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Safety of Improved Milbond-TX® when fed in broiler diets at greater than recommended levels

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Abstract

A maize-soybean meal diet was supplemented with Improved Milbond-TX® (IMTX), a hydrated sodium calcium aluminosilicate (HSCAS), in two experiments to determine product safety at elevated levels for broilers fed for 21 days. In Experiment 1, IMTX was incorporated into isonitrogenous and isoenergetic diets at 0, 2.5, 5, 10, 15 and 20 g/kg. In Experiment 2, IMTX was added to the finished feed at 10 or 20 g/kg by weight to simulate a feed mixing error. There was generally a linear ($P<0.01$) increase in feed intake and body weight of birds given IMTX in Experiment 1 but this trend did not reach significance in Experiment 2. Excreta moisture decreased linearly ($P<0.001$) in response to increasing IMTX in Experiment 2 but not in Experiment 1. There was no difference ($P>0.10$) in any blood components due to treatment. Improved Milbond-TX® appears to be safe for broilers when added at levels up to eight times the recommended amount.

Keywords: Broilers; Blood; Toe ash; Excreta moisture; Clay adsorbents; Mycotoxin

1. Introduction

Previous studies have indicated the advantages of using montmorillonite clay-based hydrated sodium calcium aluminosilicates (HSCAS) as a mycotoxin adsorbent when added

Abbreviations: IMTX, Improved Milbond-TX®; HSCAS, hydrated sodium calcium aluminosilicates; AFB_1, aflatoxin B_1

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Table 1
Typical chemical analysis of Improved Milbond-TX®

Compound	Range (g/kg)
SiO_2	546–656
Al_2O_3	145–197
Fe_2O_3	40.5–50.2
MgO	9.4–20.8
Na_2O	5.4–13.7
K_2O	6.0–11.9
TiO_2	6.3–7.7
CaO	6.4–9.7
Loss on ignition (at 1000 °C)	85–119

Osuna, 2006 (personal communication).

to the diet at levels up to 10 g/kg (Phillips et al., 1988, 1990; Desheng et al., 2005; Bailey et al., 2006).

Improved Milbond-TX® (IMTX, Milwhite Inc., 5487 South Padre Island Hwy., Brownsville, TX 78521, USA) is an inert montmorillonite clay-based adsorbent that originates from natural clay deposits mined from the earth. It is normally recommended by the manufacturer for use at a dietary concentration of 2.5 g/kg diet. The typical chemical analysis of IMTX as furnished by the manufacturer is presented in Table 1. Ledoux et al. (1999) tested IMTX to determine its efficacy to ameliorate the toxic effects of AFB_1 present in poultry diets. The researchers concluded that IMTX included in the diet at 10 g/kg was effective in preventing the toxic effects of AFB_1 that may be present in diets at levels up to 4 mg/kg feed and reported no toxicosis in broiler chicks at 10 g/kg of the diet. Kubena et al. (1993) also reported that an HSCAS fed at 5 g/kg in a diet containing no mycotoxins did not adversely affect performance of broilers raised to 21 days of age.

Although the USDA/FDA considers clay-based adsorbents, when used as dietary flow agents and carriers, to be Generally Recognized As Safe (GRAS) animal feed additives, all dietary adsorbents should be evaluated for their safety *in vivo*. The importance of continually evaluating the safety of all dietary mycotoxin enterosorbents *in vivo* was emphasized and discussed by Pimpukdee et al. (2004).

The objectives of the current experiments were to determine the safety of IMTX for broiler chicks when fed at varying concentrations from 2.5 to 20 g/kg in diets designed to be isonitrogenous and isoenergetic (Experiment 1) and when simulating an actual feed mill mixing error (Experiment 2) in which the IMTX was added directly to the balanced, complete diet. Experiment 1 tested the effect of IMTX fed at eight times the manufacturer's recommended level (2.5 g/kg) and twice the level used by Ledoux et al. (1999). Because most of the *in vivo* research published with poultry in which non-nutritive clay-based enterosorbents such as IMTX have been studied as mycotoxin binders have not reported their effect on excreta moisture content, this was also measured in these experiments. Efficacy of this product, however, has been well documented (Ledoux et al. 1999) and therefore was not studied in the present experiments so as not to inflict unnecessary stress and suffering in the birds by dosing them with AFB_1.

