

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Safety of Improved Milbond-TX[®] Mycotoxin Binder When Fed to Broiler Breeders above Recommended Levels

M.J. Schlumbohm, R. Kriseldi, J.A. England and C.N. Coon
Center of Excellence for Poultry Science, University of Arkansas,
Fayetteville, Arkansas-72701, Arkansas, USA

Abstract: A continual concern in poultry nutrition is the negative effects that mycotoxins have on animal performance when contaminated grain is used as a dietary ingredient. Mycotoxin binders have been available for decades and are used in the feed as an effective approach to decreasing the intestinal absorption of several mycotoxins, especially when present in low dietary concentrations. The research reported herein with broiler breeders was conducted to test the safety of an effective mycotoxin binder, Improved Milbond-TX[®] (IMTX), when added to the diet in concentrations higher than the 0.25% which is recommended by the manufacturer. Beginning at 21 weeks of age a typical corn-soybean meal diet was supplemented with IMTX at concentrations of 0, 0.5 and 1.0%. These three dietary treatments were fed continuously from 21 to 35 weeks of age 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. Egg production, expressed as eggs per hen housed, was not significantly different ($p>0.05$) among the three dietary treatments. Also, no significant differences ($p>0.05$) were found among the dietary treatments for egg weights, hatchability, fertility and chick weights. Results from this study show that an accidental over-supplementation of a broiler breeder diet resulting in up to 4 times the recommended dietary concentration of IMTX is not expected to result in any negative effect on broiler breeder performance or of the weight of chicks at hatch.

Key words: Broiler breeders, hatchability, fertility, mycotoxin binding agent

INTRODUCTION

Mycotoxins are produced by filamentous fungi known as molds that grow on grain and other feedstuffs either in the field, during storage and while feeding of complete feed (Fig. 1). Dangers of mold growth and mycotoxin production are known to be associated with plant stress and are especially high during drought years or if grain is allowed to hydrate during storage (CAST, 2003). Fungal contamination is often difficult to prevent due to the ubiquitous presence of mold spores. Previous studies that have reported mycotoxin levels in poultry feeds have revealed the presence of a number of different toxins simultaneously with the most prevalent being from the genera of *Fusarium*, *Aspergillus* and *Penicillium* (Croubels, 2013). For instance, Croubels (2013) reported the presence of 10 mycotoxin contaminants in various feed samples tested.

Mycotoxins, due to their toxic effects at the cellular level, exert a negative effect on every bodily system of the animal. Consequently, when mycotoxins are present in the diet of animals they compromise animal health and performance. Therefore, there is no "safe" level of dietary mycotoxin contamination in either stored feed ingredients or mixed complete feeds. The major reason for this is because the negative effects that are commonly observed in animals which are caused by mycotoxins are dependent on many factors. Such factors

are the specific mycotoxin (s), dietary mycotoxin concentration, duration of consumption, stress level in the animal as well as the animal's age and sex (CAST, 2003).

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, 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). Certain clay- based adsorbents which have been recognized by the feed industry as GRAS (Generally Recognized As Safe) feed additives, when used as flow agents and carriers, have also been shown to be effective mycotoxin binders.

Improved Milbond-TX[®] (IMTX) is an inert montmorillonite clay-based hydrated sodium calcium aluminosilicate adsorbent that originates from natural clay deposits mined directly from the earth (Miles and Henry, 2007a). IMTX has been supplemented to poultry diets successfully since 1992 when it was first introduced to the animal feed industry by Milwhite, Inc. (Brownsville, TX). The recommended concentration of IMTX in feed is 0.25% and no safety issues, with respect to animal performance, have ever been documented in any animal species when fed at this dietary concentration. In

research reported by Ledoux *et al.* (1999) which was designed to study the safety and aflatoxin B₁ binding ability of IMTX fed 4 times (1.0%) the recommended amount of IMTX in the diet without any detrimental effects on broiler performance or serum chemistry components. Also, it has been reported that feeding up to 8 times the recommended amount of IMTX did not cause any negative effects on feed consumption, body weight, feed conversion, toe ash or excreta moisture of broilers fed normal diets as well as diets deficient in available phosphorus or low in metabolizable energy (Miles and Henry, 2007bc). Similarly, 8 times the recommended level of IMTX has been fed to commercial egg-type laying hens for 5 consecutive 28-day periods without any detrimental effects on performance being reported (Miles and Henry, 2007a).

