89.7	87.6	89.0
2	82.3	83.3	87.6	88.2	87.8	88.2	85.2 <sup>b</sup>	91.3	91.3	88.9	87.4
3	85.1	85.8	87.3	88.2	87.7	88.0	91.6 <sup>a</sup>	86.7	86.7	87.0	87.7
P-value†	0.8481	0.3549	0.2958	0.9613	0.6483	0.5746	0.0306	0.1272	0.1272	0.5107	0.3152

† Standard least square reduced maximum likelihood (REML) analysis (pen treated as a random effect).

‡ Means separated by student's *t* test  $\alpha = 0.05$ .

§ Values followed by the same letter are not significantly different.

*Fertility.* The fertility among the treatments remained consistent throughout the trial and there was no significant difference between any treatments ( $P = 0.2630$ ). Overall fertility was consistent, there was a moderate increasing trend as the birds aged in treatments 2 and 3 (Table 6). It appears that feeding higher than recommended levels of Improved Milbond TX has no effect on sperm production or sperm quality of the males and does not reduce mating activity. Likewise, it had no effect on the ability of the female to store sperm and did not interfere with fertilization in the female.

*Chick Weights.* The chick weights were unaffected by feeding higher levels of Improved Milbond TX (Table 7) and there was no significant difference among the treatments ( $P = 0.6738$ ). The increase of chick weights over the trial period is normal. Older hens lay larger eggs and as a result the chick weights increased. In regard to egg weights, this same phenomenon was found in a trial done by Miles and Henry (2007a) with commercial egg-type laying hens. On average, chick weights increased 6.3 grams per chick over 10 weeks. As far as overall weights are concerned, there was only a 0.3 gram difference between treatment 3 and treatments 1 and 2. Treatments 1 and 2 were exactly the same.

Since chick weight is closely linked to egg weight (Halbersleben and Mussehl, 1922) it can be asserted that if egg weight is not affected by feeding higher than recommended levels of Improved Milbond-TX mycotoxin binder, then chick weight will also be unaffected.

### **SUMMARY**

In conclusion, the inclusion of Improved Milbond-TX mycotoxin binder at levels up to 1% in broiler breeder diets has no negative effect on bird performance in terms of egg production, egg weight, hatchability, fertility, and chick weight, and is therefore safe to use up to this level.

### **ACKNOWLEDGMENTS**

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**Table 6. Fertility of all eggs set (%). Percent of eggs set that were fertilized each week.**

Treatment	26	27	28	29	30	31	32	33	34	35	Overall
1	98.2	99.1	97.2	97.8	98.7	97.8	98.6	100	99.4	99.1	98.4
2	94.7	97.2	94.9	97.8	98.1	98.6	97.3	100	98.6	99.6	97.5
3	96.9	98.6	97.9	98.8	99.2	98.7	99.6	100	99.3	98.4	98.6
P-value†	0.2754	0.3089	0.1153	0.7397	0.6167	0.8704	0.4142	-	0.3397	0.2241	0.2630

† Standard Least Square reduced maximum likelihood (REML) analysis (pen treated as a random effect).

**Table 7. Chick Weights (g). Day old chick weights each week.**

Treatments	26	27	28	29	30	31	32	33	34	35	Overall
1	37.1	37.0	38.7	38.7	39.6	41.3	42.1	42.8	42.8	42.3	40.1
2	35.3	37.2	38.4	39.2	39.5	41.3	42.8	42.9	42.9	42.5	40.1
3	36.3	37.4	39.5	39.6	39.7	41.7	42.4	43.3	43.3	42.8	40.4
P-value†	0.5104	0.6654	0.1284	0.2850	0.9421	0.4847	0.3948	0.5099	0.5099	0.7445	0.6738

† Standard Least Square reduced maximum likelihood (REML) analysis (pen treated as a random effect).