2. Materials and methods

2.1. Animal management and diets

In Experiment 1, 480 Ross × Ross male broiler chicks were selected by weight from a larger group and allocated randomly with five birds per pen in Petersime battery brooders (Petersime Incubator Company, 300 North, Gettysburg, OH 45328, USA). Brooders were located in an environmentally controlled room with constant lighting. During the first week the room temperature was maintained at 30 °C, then decreased to 26 °C the second week and 24 °C the third week. Each pen contained approximately the same total weight of chicks to eliminate extremes in initial body weight. Feed in the form of a mash diet and tap water were available on an *ad libitum* basis for the 21-days experimental period. In Experiment 2, 240 Ross × Ross male broiler chicks were weighed from a larger group and similarly assigned randomly to pens each containing five chicks. In both experiments, there were 16 replicate pens for each dietary treatment. In both experiments diets were allotted randomly to pens. Previous experience with this facility indicated no location effects on performance such that blocking was not required. In Experiment 1, IMTX was incorporated into the corn-soybean meal basal diet (Table 2) at 0, 2.5, 5, 10, 15 and 20 g/kg diet

Table 2
Composition of the basal diets

Ingredient	Amount, as fed basis (g/kg)	
	Experiment 1	Experiment 2
Ground yellow maize	545.0	565.0
Soybean meal (485 g CP/kg)	371.5	371.5
Ground limestone	10.0	10.0
Dicalcium phosphate (220 g Ca/kg; 185 g P/kg)	17.0	17.0
DL-methionine	2.5	2.5
Maize oil	25.0	25.0
Salt (iodized)	4.0	4.0
Vitamin premix[a]	2.5	2.5
Mineral premix[b]	2.5	2.5
Variables[c]	20.0	–
Calculated composition (as-fed basis)		
ME (MJ/kg)	12.39	12.69
Crude protein	226.5	228.2
Lysine	12.4	12.4
TSAA	9.2	9.2
Calcium	9.2	9.2
Available phosphorus	4.3	4.3

[a] Ingredients supplied per kilogram of diet: biotin, 0.2 mg; cholecalciferol, 2200 IU; choline, 500 mg; ethoxyquin, 65 mg; folic acid, 1 mg; niacin, 60 mg; pantothenic acid, 15 mg; pyridoxine, 5 mg; riboflavin, 5 mg; thiamin, 3 mg; vitamin A, 8000 IU; vitamin B_{12}, 0.02 mg; vitamin E, 20 IU; vitamin K, 2 mg.

[b] Ingredients supplied per kilogram of diet: copper, 10 mg; ethoxyquin, 65 mg; iodine, 2 mg; iron, 60 mg; manganese, 90 mg; selenium, 0.2 mg; zinc, 80 mg.

[c] Improved Milbond-TX® was added in place of an equivalent weight of white builder's sand.

at the expense of white builder's sand to keep the diets isonitrogenous and isoenergetic. In Experiment 2, IMTX was added to the corn-soybean meal mash basal diet (Table 2) at 0, 10 and 20 g/kg by weight without additional sand as a variable to simulate a feed mill mixing error. In both experiments the diets were formulated to contain all nutrients recommended by the NRC (1994). Chicks were managed by methods approved by the University of Florida Institutional Animal Care and Use Committee. Total pen body weight and feed consumption were determined at 7-days intervals throughout the 21-days experimental period during both experiments. Birds were checked no less than five times per day for mortality. Feed remaining and the dead bird were weighed immediately upon discovery. These weights were then used to correct the feed and body weight estimates for that pen.