Because of the variability of known physical properties and the chemical reactions of different clay-based adsorbents numerous *in-vitro* studies have been conducted to investigate the effectiveness of each adsorbent when it interacts with mycotoxins (Huwig *et al.*, 2001). Even though *in-vitro* data collected about specific mycotoxin-adsorbent interactions is of importance, data collected *in-vivo* are even more important (Scheideler, 1993; Dwyer *et al.*, 1997; Dale, 1998). This is especially true since all clay-based adsorbents are not equal and several adsorbents have been shown to impair nutrient utilization in poultry (Chung and Baker 1990; Chung *et al.*, 1990; Kubena *et al.*, 1993; Scheideler, 1993). Ledoux *et al.* (1999) reported that IMTX fed to broilers at a dietary concentration of 1.0% totally removed all of the negative effects on performance and organ lesions associated with feeding 4 mg/kg (ppm) aflatoxin B₁. These authors concluded from examining the organs of the chicks at the end of the experiment that the absence of any signs of nutritional deficiencies in the chicks indicated that IMTX did not negatively affect the absorption of dietary nutrients such as minerals and vitamins. Furthermore, these authors also reported that feeding 4 mg/kg aflatoxin B₁ in the diet resulted in decreases in serum albumin, globulin and total protein which indicated that protein synthesis was impaired. However, these serum components as well as other serum components were totally restored to normal levels by feeding 1.0% IMTX in the diet containing the aflatoxin B₁.

Even though IMTX has been shown to be completely safe when added to broiler and commercial egg-type laying hen diets at 8 times the recommended amount of 0.25%, the safety of IMTX in broiler breeder diets when fed at higher than the recommended amount has not been investigated. Extrapolating the safety data reported in the scientific literature for broilers and commercial egg-type laying hens to broiler breeders is neither reasonable nor acceptable. This is especially true if it is kept in mind that some adsorbents have been reported

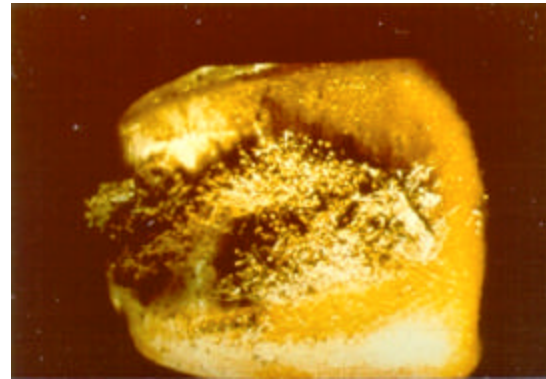


Fig. 1: Corn kernel infected by *Aspergillus flavus*. Infection by this mold can lead to mycotoxicosis

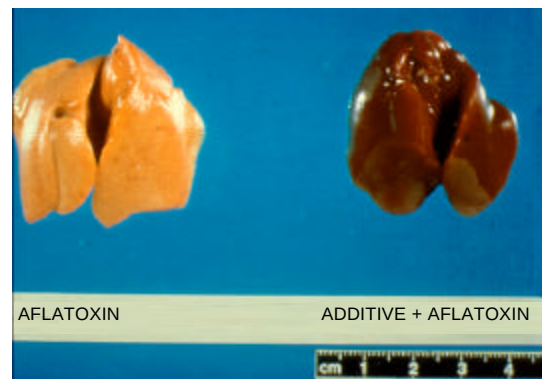


Fig. 2: Aflatoxin affected liver (left) vs healthy liver (right)

to decrease the utilization of certain nutrients in the diet. The broiler breeder hen must not only absorb and utilize nutrients from the diet to meet her own needs she absorbs nutrients for hatching egg production as well. This safety study with IMTX was conducted to assure that breeder performance would not be compromised if their breeder diet contained up to 4 times the recommended amount of IMTX.

