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Abstract

The examined fish intoxicated with aflatoxin B1 (AFB\(_1\)) showed several non-specific signs, including inadequate growth, significant losses in aquaculture industry especially resulting from aflatoxicosis. The study using Zinc oxide (ZnO) and Hematite (alpha-Fe\(_2\)O\(_3\)) nanoparticles (NPs) to assess the effect of using as on their own negative impacts of AFB\(_1\) 3 ppm/kg for 13 weeks was studied 360 healthy fingerlings of Nile tilapia, were divided into eight groups (15 fish/group) with three replicates, it has been applied in 24 aquariums. Group G\(_1\) fed only a basal diet as control. While, G2, G3, and G4 were fed on diets supplemented with 2 g/kg diet of ZnO NPs, Hematite NPs and combination of both respectively. Groups G5 was fed on diets contamination with AFB\(_1\) (3 ppm/kg diet), while G6, G7 and G8 were fed on diets containing 2 g/kg diet of (ZnO NPs, Hematite NPs and combination of both) plus AFB\(_1\) (3 ppm/kg diet) respectively.

Experimental treatments affected significantly on the Final Body Weight (FBW), Daily Gain (DG), Feed Conversion Ratio (FCR) and mortality Table (1, 2). FBW and DG reduced to 37.856 g and 0.227 g in fish feed containing AFB\(_1\), when compared with groups AFB\(_1\) which supported with feed additives (ZnO, Hematite and combination of both) NPs, which reported final body weight 45.507, 47.007, 49.762 g and daily gain 0.313, 0.328, 0.360 g respectively. The highest FBW and DG were determined in group treated with feed additives combination of (ZnO and Hematite) nanoparticles, keep it track of by the fish group fed diet supplemented with Hematite NPs then those fed diet supplemented with ZnO NPs and the recorded values were for final body weight 55.857, 61.500, 63.874 g daily gain 0.427, 0.488, 0.515 g respectively than that of the control group G1 52.784, g FBW and 0.393 g DG.

The highest survival rate was obtained in fish group treated with combination of (ZnO and Hematite) nanoparticles. The survival rate was (95.556 %) in fish fed diets combination of (ZnO and Hematite) NPs, while it was 57.778 % in fish group G1 fed AFB\(_1\) which enhanced in aflatoxicated group G5 which supplemented with combination of (ZnO and Hematite) NPs 84.444 %, and in aflatoxicated group G7 which supplemented with Hematite NPs 77.778 %, and in aflatoxicated group G8 which supplemented with ZnO NPs 75.556 %. The best FCR was obtained in same order in fish group treated with combination of ZnO NPs and Hematite NPs, Hematite NPs then ZnO NPs 1.871, 1.170, 1.253 compared to the control group which recorded 1.462. On the other hand, aflatoxicated groups significantly affected feed conversion impaired significantly affected, aflatoxicated group recorded 1.950 but when supported with ZnO NPs, Hematite NPs and combination of both recorded 1.541, 1.525 and 1.502 respectively.

Keywords: Food safety, Hematite, Mycotoxins, Nano food/ feed additives, Zinc oxide.

Introduction:

In aquaculture, the exclusive use of animal-derived proteins is not sustainable, therefore; Plant ingredients had to be replaced, whose incorporation and inclusion in the feed increased the likelihood of Mycotoxins, that increase the health riskiness to animal (Santos et al., 2010; Almeida et al., 2011; Oliva-Teles et al., 2015; Daniel 2018; Lei et al., 2018).

Aflatoxins are about twenty types, *A. flavus* has a high ability to produce six types of aflatoxins including (B, G and M), but major aflatoxins are two families (B and G), in which B family containing (B1+ B2), and G family containing (G1 + G2) usually found together in foods and feeds in various proportions. The most dangerous, virulent poison and toxicity is aflatoxin B1 (AFB1) from B family. AFB1-contaminated diets are among the commonest causes of low production and survival rate in fish farming, the performance and physiological responses of fish with regard to AFB1 toxicity are different for each species of fish (Wu et al., 2019; Abdel-Daim et al., 2020; Benkerroum 2020).

