Safety of Improved Milbond-TX When Fed to Laying Hens at Higher-Than-Recommended Levels

R. D. Miles[1] and P. R. Henry

Department of Animal Sciences, University of Florida, Gainesville 32611

Primary Audience: Nutritionists, Researchers, Commercial Egg Producers

SUMMARY

A corn-soybean meal diet was supplemented with 0, 1, or 2% Improved Milbond-TX (IMTX, a hydrated sodium calcium aluminosilicate) for Hy-Line W-36 laying hens selected for good or poor eggshell quality. Supplementing the diet with IMTX had no detrimental effects on egg weight, eggshell weight, albumen quality, feed consumption, or feed conversion during 5 consecutive 28-d periods. Increase in BW over the 5-mo experimental period was not influenced by the addition of IMTX. Results from this study show that an accidental oversupplementation of a laying hen diet with up to 8 times the recommended level of IMTX of the manufacturer, which may occur due to a feed mill mixing error, should not result in adverse performance of laying hens nor should it affect the concentration of moisture in the excreta.

Key words: layer, egg, performance, albumen quality, excreta moisture

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DESCRIPTION OF PROBLEM

Measures used by the livestock industry to protect animals from the toxic effects of aflatoxin B_1 (**AFB_1**), including grain testing, fermentation, dilution of contaminated feed, use of mold inhibitors, microbial inactivation, physical separation, thermal inactivation, irradiation, and ozone degradation, have been reviewed [1]. For the agricultural industry, most of these procedures have proven to be costly, time-consuming, impractical, potentially unsafe, and only partially effective. Currently, one of the more promising approaches is the use of adsorbents to sequester AFB_1 during the digestive process and render this mycotoxin harmless to the animal [2, 3, 4]. The major advantages of these adsorbents include cost, safety, and ease of administration, because they are added to animal feeds. When added to the feed, not all adsorbents are equally effective in protecting against the toxic effects of AFB_1, and several adsorbents have been shown to impair nutrient use [2, 5, 6]. It has also been reported that when clay-based adsorbents were added to the diet as a means of reducing toxin bioavailability by selectively binding the toxin in the digestive tract of the animal, fecal moisture and NH_3 emissions were decreased [7, 8, 9]. The poultry industry continually faces mounting scrutiny of its environmental stewardship. On-farm nutrient management plans have been developed in response to concerns about manure odor, fly problems, and fecal moisture. A reduction in fecal moisture is a first step in reducing problems associated with these concerns, as well as P loading in the environment [8]. The solubility of P and other minerals is also lessened when fecal moisture is decreased. Natural zeolites and clay-based adsorbents have moisture-absorptive properties [9], and their addition to diets or application directly

[1]Corresponding author: rdmiles@ufl.edu

Table 1. Typical chemical analysis of Improved Milbond-TX[1]

Compound	Range (%)
SiO_2	54.6 to 65.6
Al_2O_3	14.5 to 19.7
Fe_2O_3	4.05 to 5.02
MgO	0.94 to 2.08
Na_2O	0.54 to 1.37
K_2O	0.60 to 1.19
TiO_2	0.63 to 0.77
CaO	0.64 to 0.97
Loss on ignition (at 1,000°C)	8.5 to 11.9

[1]Osuna [33].

to poultry excreta or litter [10] will assist in accomplishing the goals set forth in a nutrient management plan.

Dale [11] reported that many of the adsorbents on the market have not been adequately tested for in vivo efficacy, and previous data implying their efficacy were based on in vitro testing. Therefore, it is important that adsorbent supplements be subjected to in vivo evaluation to determine their efficacy and influence on nutrient utilization and safety when fed in animal diets at higher than the recommended level of the manufacturer.

The present study was not designed to associate the finding of high dietary concentrations of a hydrated sodium calcium aluminosilicate (**HSCAS**) with an impairment of P utilization, even though natural and synthetic HSCAS contain Al, which is known to sequester P in the intestinal tract [12, 13, 14]. Hussein et al. [15] found that the addition of 0.3% Al_2SO_4 to the diets of Japanese quail resulted in a cessation of egg production within 5 d of Al supplementation. It is hypothesized that the mode of action of Al sequestering P is brought about by the complexing of its salts with phosphate and not with other minerals. This hypothesis is supported by the fact that the detrimental effects of Al can be reversed by the sole addition of P to the diet [16].