MATERIALS AND METHODS

The broiler breeders raised for this experiment were Cobb 500 females and MX males. During the growing period all of the birds were fed on a skip-a-day schedule in order to attain the point on the body weight growth curve each week that was recommended by the primary breeder.

At 21-weeks of age the birds were randomly divided among 12 floor pens with each of the 3 dietary treatments consisting of 4 replicate pens. Each floor pen contained 25 hens and 2 roosters. Also, at 21-weeks light stimulation began and the birds were switched from the grower diet to the three experimental corn soybean meal based breeder diets that included IMTX at

Table 1: Composition of experimental breeder diets containing improved milbond-TX® (IMTX)

Ingredient (%)	0% IMTX	0.5% IMTX	1.0% IMTX
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 premix	0.08	0.08	0.08
Selenium premix (0.6%)	0.02	0.02	0.02
Ethoxyquin	0.02	0.02	0.02
IMTX	0.00	0.50	1.00

*CP: Crude protein

Table 2: Calculated nutrient content of experimental diets

----- IMTX diets -----			
Nutrient	0%	0.5%	1.0%
*AME, Kcal/Kg	2.915	2.915	2.915
*CP (%)	15.50	15.50	15.50
Calcium (%)	3.25	3.25	3.25
Available phosphorus (%)	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

*AME: Apparent metabolizable energy

*CP: Crude protein

*TSAA: Total sulfur amino acids

dietary concentrations of 0, 0.5 and 1.0% (Table 1). Birds in each treatment group were fed the same amount of feed daily (g/bird/day). The amount of feed allocated daily to each pen was adjusted for pens with mortality. The roosters were also fed the experimental diets in amounts recommended by the primary breeder.

All diets were formulated to an ideal amino acid profile and contained 15.5% crude protein and 2915 kcal AME/kg (Table 2). Hens were allocated a quantity of feed each day according to the primary breeder's recommendations for feeding hens into production. At peak feed consumption, hens were receiving 154 g feed/bird/day which provided approximately 450 kcal AME/bird/day and 23.9 g crude protein/bird/day. The quantity of feed allocated to the birds each day was decreased gradually post-peak egg production. Samples of the feed representing each dietary treatment were submitted to the Central Analytical Laboratory (University of Arkansas Poultry Science Center, Fayetteville, AR) for analysis (Table 3).

Eggs were collected three times daily once egg production began and this collection regime continued until peak egg production was attained. After the hens reached peak production, eggs were collected twice daily. At each collection the number of eggs per pen was recorded. All eggs were saved for hatching and a marker was used to write the pen number on each egg in order to track the hatchability and fertility by pen. Average egg

Table 3: Actual Nutrient Analysis, Nutrient content of the diets as determined by laboratory analysis

----- Experimental diets -----			
Analyzed nutrient	0% IMTX	0.5% IMTX	1.0% IMTX
*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 phosphorus (%)	0.60	0.64	0.60
Calories/g	3.788	3.787	3.772

*DM: Dry matter

*CP: Crude protein

weights were determined using a digital scale and recorded for all eggs collected on two days of each week.

Starting the week after the hens came into production the eggs were set in an incubator weekly for 12 consecutive weeks. At transfer on the 18th day of incubation the eggs were candled and the number of infertile, contaminated and early dead embryos recorded after the eggs were broken-out. When the hatch from each dietary treatment was pulled each chick was weighed individually using a digital scale.

At the termination of the study all of the data collected were analyzed using JMP Pro 10 using standard least square analysis REML method. Pen was considered as a random effect. When significant differences were found, means were separated using Tukey HSD, $\alpha = 0.05$.

