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Alleviation of Aflatoxin-B_1 Toxicity by using Clay Adsorbent in Nile Tilapia (*Oreochromis niloticus*) Diets

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ABSTRACT

The present study was designed to alleviate the aflatoxin-B_1 toxicity by the use of 0.5% calcium bentonite clay and to evaluate its effect on the growth performance of Nile tilapia over a period of 10 weeks. Inclusion of AFB_1 at both 2 and 4 ppm levels significantly ($p<0.05$) decreased specific growth rate (SGR), net weight gain (NWG), average daily gain (ADG), survival, feed intake, feed efficiency ratio (FER) and protein efficiency ratio (PRE), irrespective the addition of the 4TX in the diets. Among different dietary groups of the fish, % survival was not affected significantly ($p<0.05$). T_1 showed maximum NWG (45.49±3.85), FER (0.739±0.02) and PER (36.36±1.83) when compared to other dietary treatment groups. The addition of 4TX clay in the diets at both 2 and 4 ppm AFB_1 concentrations have almost the same effect on the growth parameters tested except in the case of PER. T_3 (0.5% 4TX+2 ppm AFB_1) showed better PER (31.42±1.74) when compared to T_4 (0.5% 4TX+ 4 ppm AFB_1) group (27.76±0.67). Interaction between different growth parameters of tilapia fed AFB_1 and 4TX supplemented diets showed that net weight gain (NWG) was significantly ($p<0.01$) correlated with average daily gain (ADG), specific growth rate (SGR), feed intake (FI) and protein efficiency ratio (PER). In conclusion, aflatoxin-B_1 negatively impacted the growth performance of Nile tilapia regardless the addition of the 4TX clay. Addition of 4TX in the diets has significant effect on some of the parameters tested.

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DH and AM conceived and designed the study. DH collected and analyzed data and wrote the article.

Key words
Nile tilapia, Calcium bentonite clay, Aflatoxin-B_1, Growth performance, Correlation matrices.

INTRODUCTION

Aflatoxins are toxic compounds that are produced mainly by two naturally present mold species (*Aspergillus flavus* and *Aspergillus parasiticus*). These fungal species are abundant in the soil and are a common contaminant of feed crops in warm and humid environments (Fowler *et al*., 2014). Oilseed crops such as corn, cottonseed and peanut meal are mostly contaminated with aflatoxins. In warm and humid conditions, fish meal, soybean meal, sunflower and other nutritionally complete feeds are also at a risk of aflatoxin contaminations (Hutanasu *et al*., 2009; Kitya *et al*., 2010; Kumagai *et al*., 2008; Tchana *et al*., 2010). Among all the documented aflatoxins, aflatoxin-B_1 (AFB_1) is the most widespread and toxic for all the animal species including humans due to its strong mutagenic, teratogenic and carcinogenic effects on these species (Han *et al*., 2009; Santacroce *et al*., 2008). Being a wide distribution in tropical and sub-tropical areas of the world where a large number of studies reported the frequent prevalence of aflatoxin contamination, tilapia is repeatedly studied to examine the deleterious effects of AFB_1 on its physiological properties and health (Deng *et al*., 2010). Chen and Rawlings (2008) collected commercial feeds and raw materials from Asia and reported the fact that aflatoxins could be detected in 96.1% of the 334 tests.

Negative effect of AFB_1 on the health performance of Nile tilapia were observed in many studies as Zychowski *et al*. (2013b) reported the deleterious effects of AFB_1 in tilapia at lower concentrations such as 1.5 ppm. Mehrim and Salem (2013) have revealed serious lethal effects of incorporating 150 ppb AFB_1 in the diets of Nile tilapia with addition to the hepatotoxic effects on the liver than the control group. In another study by El-Banna *et al*. (1992) it is reported that the growth of Nile tilapia decreases significantly when fed 100 µg AFB_1 / kg for a period of 10 weeks, and was observed 16.70% mortality when exposed to a dose of 200 µg / kg.

Extensive research has been designed and carried out to prevent mycotoxicosis in different animal species, including fish that mainly consists of different physical, nutritional chemical or biological means. Vast use of mycotoxin adsorbents that can capture and adsorb the toxin molecules by ion exchange process, thereby delaying their entry into the blood from the gastrointestinal tract has been made considerable attention in the prevention of mycotoxins. Hydrated sodium calcium aluminosilicate (HSCAS), bentonite, zeolite ore-based compound, canola

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oil, activated carbon, spent bleaching clay, inorganic adsorbents, some organic acids and alumina-silicates have been disclosed in the prevention of aflatoxicosis (Devegowda and Murthy, 2005).