Nile tilapia (*O. niloticus*) is an important cultured fish species in Egypt and one of highly sensitive fish species to AFB1 (Kenawy et al., 2009). The exposure of fish to AFB1 causes many risks such as the decrease in growth performance, increase susceptibility to disease and high mortality Santacroce et al., (2008). Nanotechnology, one of the most dynamically developing sciences, it is the main base for many innovative branches, and major opportunity for the economy and sustainable.
development, and an emerging avenue employed in disease prevention and treatment. Although the application of it in an emerging stage, it may have the potential to solve most of the problems and the obstacles in aquaculture (Umaltha et al., 2016; Thangapandiyan and Monika 2020). Among the nanomaterials (NMs), Zinc oxide and Hematite have garnered a wide area of attention due to their unique properties as well as safe significantly on the environment.

Hassan et al., (2014) evaluated zinc oxide nanoparticles against fungi in culture media. As for the results of the antifungal activities of Zinc oxide, molds as Aspergillus flavus and Aspergillus ochraceus needed a higher dose of Zinc oxide, compared to Aspergillus niger which required lower concentration to inhibit their growth.

Hassan et al., (2012) noted that the growth of aflatoxicogenic fungi mold and the toxins they produce were prevent by supplemented of 10 µg /ml of Zinc oxide nanoparticles, while that of ochratoxin (OTA) and fumonisin B1 (FUMB1) producing molds and mycotoxins production were prevented and restricted by supplement of 10 µg/ml on Zinc oxide nanoparticles to examined media.

The anti-fungal potential of prepared Zinc oxide NPs and Hematite NPs were estimate against isolated aflatoxicogenic and non-aflatoxicogenic Aspergillus flavus that were reconquest from animal and poultry feeds associated with animal diseases using well and disc diffusion tests, the zone of A. flavus growth present appeared at lower concentration 50 µg /ml of Zinc oxide NPs and Hematite NPs, and the production of AFB1 by toxigenic strains on synthetic or natural medium was affected by all used nanoparticles (Nabawy et al., 2014).

The main target of the study is to evaluate the effect of the dietary supplementation of nanoparticles of Zinc oxide and Hematite or combination of both as trails to control aflatoxocosis (AFB1) and to evaluate the effect of those nanoparticles in improving the growth performance of Nile tilapia.

Materials and methods

The present study was carried at Department of Aquatic animal disease and Management, Faculty of Veterinary Medicine, Benha University, Egypt. The experimental period lasted (13 Weeks) from 1/June to 1/September, 2018.

Fish diet

All fish groups were fed on basal pelleted diet composed of: yellow corn 40%, fish meal 16%, soybean meal 28%, and wheat bran 10.5%. These diets were containing: crude protein (C.P) 30.18%, Lipids (E.E) 4.44%, crude fiber (C.F) 9.33% and metabolism energy (ME) 2610 Kcal /Kg diet.

The feeding rate was 3 % of total biomass during the experimental period. Bi- weeks the feed weight changes according to the actual body weight at that time. The feed was offered for 6 day/week. Fish were fed 3 times/ day at 7.30 to 8.30 Am., then at 11:30 to 12:30 pm., and the last meal at 3:30 to 4:30 pm. for 13weeks.

Fish and experimental conditions:

Healthy fingerlings of Nile tilapia (O. niloticus) obtained from the fish Hatchery of Central Laboratory for Aquaculture Research at Abbassa, Sharqia, Egypt. 360 fingerlings (weight with average 16.70±0.45 g/ fish) after acclimation in well prepared fiber glass tanks (1000 liter, each tank was filled by 800 liter dechlorinated tap water) for two weeks under normal laboratory conditions.

Fish were randomly distributed into well prepared 24 glass aquaria 100 X 100 X 50 cm (500 liter capacity) were used in the study and each aquarium was filled by 400 liter dechlorinated tap water, representing 8 groups (three replicates/treatment) maintained aerated continuously from storage tank. The experiment installed in an environmentally controlled laboratory, a photoperiod of twelve hour light and twelve hour darkness, and aeration by an air blower of 5 watt aquarium.