Improved Milbond-TX (**IMTX**) [17] 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 concentration of 0.25% of the diet. The typical chemical analysis of IMTX as furnished by the manufacturer is presented in Table 1.

Ledoux et al. [18] 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 1% 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 due to IMTX in broiler chicks when fed at 1% of the diet. Kubena et al. [2] also reported that a clay-based HSCAS fed at 0.5% in the control diet containing no aflatoxin or diacetoxyscirpenol did not adversely affect performance of broilers raised to 21 d of age.

Previous studies have indicated the advantages of using a clay-based montmorillonite (HSCAS) as a mycotoxin adsorbent when added to the diet at levels up to 1% [3, 4, 19, 20]. The adsorbent activities of these HSCAS products have raised questions regarding their effect on the utilization of nutrients such as vitamins and minerals [5, 21, 22, 23]. Data collected from in vitro tests alone can be misleading when testing clay adsorbents in relation to their ability to bind and detoxify mycotoxins, especially AFB_1 [6, 24]. These authors emphasized that any prediction made from in vitro data concerning the ability of inorganic adsorbents to protect poultry from the detrimental effects of mycotoxins should be approached with caution and should be confirmed in vivo, paying particular attention to the potential for nutrient interactions.

Although the USDA and Food and Drug Administation consider clay-based adsorbents, when used as dietary flow agents and carriers, to be Generally Recognized as Safe 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. [25].

The objective of this experiment was to evaluate the safety of IMTX when supplemented to laying hen diets at concentrations greater than recommended by the manufacturer. Laying hens, which had been selected for good and poor eggshell quality, were used in the study. Because most of the in vivo research published with poultry in which nonnutritive zeolites and 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 with hens laying eggs of good shell quality. Also, no detrimental effects on laying hen performance were expected due to the possibility that Al in IMTX was binding phytate or inorganic P. Scheidler [6], Chung and Baker [21], and Plunske [26] reported no detrimental effects in broiler chicks from HSCAS in studies specifically designed with low dietary available P and containing up to 2% HSCAS.

MATERIALS AND METHODS

Bird Management and Experimental Treatments

Before the initiation of the study, 3 eggs from each of 300 thirty-five-week-old Hy-Line W-36 [27] laying hens were collected, weighed, broken out, washed of excess albumen, and dried to determine the average shell weight of the individual hen. All hens were ranked by shell quality based on mean shell weight. The 75 hens with the highest and the 75 hens with the lowest shell weights were allocated randomly in 5 replicate pens of 5 birds with either good or poor shell quality for the experiment. Each pen of 5 hens was assigned randomly to 1 of 3 dietary treatments, which were the corn-soybean basal diet (Table 2) supplemented with 0, 1, or 2% IMTX. The control diet was formulated to be slightly below NRC requirements [28] for all nutrients except Ca for hens consuming 100 g of feed/d. The 5-bird replicates were individually caged but fed from a single feed trough and had individual waterers. Feed and tap water were available on an ad libitum basis throughout the experimental period. Hens were weighed at the beginning and end of the study. The birds were managed by procedures approved by the University of Florida Institutional Animal Care and Use Committee.

The experimental diets were fed for 5 consecutive 28-d periods. Total egg production and feed consumption for each 28-d period were measured. On d 7 of each week of the first period, all eggs were individually marked and collected. Eggs were weighed (g), broken out, and albumen height (mm) measured on a level surface with a micrometer [29]. Haugh units (**HU**) were calculated based on the formula HU = 100 log (H + 7.57 – $1.7^{0.37}$) [30]. The shells were cleaned of excess albumen, air-dried for 48 h, and weighed. During the second through the fifth periods, eggs were collected once every 14 d and analyzed as described above.

Table 2. Composition of basal diet

Ingredient	Amount[1] (%)
Ground yellow corn	71.55
Soybean meal (48.5% CP)	15.80
Ground limestone	8.90
Dicalcium phosphate (22% Ca, 18.5% P)	0.79
DL-Met	0.08
Iodized salt	0.38
Vitamin premix[2]	0.25
Mineral premix[3]	0.25
Variables[4]	2.00
Calculated nutrient content[1]	
ME (kcal/kg)	2,782
CP (%)	14.70
Met (%)	0.32
Ca (%)	3.65
Total P (%)	0.45
Available P (%)	0.23

[1]As-fed basis.
[2]The vitamin premix supplied per kilogram of diet: biotin, 0.2 mg; cholecalciferol, 2,200 ICU; choline chloride, 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 palmitate, 8,000 IU; vitamin B_{12}, 0.02 mg; vitamin E, 20 IU; and menadione dimethylpyrimidinol bisulfite, 2 mg.
[3]The mineral premix supplied per kilogram of diet: Cu, 10 mg; ethoxyquin, 65 mg; I, 2 mg; Fe, 60 mg; Mn, 90 mg; Se, 0.2 mg; and Zn, 80 mg.
[4]Variables consisted of Improved Milbond-TX calculated for experimental diet level and white builder's sand (both nonnutritive).