Deleterious effects of aflatoxins in many animal species has alleviated by the use of clay based, non-nutritive adsorbents. Several studies reported the efficacy of these bentonites and aluminosilicate clays to adsorb and mitigate the harmful effects of aflatoxins by *in vitro* binding of these toxins in the interlayers and on the edge of the clay structure with relatively high attraction (Phillips, 1999; Desheng *et al*., 2005; Kannewischer *et al*., 2006). As a result of this absorbance, the aflatoxins molecules passes through the gastrointestinal tract unabsorbed, in that way decreasing the lethal effects.

The 4TX calcium bentonite clay was selected on the basis of presence of 81.4% smectite which shows its purity level and strong adsorption capacity with aflatoxin-B_1 (13.9% w/w) (Velazquez, 2011). Considering the fact that commercial bentonites used in feeding industry are dried and ground to fine powders, we obtained commercial bentonite (4TX) from Southern Clay Products Inc. (Gonzales, Texas, USA). The current study is therefore designed to alleviate the AFB_1 induced toxicity by the use of 0.5% 4TX clay against 2 and 4 ppm AFB_1 in the diets and to evaluate the potential effects of the clay on growth parameters of Nile tilapia.

MATERIAL AND METHODS

Experimental diets

Controlled basal diet was formulated having 33.8 g of protein, 8.2 g of lipid, and an estimated 290 KCal of digestible energy $100g^{-1}$. This diet fulfils all published nutrient requirements of Nile tilapia (Table I) (Lim and Webster, 2006). Purified aflatoxins B_1 was purchased from Sigma-Aldrich, USA and prior to the addition into the remaining ingredients of the diets it is dispersed in chloroform and thoroughly mixed with Celufil. All dry ingredients accurately weighed and then mixed for 40 min in a mixer (V-mixer) then oil was added slowly to mix thoroughly and at the end 400 mL of water was added and mixed in mixer (Hobart mixer) for 50-60 min. The mixer of all the ingredients then passed through a 3-mm die that is attached with a meat grinder. The diets were dried, labelled and then stored at -20 °C for use in experiment. The four experimental diets consisted of: T_1, 0% 4TX + 0 ppm AFB_1 (Negative control); T_2, 0.5% 4TX + 0 ppm AFB_1 (Positive control); T_3, 0.5% 4TX + 2 ppm AFB_1; T_4, 0.5% 4TX + 4 ppm AFB_1.

Table I.- Basal diet Composition (% dry matter basis) fed tilapia for 10 weeks.

Ingredients	(%) dry matter basis
Fish meal	11
Soybean meal	46.68
Starch	20.25
Vitamin premix[a]	3
Mineral premix	4
Carboxymethyl cellulose	2
Soy oil	4.64
$CaPO_4$, dibasic	1
Glycine	1
DL-methionine	0.15
4TX	0
AFB_1 (µg)	0
Celufil	6.28
Total	**100**

[a] Contains (as g kg−1): Ca(C6H10O6) 5H2O, 348.49; Ca(H2PO4)2 H2O, 136.0; FeSO4 7H2O, 5.0; MgSO4 7H2O, 132.0; K2HPO4, 240.0; NaH2PO4 H2O, 88.0; NaCl, 45.0; AlCl3 6H2O, 0.15; KI, 0.15; CuSO4 5H2O, 0.5; MnSO4 H2O, 0.7; CoCl2 6H2O, 1.0; ZnSO4 7H2O, 3.0; Na2SeO3, 0.011.

Stocking and culturing of tilapia

Nile tilapia fingerlings were imported from a local Hatchery operated in Louisiana State, USA. Before the start of the experiment, tilapia were stocked and conditioned with a commercial diet for 15 days in round tanks and conditioned on basal diet for 7 more days in glass aquaria. A closed, recirculating system consisting on glass aquaria 110-L were used for the feeding trial, where salinity was maintained at 5 ppt by the addition of saline water and temperature was controlled at 26 °C. Water quality parameters (Temperature, pH, Ammonia, nitrite, disolved oxygen and salinity) monitored weekly and keeping below the toxic levels using a biofilteration unit. A total of 144 fish of equal size (4.5±0.4 g) were randomly divided into 4 experimental units (12fish/unit) with three replicates for a period of 10 weeks. Fish were fed at 5% of body weight daily their assigned diet at 8:00 AM and 4:00 PM. The system was monitored daily to check any abnormalities and mortalities and if any, was removed immediately and recorded.