Group1 on a basal diet only (D1), while G2, G3 and G4 treated with supplemented diets (2 g/kg) (ZnO, Hematite and combination of both) fed without AFB1 (D2, D3 and D4) respectively.

G5 was fed on AFB1 (3 ppm/Kg) contaminated diet (D5), G6, G7 and G8 were fed on (ZnO, Hematite and combination of both) respectively supplemented diets (2 g/kg) with AFB1 (3 ppm/Kg).

The diet residual and fish wastes of each aquarium were collected by siphoning before the 2nd daily feeding, each aquarium was partially cleaned including the fish feces and the water partially changed (near 33.33%).

Water quality

The aquariums were supplied with de-chlorinated tap water. Aeration was continuously provided using an air blower (2 outlets 5 watt/ aquarium). The remaining wastes in each aquarium was removed, was changed approximately 1/3 of the water in the aquarium, dissolved oxygen was maintained at above 5.9 mg/L, by continuous aeration (estimated by using dissolved oxygen meter: HI 93732N HANNA, Hungary) and water temperature at 28±1 °C. Ammonia concentration was 0.53±0.07 mg /L and pH was in range of 7.7-7.50 during the experiment (estimated by using pH meter 211, HANNA instruments, U.S.A.). Photoperiod was natural by the sunlight.
Fish weight and growth

The average fish weight was 16.7±0.45 g/fish at the beginning of the experiment and bi-weekly intervals throughout the experimental duration 13 weeks; Food consumed was calculated as (g /fish /day) by dividing the amount of food consumed each day by the number of fish in the aquarium. Weight Gain (WG), Mortality, Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Specific Growth Rate (SGR), were measured according to formulae of Altunoglu et al., (2017); Elumalai et al., (2019).

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) followed by Duncan, (1955) using XLSTAT, (2014) to compare results obtained from treatments groups with results obtained from the control group, and differences were considered statistically significant at p < 0.05. Values were expressed as means ± standard error.

Results and discussion

Growth performance and feed efficiency:

Economically, aflatoxins contamination is one of the most severe problems for the livestock and feed industries (Souza et al., 2020). Nile tilapia (O. niloticus) is an important cultured fish species in Egypt and one of highly sensitive fish species to AFB1 (Kenawy et al., 2009).

In our present study; it has been observed that aflatoxin has a negative impact on the growth and survival rate of the studied fish. It was found that the weight gain significantly decreased in aflatoxin treated fish as compared to the fish kept at control condition, the Highest average BW 63.874 g was recorded in fish under the G1 (ZnO and Hematite) group, The lowest average body weight 37.856 g was observed in group G5. On the contrary, a similar trend was also demonstrated in specific growth rate.

The growth rate, specific growth rate was high in group G4 it reported 1.473, but decreased to 0.868 in group G9 (Table1).

The mortality rate was increased in aflatoxicated dietary feed; the survival rate of different groups was significantly different. The lowest survival rate was found in aflatoxicated group 57.778%, on the other hand group G4 was exhibited about 95.556% of the survival rate.

At the end of the feeding trial, Nile tilapia fingerlings fed concentrations of 3 ppm AFB1/kg for 13 weeks, showed significantly depressed growth rate, average weight gain and average daily gain, and mean final body weight. All these parameter was higher in treatments (ZnO, Hematite nanoparticles and combination of both) without aflatoxins in diets, as showed in (Table 1, 2).

These findings were closely similar to those mentioned by Ayyat et al., (2018) who noted a decrease in the FBW, DG and FCR, in Nile tilapia (O. niloticus) fed a diet contaminated with two thousand µg /kg feed of aflatoxin B1 compared with a control diet (without fungus toxins). And also agreement in with those recorded by Mahfouz and Sherif (2015), fed Nile tilapia with meals containing 120 µg/kg feed of aflatoxin B1 for twelve weeks. They reported a significant drooping in WG, DG and relative growth rate, but not in the survivability in comparison with the exposure to 20 ppb. In despite of the wide difference in growth performance and mortality rates, these may be attributed to the difference in the toxin dose and/or the exposure time.