Excreta moisture was determined for all birds from the group of good shell hens during the fourth period. Collecting trays were lined with plastic sheeting and hung under each replicate pen of 5 hens. Excreta was allowed to collect for a period of 2 d, then the total sample was cleaned of debris, homogenized, and 3 subsamples were collected individually, weighed, dried in an oven at 100°C for 48 h, and reweighed for determination of moisture content by difference.

Statistical Analysis

Performance and production data at each individual time point were first analyzed by a 2-way ANOVA with the GLM procedure of SAS [31] with IMTX and shell quality as main effects and their interaction. Treatment means were compared using Duncan's multiple range test [31]. The percentage hen-day egg production data were

Table 3. Effect of Improved Milbond-TX (IMTX) on performance of Hy-Line W-36 laying hens selected for good or poor eggshell quality (EQ)[1]

	Good shell quality			Poor shell quality				ANOVA		
Time (wk)	Control	1% IMTX	2% IMTX	Control	1% IMTX	2% IMTX	Pooled SE	IMTX	EQ	IMTX × EQ
Feed intake (g/bird per d)										
1 to 4	102.7	106.8	105.6	99.9	100.3	98.6	0.88	NS	<0.01	NS
5 to 8	105.9	105.5	107.4	100.7	107.6	98.5	1.27	NS	NS	NS
9 to 12	105.5	104.0	106.3	98.0	101.2	100.3	0.84	NS	<0.01	NS
13 to 16	103.2	110.6	104.3	98.5	102.4	98.6	0.87	NS	<0.01	NS
17 to 20	91.9	93.2	98.1	91.4	97.1	97.4	1.25	NS	NS	NS
Feed conversion (kg/dozen eggs)										
1 to 4	1.38	1.43	1.41	1.29	1.29	1.26	0.013	NS	<0.01	NS
5 to 8	1.44	1.49	1.45	1.34	1.43	1.33	0.021	<0.10	<0.01	NS
9 to 12	1.44	1.53	1.49	1.30	1.38	1.36	0.014	NS	<0.05	NS
13 to 16	1.48	1.55	1.55	1.36	1.34	1.34	0.015	NS	<0.01	NS
17 to 20	1.30	1.37	1.47	1.26	1.44	1.43	0.029	NS	NS	NS
Hen-day egg production[2] (%)										
1 to 4	89.0	89.4	90.0	93.3	93.1	93.9	0.519	NS	<0.001	NS
5 to 8	88.3	85.6	88.6	90.4	90.3	89.1	0.660	NS	<0.10	NS
9 to 12	87.7	81.7	85.6	90.6	88.4	88.7	0.564	<0.05	<0.001	NS
13 to 16	83.7	85.9	81.1	87.0	86.7	88.4	0.673	NS	<0.01	NS
17 to 20	85.0	81.4	80.2	87.4	85.9	82.1	0.909	NS	NS	NS

[1]Each value represents the mean of 5 pens of 5 individually caged hens.
[2]ANOVA conducted on the arc sine-transformed variable.

analyzed after an arc sine transformation to equalize variances. Then, egg weight, HU, and shell weight were analyzed by repeated measures with GLM. Helmert and polynomial options were used to evaluate time and treatment effects on these production parameters. The Helmert option calculated the probability of a difference between any given time and the mean of the remaining times to determine breaks (changes) in the response curves over time. The polynomial option evaluated the nature of the time response to dietary IMTX and shell quality selection.