Fish growth response

After terminating the trial, net weight gain (NWG), average daily gain (ADG), % survival, % specific growth rate (SGR), protein efficiency ratio (PER), feed efficiency ratio (FER), feed intake (FI), and correlation matrices were calculated.

Statistical analysis

All the parameters were computed by analysis of variance (ANOVA) first and then differences among different treatment groups and means were compared by Least Significant Difference (LSD) test. To compute all the statistical data, Statistix, software program version 8.1 (Analytical Software, Tallahassee, FL) was used. The significance level was set at P≤0.05.

RESULTS

Growth performance

After 10 week exposure of AFB_1 and 4TX clay, there observed a non-significant difference (P>0.05) in % survival among all the treatments. While the growth parameters of tilapia regarding the initial body weight (IBW), final body weight (FBW), average daily gain (ADG), net weight gain (NWG) and specific growth rate (SGR) was significantly (p<0.05) affected after the exposure of AFB_1 and 4TX supplemented diets (Table II). Addition of AFB_1 in the diets at both 2 and 4 ppm negatively affected the health performance of tilapia, irrespective the supplementation of 4TX in the diets. When supplemented alone, 4TX has significantly higher net weight gain (45.03±5.10) and specific growth rate (2.202±0.07) as compared to 2 ppm (31.63±3.03) and (1.998±0.06) and 4 ppm AFB_1 (31.23±5.73) and (1.986±0.11) exposed fish, respectively. The efficacy of 4TX clay in alleviating the AFB_1 toxicity at both 2 and 4 ppm AFB_1 concentrations remains non-significant (P>0.05).

Feed and protein utilization

Feed efficiency ratio (FER) and protein efficiency ratio (PER) exhibited a significant difference (p<0.05) in all the treatment groups (Table III). T_1 showed maximum (0.739±0.02) while T_4 showed minimum (0.584±0.03) FER. 4TX clay showed better PER (31.42±1.74) at 2 ppm AFB_1 concentration as compared to 4 ppm AFB_1 exposed group (27.76±0.67).

Correlation matrices

Interaction between different growth parameters of tilapia fed AFB_1 and 4TX supplemented diets are shown in Table IV. Net weight gain (NWG) was significantly (p<0.01) correlated with specific growth rate (SGR), protein efficiency ratio (PER), average daily gain (ADG) and feed intake (FI).

Water quality parameters

All the water quality parameters were found within the range (temperature, 25.5–27.2°C; pH, 7.89–8.61; ammonia, 0.11-0.20 mg/1N NH_3; nitrite, 0.03-0.09 mg/1N NO_2 $^-$L; dissolved oxygen, 5.42–6.51 mg$^-$L and salinity, 0.41-0.48 ppt) optimum for tilapia growth (Table V).

Table III.- Feed intake, feed efficiency ratio (FER) and protein efficiency ratio (PER) of tilapia AFB_1 and 4TX supplemented diets for 10 weeks.

Treatment	FI (g/fish)	FER	PER
T_1	61.58±5.56AB	0.739±0.02A	36.36±1.83A
T_2	65.70±10.15A	0.689±0.09A	34.53±0.51A
T_3	52.14±3.51B	0.624±0.03B	31.42±1.74B
T_4	53.00±4.82B	0.584±0.03B	27.76±0.67C

Means sharing similar letters in a column are statistically non-significant (p>0.05). T_1, 0%4TX+0 ppm AFB_1 (Negative control); T_2, 0.5% 4TX+ 0 ppm AFB_1 (Positive control); T_3, 0.5% 4TX+2 ppm AFB_1; T_4, 0.5% 4TX+4 ppm AFB_1; FI, Feed intake; FER, Feed efficiency ratio; PER, Protein efficiency ratio. Feed efficiency ratio (FER) = Live weight gain (g) / Feed intake (g). Protein efficiency ratio (PER) = Live weight gain (g) / Protein intake (g).

Table II.- Means ± SD of growth performance of tilapia fed AFB_1 and 4TX supplemented diets for 10 weeks.