2.2. Collection and analysis of samples

At the termination of Experiment 1, two chicks from each of five pens fed the control, 10 and 20 g/kg IMTX were chosen at random, weighed and bled via cardiocentesis for serum analyses and then were killed by cervical dislocation. The serum chemistry was determined on 7–10 mL samples of blood using a Hitachi 911 blood/urine analyzer (Boehringer GmbH, Mannheim, Germany) at the Department of Physiological Sciences at the University of Florida Veterinary Medical Teaching Hospital. The chemistry panel included calcium (Ca), phosphorus (P), sodium (Na), potassium (K), chloride (Cl), glucose, triglycerides, cholesterol, total protein, albumin and uric acid.

All remaining chicks were then weighed and killed by cervical dislocation. In Experiment 1, the middle toes of all chicks were removed at the tarsometatarsal/P3 joint with the skin intact. The tip of the toe containing the nail was removed and discarded, then the toes were cleaned of any adhering foreign material with wet paper towels and pooled by pen for toe ash analysis. Each set of toes was dried in an oven for 48 h at 100 °C, then ashed in a muffle furnace at 550 °C for 14 h to determine the toe ash weight. Toes were ashed without lipid extraction. In Experiment 2, all the chicks were killed and toes collected and treated as described previously.

Moisture in excreta was determined during the last week of both experiments. Excreta samples were collected separately from each pen during a 2-day period using aluminum foil placed on the pans under each pen of chicks. These samples were homogenized by hand for 2 min in a 2 L beaker using a spoon. Three sub-samples, approximately 2 g each, were weighed into individual aluminum dishes, dried for 48 h at 100 °C and weighed for calculation of moisture by difference.

2.3. Statistical analysis

Data for both experiments were analyzed using one-way ANOVA in the General Linear Models (GLM) procedure of SAS (1994) Pen was the experimental unit for all analyses. Data were further analyzed by regression using Proc Stepwise (SAS, 1994) to determine significant linear and quadratic components in Experiment 2 and these terms as well as higher order components in Experiment 1.

Table 3
Average weekly body weight, feed consumption and feed conversion for broiler chicks supplemented with Improved Milbond-TX® (IMTX) in Experiment 1[a]

IMTX (g/kg)	Body weight (g)			Feed consumption (g)			Feed conversion (g/g)		
	7 d[b]	14 d[b,c]	21 d[b]	7 d	14 d[d]	21 d[b]	7 d[b]	14 d	21 d
0	108	285	628	113	381	875	1.06	1.34	1.41
2.5	108	276	582	118	362	847	1.11	1.31	1.46
5	116	316	669	116	401	945	1.02	1.27	1.42
10	118	324	662	119	404	935	1.02	1.25	1.41
15	114	317	637	108	397	898	0.95	1.26	1.41
20	119	317	668	110	410	978	0.93	1.30	1.47
Pooled SE	1.1	3.3	5.4	1.3	4.6	8.5	0.016	0.013	0.012
ANOVA, P	<0.01	<0.001	<0.001	n.s.[e]	<0.05	<0.001	<0.05	n.s.	n.s.

[a] Each value represents the mean of 16 pens of five birds.
[b] Significant linear effect (P<0.01).
[c] Significant quadratic effect (P<0.01).
[d] Significant linear effect (P<0.05).
[e] n.s., Not significant.

3. Results

In both experiments, mortality was less than 2% throughout the experimental period and was not related to dietary treatment. In Experiment 1, significant linear effects ($P<0.01$) were found on body weight at all three ages (Table 3). A significant ($P<0.01$) quadratic component was also found for the response of body weight to IMTX at 14 days of age. Feed intake did not differ among treatments at 7 days (Table 3). At 14 days there was a linear effect ($P<0.05$) of IMTX on feed intake. At 21 days feeding IMTX also resulted in a linear ($P<0.01$) effect on feed consumption. Feed conversion, which did not take into account the initial weight of the chicks, differed ($P<0.05$) among the dietary treatments only at 7 days of age, with chicks given the highest level of IMTX more efficient than birds given the control diet or 2.5 g IMTX/kg (Tables 3 and 4).