RESULTS AND DISCUSSION

Performance

Mortality was less than 3% for all birds throughout the experiment and was not related to treatment (data not presented). Feed consumption was not affected by the IMTX but was greater ($P < 0.01$) during wk 1 to 4, 9 to 12, and 13 to 16 for birds laying eggs with good shell quality compared with those laying eggs with poorer-quality shells (Table 3). Feed conversion was not influenced by addition of IMTX to the diet; however, hens laying eggs with better shell quality required more ($P < 0.01$) feed per dozen eggs during the first 4 periods than those with poorer-quality eggshells (Table 3). Hen-day egg production was greater ($P < 0.01$) for birds with poorer-quality eggshells during wk 1 to 4, 9 to 12, and 13 to 16 (Table 3), and this same tendency was observed ($P < 0.10$) during wk 5 to 8. The hen-day egg production during wk 9 to 12 averaged 89.1, 85.0, and 87.1% for 0, 1, and 2% IMTX, respectively, with the value for the birds fed the control diet being greater ($P < 0.05$) than that for hens fed 1% IMTX. The value for hens fed 2% IMTX was intermediate. A deficiency of Ca or P has been shown to cause a reduction in egg production [13, 14]. If IMTX had caused a significant decrease in feed intake, this may have

Table 4. Effect of Improved Milbond-TX (IMTX) on change in BW (kg) of Hy-Line W-36 laying hens selected for good or poor eggshell quality[1]

Treatment	Good shell quality	Poor shell quality
Control	0.132 ± 0.024	0.136 ± 0.025
1% IMTX	0.128 ± 0.027	0.134 ± 0.021
2% IMTX	0.139 ± 0.043	0.182 ± 0.031

[1]Each value represents the mean ± SE of 25 individually caged hens fed for five 28-d periods.

Table 5. Effect of Improved Milbond-TX (IMTX) on egg weight (g) of Hy-Line W-36 laying hens selected for good or poor eggshell quality (EQ)[1]

		Good shell quality			Poor shell quality				ANOVA		
Time (wk)	Eggs (n)	Control	1% IMTX	2% IMTX	Control	1% IMTX	2% IMTX	Pooled SE	IMTX	EQ	IMTX × EQ
0	141	62.0	61.9	61.1	55.9	56.7	55.5	0.291	NS	<0.0001	NS
1	137	62.9	62.0	61.5	56.1	56.4	55.9	0.308	NS	<0.0001	NS
2	140	62.8	62.6	61.6	57.1	57.5	56.1	0.283	NS	<0.0001	NS
3	139	63.6	64.7	62.5	57.4	57.9	56.8	0.312	NS	<0.0001	NS
4	132	65.3	64.6	63.6	58.1	58.9	57.5	0.292	NS	<0.0001	NS
6	137	64.0	64.1	62.1	58.5	58.8	58.1	0.319	NS	<0.0001	NS
8	130	64.1	64.8	65.0	60.3	59.6	58.3	0.298	NS	<0.0001	NS
10	131	64.1	65.1	64.8	60.0	60.3	59.3	0.339	NS	<0.0001	NS
12	123	64.2	65.7	66.0	60.1	59.4	59.1	0.338	NS	<0.0001	NS
14	131	65.4	64.8	65.3	60.2	59.0	58.4	0.358	NS	<0.0001	NS
16	120	66.7	66.0	64.3	60.5	60.6	58.8	0.338	<0.05	<0.0001	NS
18	122	65.3	65.8	64.5	58.7	60.3	58.7	0.397	NS	<0.0001	NS
20	119	66.9	66.7	63.6	60.7	61.4	59.6	0.364	<0.05	<0.0001	NS

[1]Each value represents the mean of 5 pens of 5 individually caged hens.

resulted in a decline in egg production depending on the magnitude of the decrease in feed intake. However, this did not occur during the 5-mo experimental period. Similarly, if the Al in IMTX was available for binding P and not allowing it to be absorbed, then a decrease in egg production would also have been expected if a deficiency of P was present. This did not occur even when IMTX was fed at the highest dietary concentration.

Change in BW during the entire length of the experiment did not differ among treatments for hens in the good shell or poor shell quality groups (Table 4), nor was there an effect due to IMTX.

Egg Characteristics

Egg weight was consistently lower ($P < 0.0001$) during the entire experiment for hens selected for poor eggshell quality than those with good shell quality (Table 5). When separate times were analyzed independently, there was no difference ($P > 0.10$) among IMTX treatments for egg weight except during wk 16 and

Table 6. Effect of Improved Milbond-TX (IMTX) on albumen quality (Haugh units) of eggs from Hy-Line W-36 laying hens selected for good or poor eggshell quality (EQ)[1]