Treatment	Body weight (g/fish)		Body gain		SGR (%)	Survival (%)
	IBW	FBW	NWG (g/fish)	ADG (g/fish/d)		
T_1	4.657±0.20B	50.14±4.02A	45.49±3.85A	0.607±0.05A	2.209±0.05A	94.44±4.81A
T_2	5.050±0.15A	50.07±5.24A	45.03±5.10 A	0.600±0.07A	2.202±0.07A	94.44±4.81A
T_3	4.467±0.11B	36.10±3.13B	31.63±3.03B	0.422±0.04B	1.998±0.06B	97.22±4.81A
T_4	4.710±0.12B	35.94±5.83B	31.23±5.73B	0.416±0.08B	1.986±0.11B	97.22±4.81A

Means sharing similar letters in a column are statistically non-significant (p>0.05). T_1, 0%4TX+0 ppm AFB_1 (Negative control); T_2, 0.5% 4TX+ 0 ppm AFB_1 (Positive control); T_3, 0.5% 4TX+2 ppm AFB_1; T_4, 0.5% 4TX+4 ppm AFB_1; IBW, Initial body weight; FBW, Final body weight; NWG, Net weight gain; ADG, Average daily gain; SGR, Specific growth rate.
Net weight gain (g/fish) NWG = Average final weight (g) – Average initial weight (g).
Average daily gain, (g/fish/day) ADG = AWG (g)/Experimental period (days).
Specific growth rate (SGR, %/day) = [In final weight – In initial weight] x 100/Experimental period (d).
Survival rate (SR %) = End number of the alive fish/The beginning number of the fish x 100.

Table IV.- Interaction between growth responses of tilapia fed different levels of AFB_1 and 4TX supplemented diets for 10 weeks.

Control Variables	IBW	FBW	NWG	ADG	SGR	Survival	FI	FER
FBW	0.7367** 0.0063							
NWG	0.7222** 0.0080	0.9998** 0.0000						
ADG	0.7222** 0.0080	0.9998** 0.0000	1.0000** 0.0000					
SGR	0.7039* 0.0106	0.9931** 0.0000	0.9937** 0.0000	0.9937** 0.0000				
Survival	-0.1242 0.7005	-0.1255 0.6976	-0.1243 0.7004	-0.1243 0.7004	-0.0555 0.8641			
FI	0.7933** 0.0021	0.9359** 0.0000	0.9324** 0.0000	0.9324** 0.0000	0.9107** 0.0000	-0.2962 0.3500		
FER	-0.1513 0.6389	0.3732 0.2322	0.3865 0.2146	0.3865 0.2146	0.3954 0.2034	0.0224 0.9449	0.1730 0.5907	
PER	0.2993 0.3446	0.7287** 0.0072	0.7359** 0.0064	0.7526** 0.0047	0.7171** 0.0087	-0.4257 0.1676	0.6202 0.0315	0.5487 0.0647

Upper values indicated Pearson's correlation coefficient; lower values indicated level of significance. *, Significant ($P<0.05$); **, Highly significant ($P<0.01$).

Table V.- Physico-chemical analysis of control and AFB_1 and 4TX treated aquaria.

Week	Temp. (°C)	pH	Ammonia	Nitrite	Dissolved Oxygen	Salinity
Initial	26.7	8.52	0.14	0.03	6.45	0.42
1.	26.7	7.89	0.11	0.03	6.51	0.43
2.	26.6	8.45	0.15	0.03	5.99	0.41
3.	27.2	8.46	0.12	0.04	6.32	0.48
4.	26.7	7.96	0.12	0.05	6.30	0.46
5.	26.8	8.39	0.13	0.04	6.31	0.47
6.	26.9	8.48	0.18	0.03	6.36	0.42
7.	25.5	8.53	0.14	0.03	6.39	0.44
8.	26.7	8.61	0.13	0.04	5.87	0.46
9.	26.8	8.54	0.20	0.09	5.42	0.42
10.	26.7	8.46	0.12	0.03	6.35	0.41

Ammonia, (Mg/1N NH_3); Nitrite, (Mg/1N NO_2^{-}L); Dissolved Oxygen, (Mg/L); Salinity, (Ppt).