RESULTS AND DISCUSSION

Mortality: Total mortality (data not shown) during the entire experimental period averaged only 1.7% and was not related to dietary treatment. Similarly, in numerous experiments specifically designed to determine the safety of IMTX in broiler and commercial egg-type laying hens, Miles and Henry (2007abc) reported total mortality did not exceed 3% in any experiment and the mortality was not attributed to a specific dietary treatment when IMTX was fed in the diet at concentrations up to 2.0%. Ledoux *et al.* (1999) also reported that the mortality (4.1%) which occurred in their study was not related to treatment when IMTX was fed to broilers at the same dietary concentration of 1.0% which was used in this broiler breeder study.

Egg production: In the current trial the number of eggs per hen housed was not significantly different among the three dietary treatments. The numbers of eggs per hen housed were 55.5, 57.8 and 56.9 for treatments containing 0, 0.5 and 1.0% IMTX, respectively ($p = 0.4233$, $SEM = 1.2$).

In the study reported with commercial egg-type laying hens, Miles and Henry (2007a) fed diets containing up to 2.0% IMTX and also reported no decline in egg production during an experimental period of 5 consecutive 28-day periods. These authors concluded

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	0.562	1.126	0.384	0.994	1.547	0.561	0.595	0.689

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 ^a	89.7	89.7	87.6	89.0
2	82.3	83.3	87.6	88.2	87.8	88.2	85.2 ^b	91.3	91.3	88.9	87.4
3	85.1	85.8	87.3	88.2	87.7	88.0	91.6 ^a	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 REML analysis (pen treated as a random effect)

Means separated by student's t test $\alpha = 0.05$ ^aMeans with different superscripts are significantly different

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 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 REML analysis (pen treated as a random effect)

that if a feed mill error occurred and IMTX was accidentally over-supplemented to a laying hen diet no adverse effects on any egg production or any other attribute of layer performance should be expected.

Egg weights: Average egg weights were not significantly different among the three dietary treatments at any week during the entire experimental period (Table 4, $p = 0.759$, SEM = 0.689). The egg weights at 35 weeks of age were within 0.6 grams when comparing the un-supplemented control diet with the diets containing either 0.5 or 1.0% IMTX. Likewise, Miles and Henry (2007a) in their research with egg-type laying hens found no significant differences ($p > 0.10$) in egg weights among the control diet and diets containing IMTX up to the highest dietary concentration of 2.0%. Only at weeks 16 and 20 in the study of Miles and Henry (2007a) did egg weights for the IMTX treatments decrease in comparison to the weight of those from hens fed the control diet and this was attributed to an unexplained anomaly.

Hatchability: Hatchability (Table 5) was calculated from total eggs collected and set for each week during weeks 26-35. Overall egg hatchability in this study was not significantly different among treatments ($p = 0.3152$). Only in the hatch for week 32 was there a significant difference and this is considered to be an anomaly since the significant decrease in hatchability, when compared

to that for the control diet, occurred only in the treatment containing 0.5% IMTX and not in the treatment containing the higher level of 1.0%. IMTX. Hatchability of eggs throughout the trial was considered to be consistent and was not affected by feeding IMTX.

Fertility: Fertility (Table 6) among treatments remained consistent throughout the trial and there were no significant differences among any treatments ($p = 0.2630$). Data collected indicate that feeding higher than the recommended level of 0.25% IMTX has no obvious effect on sperm production or sperm quality of the roosters and apparently does not reduce mating activity. Likewise, adding 1.0% IMTX to the diet did not seem to affect the ability of the female to store sperm and also did not interfere with fertilization in the female.

Chick weights: Chick weights each week from 26 to 35 weeks were unaffected by feeding higher than the recommended level of IMTX (Table 7). Overall, no significant differences among the treatments (p -value = 0.6738) occurred during the entire experimental period. The increase of chick weights over the trial period was expected since older hens lay larger eggs and as a result the chick weights increase. On average for all treatments chick weight increased an average of 6.3 grams per chick over the 10 week period from 26 to 35 weeks in which the eggs were collected and set.