	Good shell quality			Poor shell quality				ANOVA		
Time (wk)	Control	1% IMTX	2% IMTX	Control	1% IMTX	2% IMTX	Pooled SE	IMTX	EQ	IMTX × EQ
0	87.7	88.1	87.5	87.7	88.3	87.7	0.463	NS	NS	NS
1	88.3	88.4	88.2	88.0	87.5	87.6	0.482	NS	NS	NS
2	88.1	89.9	89.0	87.9	87.4	89.2	0.524	NS	NS	NS
3	87.3	87.5	88.0	87.6	87.3	88.3	0.487	NS	NS	NS
4	86.7	87.3	86.1	85.7	87.3	87.1	0.456	NS	NS	NS
6	88.4	85.4	87.1	87.6	87.6	87.9	0.524	NS	NS	NS
8	84.2	86.7	83.9	84.3	83.6	85.4	0.511	NS	NS	NS
10	82.1	83.8	81.0	80.0	82.0	81.4	0.583	NS	NS	NS
12	81.9	83.2	81.0	83.1	81.8	79.8	0.616	NS	NS	NS
14	80.5	83.9	81.0	82.5	82.8	81.3	0.604	NS	NS	NS
16	78.4	80.8	79.1	81.0	79.2	80.1	0.665	NS	NS	NS
18	80.6	83.9	78.9	82.6	80.6	81.9	0.675	NS	NS	NS
20	82.0	85.5	79.2	81.6	84.9	81.6	0.612	<0.01	NS	NS

[1]Each value represents the mean of 5 pens of 5 individually caged hens.

Table 7. Effect of Improved Milbond-TX (IMTX) on shell weight (g) of eggs from Hy-Line W-36 hens selected for good and poor eggshell quality (EQ)[1]

	Good shell quality			Poor shell quality				ANOVA		
Time (wk)	Control	1% IMTX	2% IMTX	Control	1% IMTX	2% IMTX	Pooled SE	IMTX	EQ	IMTX × EQ
0	5.61	5.74	5.65	4.86	4.84	4.82	0.032	NS	<0.0001	NS
1	5.66	5.74	5.69	4.84	4.83	4.87	0.030	NS	<0.0001	NS
2	5.54	5.56	5.54	4.85	4.81	4.83	0.030	NS	<0.0001	NS
3	6.12	6.23	5.90	5.24	5.18	5.11	0.030	<0.10	<0.0001	NS
4	6.10	5.94	6.00	5.21	5.12	5.08	0.034	NS	<0.0001	NS
6	5.93	6.00	5.72	5.08	5.11	5.07	0.032	NS	<0.0001	NS
8	5.73	5.78	5.88	5.30	5.16	5.11	0.039	NS	<0.0001	NS
10	5.74	6.00	5.94	5.00	5.06	5.06	0.032	NS	<0.0001	NS
12	5.71	5.92	5.65	5.10	4.99	5.07	0.030	NS	<0.0001	<0.05
14	5.62	5.53	5.49	4.93	4.77	4.70	0.038	<0.10	<0.0001	NS
16	5.82	5.69	5.63	5.13	4.99	4.94	0.031	<0.10	<0.0001	NS
18	5.36	5.58	5.43	4.69	4.80	4.84	0.040	NS	<0.0001	NS
20	5.74	5.89	5.73	5.02	5.14	5.09	0.035	NS	<0.0001	NS

[1]Each value represents the mean of 5 pens of 5 individually caged hens.

20. Egg weight averaged 63.6, 63.4, and 61.4 g during wk 16 and 63.8, 63.8, and 61.5 g during wk 20 for 0, 1, and 2% IMTX, respectively. The values for 2% IMTX were lower ($P < 0.05$) than those for the other 2 treatments. Repeated measures analysis indicated no effect ($P > 0.10$) of IMTX during the 20 wk of the experiment, but there was a difference ($P < 0.0001$) in egg weight between hens laying eggs with good and poor shells. The Helmert option indicated a significant interaction between shell quality and IMTX ($P < 0.05$) at wk 12 for egg weight, which appears to be an anomaly. For control hens laying eggs with good shells, the difference in egg weight from those collected at wk 12 with the mean of the remaining week was an increase of 1.8 g, whereas control hens laying eggs with poor shells had a 0.1-g decrease in egg weight for the mean of the remaining collections. Hens fed 1% IMTX and laying eggs with good shells increased egg weight by 0.1 g, whereas those with poor shells increased egg weight by 0.9 g. In hens fed 2% IMTX, there was a 1.6-g decrease in egg weight in hens with good shells but only a 0.3-g decrease in egg weight for those laying eggs with poor shells. There was no apparent explanation for these findings. The polynomial option indicated highly significant linear and quadratic ($P < 0.0001$) and cubic ($P < 0.01$) order time effects on egg weight independent of either shell quality or supplementation with IMTX. As commercial egg-type laying hens mature, egg weight increases [32]. In this experiment, the increase in egg weight was observed to occur in 3 stages.