DISCUSSION

In tropical and subtropical regions of the world, a high risk of aflatoxin contamination has been observed in many studies due to the higher use of plant based alternatives in animal diets that ultimately have lethal effects on fish health. Overall, aflatoxin-B_1 negatively affected the tilapia over a course of 10 weeks. The results of current study showed a reduction in specific growth rate (SGR), average daily gain (ADG) and net weight gain (NWG), and at both the supplemented levels of AFB_1. Fish exposed to 4 ppm AFB_1 affected the most comparing with 2 ppm AFB_1 offered fish and control groups. This negative effect of AFB_1 on growth parameters at 2 and 4 ppm proved anti-nutritional nature of AFB_1 as described in previous findings (Al-Faragi, 2014; Ayyat *et al*., 2013; Chavez-Sanches *et al*., 1994; Deng *et al*., 2010; El-Banna *et al*., 1992; Encarnacao *et al*., 2009; Salem *et al*., 2010; Sepahdari *et al*., 2010; Shehata *et al*., 2009; Zaki *et al*., 2008; Zychowski *et al*., 2013a, b).

In the present study, % survival remained almost the same ($p<0.05$) among tilapia exposed to 2 and 4 ppm AFB_1 concentrations along with controls. Our results was confirmed by the study of Tuan *et al*. (2002) who concluded that when tilapia exposed to 10 ppm AFB1/kg or less have not increased the mortality over a period of 8 weeks. In another study by Chavez-Sanchez *et al*. (1994), it is reported that even when exposed to 30 ppm AFB1/kg did not cause death in tilapia. Our results regarding the survival confirmed this trend.

In the present study, protein efficiency ratio (PER) and feed efficiency ratio (FER) was also decreased significantly

($p<0.05$) with the increasing AFB_1 concentration. Our results regarding the feed and protein utilization are in line with the previous findings by Abdelhamid *et al*. (2004), Hussein *et al*. (2000), Nguyen *et al*. (2002) and Salem (2002). The possible explanation of this toxicity and deleterious effect of AFB_1 may be because of pathological modifications in the gastro-intestinal tract of the fish (Murjani, 2003). Also, our results was in line with the results of Nguyen *et al*. (2002) who proposed that when fish offered a diet ranging from 10 and 100 ppm AFB_1/kg was expelled out when ingested. When fish administrated a diet having 100 ppm AFB_1/kg diet, only consumed 59 ppm AFB_1/kg of its body weight, the three times than the amount for fish fed the 10 mg AFB_1/kg. Additionally, the results of the study of Salem (2002) reported that a significant reduction ($P<0.05$) was observed in protein and feed efficiency feed in tilapia when fed with dietary AFB_1. Similar findings regarding the protein and feed utilization were reported by Abdelhamid *et al*. (2002b).

Addition of 4TX clay in the diets did not show any significant difference regarding the growth parameters at both 2 and 4 ppm AFB_1 exposed groups except in protein efficiency ratio in which 0.5% 4TX+2ppm AFB_1 group (T_3) showed higher (31.42±1.74) PER as compared to 0.5%4TX+4ppm AFB_1 (27.76±0.67) group (T_4). The theory behind this effectiveness is that mycotoxin adsorbents such as 4TX strongly binds with the AFB_1 molecules present in the feed that it prevents the absorption of AFB_1 in the digestive tract of the animals. The efficacy of 4TX to adsorb and remove the intoxication of AFB_1 in animal species was also reported by Fowler *et al*. (2014), (2015) and Velazquez (2011). Bentonite clays were found active in minimizing the bioavailability of aflatoxins (Chaturvedi *et al*., 2002; Desheng *et al*., 2005; Magnoli *et al*., 2008). All the water quality parameters were found suitable for the optimum growth of Nile tilapia as described in previous studies (Abdelhamid *et al*., 2002b).

CONCLUSIONS

The results showed significant difference ($p<0.05$) regarding the growth parameters among all the dietary treatments. Fish exposed to 4 ppm AFB_1 performed poorly in terms of growth performance when compared to 2 ppm exposed fish and the control groups. Both negative (T_1) and positive (T_2) control groups showed maximum growth performance over AFB_1 offered fish. Supplementation of 4TX in the diets has positive effects on fish growth with better PER in 2 ppm AFB_1 group compared to 4 ppm AFB_1 exposed fish. So, a clay binder such as 4TX if used efficiently have the potential to reduce AFB_1 exposure, thereby preventing bioavailability and consequent effects, such as decreases in growth parameters and immunosuppression and in Nile tilapia.

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Conflict of interest statement

We declare that we have no conflict of interest.

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