Average chick weights during the entire experimental period for the control and treatments containing 0.5 and 1.0% IMTX were 40.1, 40.1 and 40.4 g, respectively.

Conclusion: As has been previously documented with broilers and commercial egg-type laying hens in experiments conducted by other investigators and cited in the scientific literature, the inclusion of IMTX 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 in the diet of broiler breeders at the recommended concentration of 0.25% and up to a concentration of 1.0%.

ACKNOWLEDGMENTS

The authors thank Cobb-Vantress for providing the birds used in this study and Milwhite Inc. for their financial support of this study. Also, the authors acknowledge Dr. Richard Miles (Professor Emeritus, University of Florida) for reviewing this paper and contributing information regarding IMTX associated research related to the safety of IMTX in poultry.

REFERENCES

- CAST, (Council for Agricultural Science and Technology), 2003. Mycotoxins: Risks in Plant, Animal and Human Systems. Task Force Report No. 139. Ames, Iowa.
- Chung, T.K. and D.H. Baker, 1990. Phosphorus utilization in chicks fed hydrated sodium calcium aluminosilicate. *J. Anim. Sci.*, 68: 1992-1998.
- Chung, T.K., J.W. Erdman, Jr. and D.H. Baker, 1990. Hydrated sodium calcium aluminosilicate. Effects of zinc, manganese, vitamin A and riboflavin utilization. *Poult. Sci.*, 69: 1364-1370.
- Croubels, S., 2013. Mycotoxins and Their Effects on the Intestinal Health of Poultry. The Poultrysite Digital. 5M Publishing. <<http://www.thepoultrysite.com/>> Accessed 10 November 2013.
- Dale, N., 1998. Mycotoxin binders. It's time for real science. *Poult. Digest*, 57: 38-39.
- Dwyer, M.R., L.F. Kubena, R.B. Harvey, K. Mayura, A.B. Sarr, S. Buckley, R.H. Bailey and T.D. Phillips, 1997. Inorganic adsorbents and cyclopiazonic acid in broiler chickens. *Poult. Sci.*, 76: 1141-1149.
- Huwig, A., S. Freimund, O. Kappeli and H. Dutler, 2001. Mycotoxin Detoxication of Animal Feed by Different Adsorbents. *Toxicol. Lett.*, 122: 179-188.
- Kabak, B., A.D. Dobson and I. Var, 2006. Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed: A Review. *Crit. Rev. in Food Sci. and Nutr.*, 46: 593-619.
- Kubena, L.F., R.B. Harvey, W.E. Huff, M.H. Elissalde, A.G. Yersin, T.D. Phillips and G.E. Rottinghaus, 1993. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol. *Poult. Sci.*, 72: 51-59.
- Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez and M. Alonso-Bebolt, 1999. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol. *Poult. Sci.*, 78: 204-210.
- Miles, R.D. and P.R. Henry, 2007a. Safety of Improved Milbond-TX When Fed to laying Hens at Higher-Than-Recommended Levels. *J. Appl. Poult. Res.*, 16: 404-411.
- Miles, R.D. and P.R. Henry, 2007b. Safety of Improved Milbond-TX When Fed in Broiler Diets at Higher-Than-Recommended Levels. *Anim. Feed Sci. and Technol.*, 138: 309-317.
- Miles, R.D. and P.R. Henry, 2007c. Safety of Improved Milbond-TX When Fed in Broiler Diets Limiting in Available Phosphorus or Containing Variable Levels of Metabolizable Energy. *J. Appl. Poult. Res.*, 16: 412-419.
- Scheideler, S.E., 1993. Effects of various types of aluminosilicates and aflatoxin B₁ on aflatoxin toxicity, chick performance and mineral status. *Poult. Sci.*, 72: 282-288.
- Wu, F., 2006. Mycotoxin Reduction in Bt Corn: Potential Economic, Health and Regulatory Impacts. *Transgenic Res.*, 15: 277-289.