Albumen quality did not differ ($P > 0.10$) in eggs from hens laying eggs with either good or poor shell quality (Table 6). With the exception of wk 20 in the individual analyses, there was no difference ($P > 0.10$) in albumen quality due to feeding IMTX. At wk 20, HU averaged 81.8, 85.2, and 80.5 for 0, 1, and 2% IMTX, respectively. The value for 1% IMTX was greater than those for the other 2 treatments. The repeated measures analysis indicated a significant ($P < 0.0001$) effect of time on albumen quality but no influence ($P > 0.10$) due to IMTX or shell quality. The Helmert option indicated a difference ($P < 0.05$) due to IMTX between HU at wk 6 and the mean of the remaining egg collections. Hens fed the control diet or 2% IMTX had an average decrease in albumen quality of 6.2 and 6.35 HU, respectively, between wk 6 and the mean of the remaining week. Those hens fed 1% IMTX had an average decline of only 3.43 HU between the same periods. The polynomial option indicated that the decline in HU over time had first, second, and third order components ($P < 0.0001$).

Eggshell weight was lower ($P < 0.0001$) at all collection times for the hens selected for poor shell quality than those with good shells when analyzed individually (Table 7). These data are in agreement with those of Roland et al. [32]. These researchers reported that shell quality of eggs from individual hens at the end of the laying cycle was directly related to the shell quality of eggs at the beginning of the laying cycle. The repeated measures analysis indicated effects of time, shell quality ($P < 0.0001$), and IMTX ($P < 0.05$) on eggshell weight, but no interactions. Regarding the effect of shell quality, the Helmert option detected differences ($P < 0.05$) at wk 3, 4, and 10 with the means of the remaining week. At wk 3, the decrease in shell quality for hens laying eggs with good shells averaged 0.32 g, whereas that for layers of poor shell eggs was 0.16 g. Respective differences at 4 wk were 0.28 and 0.13 g, and those at 10 wk were 0.24 and 0.08 g. Thus, hens laying shells with good quality initially saw a greater decrease in eggshell weight than hens with poorer shell quality initially. Two break points were detected by the Helmert option for eggshell weight resulting from IMTX ($P < 0.05$): wk 10 and 16. At wk 10, the difference between that week and the mean of the remaining week was 0.06, 0.18, and 0.24 g for 0, 1, and 2% IMTX, respectively. At 16 wk, respective values were 0.28, 0.02, and 0.01 g. Hens fed the control diet maintained shell weight for a longer period than hens fed 2% IMTX. Linear ($P < 0.05$), quadratic, and cubic ($P < 0.0001$) were found for shell weight.

When time was considered as a main effect in the statistical analysis with the repeated measures analysis, rather than running separate ANOVA for each period, the effect of IMTX on all egg characteristics was nonsignificant. It should be kept in mind that any data set collected over time will have a covariance structure that should be taken into consideration through repeated measures analysis or with a newer mixed model procedure [31] to avoid reporting possibly erroneous results.

Excreta Moisture

Values for excreta moisture of hens in the present study were similar to those reported by Nakaue and Koelliker [7]. However, no difference ($P > 0.10$) in percentage of excreta moisture was observed among the hens having good shell quality at wk 16. Means were 73.1 ± 0.56, 72.5 ± 0.49, and 72.6 ± 0.46% for 0, 1, and 2% IMTX, respectively. Nakaue and Koelliker [7] supplemented a naturally occurring zeolite, clinoptilolite, to laying hen diets at 0, 2.5, 5, and 10%. They reported a significant decrease in fecal moisture and volatile solids from hens fed the highest dietary concentration of clinoptilolite compared with hens fed the control diet. Fecal moisture values reported for the 4 above treatments were 75.6, 75.9, 73.4, and 71.0%, respectively.

CONCLUSIONS AND APPLICATIONS

1. Feeding IMTX to Hy-Line W-36 laying hens, grouped according to their eggshell quality, at 8 times the recommended amount of the manufacturer to simulate a feed mill mixing error had no detrimental effect on the overall performance over an experimental period of 5 mo.
2. Excreta moisture was not affected by the supplementation of IMTX to laying hens with good shell quality.

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