There were no differences ($P>0.10$) in the serum components analyzed in Experiment 1 (Table 5) when IMTX was incorporated at levels above those recommended by the manufacturer. In Experiment 1, toe ash tended ($P<0.10$) to increase as the level of supplemental

Table 4
Average weekly feed weight, feed consumption and feed conversion for broiler chicks supplemented with Improved Milbond-TX® (IMTX) in Experiment 2

IMTX (g/kg)	Body weight (g)			Feed consumption (g)			Feed conversion (g/g)		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
0	126	335	687	115	384	885	0.91	1.15	1.29
10	130	354	711	115	404	924	0.89	1.15	1.31
20	131	355	710	119	410	935	0.92	1.16	1.32
Pooled SE	2.0	6.5	10.5	1.6	6.3	13.4	0.009	0.007	0.006
ANOVA, P	n.s.[a]	n.s.	n.s.	n.s.	n.s	n.s.	n.s.	n.s.	n.s.

[a] n.s., Not significant. Each value represents the mean of 16 pens.

Table 5

Serum components of broiler chicks supplemented with Improved Milbond-TX® (IMTX) for 21 days in Experiment 1

Serum component	IMTX (g/kg)			Pooled SE	P
	0	10	20		
Calcium (mg/dL)	9.6	10.2	9.7	0.13	n.s.[a]
Phosphorus (mg/dL)	7.0	7.0	6.8	0.21	n.s.
Sodium (mEq/L)	154	155	153	1.1	n.s.
Potassium (mEq/L)	6.3	6.6	6.6	0.18	n.s.
Chloride (mEq/L)	115	116	115	0.6	n.s.
Glucose (mg/dL)	244	245	238	6.1	n.s.
Triglycerides (mg/dL)	40	47	41	3.4	n.s.
Cholesterol (mg/dL)	126	134	130	3.5	n.s.
Uric acid (mg/dL)	5.9	5.3	5.7	0.44	n.s.
Total protein (g/dL)	2.62	2.95	2.69	0.065	n.s.
Albumin (g/dL)	1.41	1.55	1.46	0.035	n.s.

[a] n.s., Not significant. Each value represents the mean of five pens.

IMTX increased (Table 6), but this was not observed in Experiment 2. In Experiment 1, there were no differences ($P>0.10$) in litter moisture among the dietary treatments. In Experiment 2, excreta moisture decreased linearly ($P<0.001$) from 685 g/kg for chicks fed the control diet to 671 and 658 g/kg for those given 10 and 20 g/kg IMTX, respectively. At the end of the second trial, excreta from chicks given 20 g/kg IMTX could be easily identified visually by all of the investigators as being dryer than droppings from the other birds on the two treatments.

4. Discussion

Feed intake and body weight either remained the same or increased with supplemental IMTX up to 20 g/kg, whether the diets were isoenergetic and isonitrogenous (Experiment 1) or the IMTX was added by weight to the finished feed (Experiment 2). The improvement in

Table 6

Average toe ash and excreta moisture of broiler chicks supplemented with Improved Milbond-TX® (IMTX) for 21 days in Experiments 1 and 2

Item	IMTX (g/kg)			Pooled SE	P
	0	10	20		
Experiment 1					
Toe ash (g/kg)	139	141	145	1.1	<0.10
Excreta moisture (g/kg)	661	671	678	6.4	n.s.[a]
Experiment 2					
Toe ash (g/kg)	139	142	137	1.0	n.s.[a]
Excreta moisture[b] (g/kg)	685	671	658	2.7	<0.001

[a] n.s., Not significant. Each value represents the mean of 16 pens.

[b] Significant linear effect ($P<0.001$).

body weight and feed conversion in chicks fed IMTX in the isocaloric and isonitrogenous diets was not unexpected. The literature contains numerous reports that document a promotion in performance when various clay-based enterosorbents were fed to animals, especially poultry. Xia et al. (2004) cited many such instances of improvement in performance in their discussion, which echoed their own results with a montmorillionite clay-based enterosorbent fed to poultry. These authors reported positive changes in intestinal morphology and intestinal enzyme concentrations that they proposed as possible mechanisms responsible for the improved performance of chicks fed the montmorillonite. Xia et al. (2004) reported that dietary addition of a montmorillonite clay product to chick diets resulted in measurable changes in mucosal morphology of the intestine that improved general gut health. This may be one explanation for the significantly lower moisture in the excreta of chicks fed IMTX in the present trial.

Ledoux et al. (1999) reported that serum chemistry measurements were not affected by the addition of 10 g IMTX/kg, in the absence of AFB_1. Their data indicated that IMTX did not have a negative effect on dietary utilization at 10 g/kg, while at the same time completely preventing the reduced performance, changes in organ weights, serum chemistry and gross pathology observed in chicks fed a corn-soybean meal basal diet supplemented with AFB_1 at 4 mg/kg feed. Kubena et al. (1993) also reported that feeding 5 g HSCAS/kg had no effect on organ weight, serum biochemical values or enzyme activities. The lack of effect on serum chemistry from feeding IMTX at dietary concentrations up to 20 g/kg in the present study is in agreement with results from other studies with clay-based HSCAS in diets for poultry when fed at concentrations no greater than 10 g/kg (Kubena et al., 1993; Dwyer et al., 1997; Rosa et al., 2001; Bailey et al., 2006). In general, the literature suggests clay-based enterosorbents fed at recommended levels have not resulted in any significant changes in serum chemistry in trials with chicks.

In the present study, toe ash was chosen as a variable rather than tibia ash. Since the early 1940s, toe ash has been used by numerous investigators as a rapid, economical and acceptable means to quantify bone mineralization of chicks (Potter, 1988; Potter et al., 1995; Ravindrin et al., 1995; Garcia and Dale, 2006). In fact, Ravindrin et al. (1995) found that body weight and toe ash percentage were equal or superior in sensitivity compared to tibia ash as criteria to estimate relative bioavailability of P for broilers. Yan et al. (2005) compared various methods to evaluate bone mineralization in 21-day-old male chicks and concluded that use of toe ash, foot ash or tibia ash without lipid extraction were all valid criteria that will enable more rapid evaluation of factors that influence bone mineralization.

One factor that has a profound negative effect on bone mineralization is aluminum (Al) because it has been shown to complex with P in the gastrointestinal tract and reduced the relative bioavailability of P to the animal (Storer and Nelson, 1968; Edwards, 1988). Because IMTX (Table 1) contains from 145 to 197 g Al_2O_3 (100 to 136 g/kg Al)/kg, bone mineralization was of special interest in these earlier studies. If the Al in the IMTX complexed P in the gastrointestinal tract, preventing absorption and utilization of the P for bone formation, a decrease in serum P and toe ash would have been expected, especially with the highest levels of IMTX in the diet. This, however, was not observed in either study reported herein.

The toe ash values in these two experiments, which averaged from 130 to 140 g/kg, are greater by 10 to 20 g/kg than values reported by other researchers using similar maize-soybean meal diets (Potter, 1988; Potter et al., 1995; Yan et al., 2005). One explanation may be due to larger birds in the present study, eating more feed and consuming a diet with slightly more available P up to 3 week of age. Also, removing the toe tip with the nail may have contributed to overall ash concentration being somewhat greater. Apparently the Al in IMTX remains in a form that does not complex dietary P.

Because there are many different types of clay-based products available commercially for use in animal feeds, establishing their safety under a variety of feeding regimens should be a top priority. The feed and animal industries are interested in any additive that will alleviate the detrimental effects that mycotoxins impart to animals. The industry must have a high degree of confidence that these additives are safe, as well as effective, if accidentally incorporated into diets at levels greater than the recommended concentrations.

5. Conclusion

Data reported herein demonstrate that IMTX is safe for broilers when added at levels up to eight times the recommended amount.

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