Our results agree in harmony on the content of what recorded by Ahmed et al., (2020) who concluded that the aflatoxin contaminated feed has a negative impact on the growth and mortality rate of tilapia fingerling which may accelerate the loss of productivity in the aquaculture system. Tuan et al., (2002); Cagauan et al., (2004); Abdelhamid (2008); Selim et al., (2014) and Mahfouz (2015) reported that reduced growth, weight and feed efficacy resulted from exposure to food contaminated with aflatoxin B1 at a low to moderate concentration in a not short period of time. High dose and long-time exposure are mostly responsible for aflatoxin toxicity in tilapia fingerling. The high mortality and haematological changes in Nile tilapia (O. niloticus) are a strong predictor of toxic effects of the AFB1 contaminated diet (Selim et al., 2014). According to Santacroce et al., (2008); Selim et al., (2014) the exposure of fish to AFB1 causes many risks such as the decline in growth performance; increase the chance of disease and high mortality, and cause a negative impact on tilapia WG and feed efficiency over a relatively short period of ten weeks (Zychowski et al., 2013). Likewise Deng et al., (2010) reported, a gradual decrease in growth parameters, occurs when fish are exposed to aflatoxicated diet contaminated with aflatoxin B1.

On the other hand, our study and many other were dis agree with the work carried out by Anater et al., (2020) they found no significant decreases were noted in Daily weight gain (DWG), Body gain (BG) and Feed conversion ratio (FCR), and found significant increases in the standard and overall sizes of silver catfish fed with180 µg /kg feed. Likewise, we contradict with Huang et al., (2011) and Huang et al., (2014) that did not report any changes in growth of Gibel carp, fed aflatoxicated diet Contain type B1 at rates from 1000 and 2000 µg/kg feed at a period of twelve and twenty four weeks, respectively. This different may be due to they cared out work on different fish species cat fish and (Gibel carp) respectively, indicating that the sensitivity of fish to
AFB₁ difference according to fish species. Anater et al. (2016) reported that there are factors that play a role in the toxic effects of aflatoxins: dose and type of toxin, type and sex of animal, and duration of exposure to the mycotoxins.

The adverse effect of AFB₁ may be attributed to the reasons mentioned by Zhao et al. (2017); Souto et al. (2018); Souza et al. (2020). Where reactive oxygen is produced as a result of stimulation of aflatoxin B₁, which causes direct damage to cells and tissues. Marin and Taranu (2012) reported that continuous exposure to aflatoxin pollution causes immune suppression and increase the susceptibility of fish to infectious diseases resulting from deterioration of blood status and overproduction of reactive oxygen species (ROS).

Zinc is an indispensable ingredient element in finfish nutrition (Wei et al., 1999). Adding Zinc in trace amounts has a pivotal role in many vital processes, as it is important for improving growth and regulating enzymes (Halver and Hardy 2002; Jiang et al., 2016; Munir et al., 2020).

In the present study; it has also been observed that ZnO NPs have a positive impact on the growth and survival rate of studied fish. It was found that the weight gain significantly increased in additives nanoparticles treated fish as compared to the fish kept in control condition, the highest average body weight (63.874 g) was recorded in fish under the G4 (ZnO and Hematite) NPs, and G2 which treated with ZnO 55.857 g compare control group which recorded 52.784 g A similar trend was also demonstrated in specific growth rate.

The growth rate, specific growth rate was high in group G4 1.473 while G2 reported 1.319 compared control group which recorded 1.254 Table (1).

The mortality rate was decreased in nanoparticles dietary feed; the survival rate of different groups was significantly different, the highest survival rate was found in G4 which treated with combination of ZnO and Hematite recording 95.556 % on the other hand G2 was exhibited about 86.667 % of the survival rate.

Our results agree in harmony on the content of what recorded by Shiau and Su (2003) who found a Significant increase in weight and feed conversion efficiency has been reported in juvenile hybrid tilapia when supplementing the diet with iron at a dose of 149 mg/kg. Other studies have reported that dietary iron deficiency could be the cause of decreased growth performance in trials of O. niloticus X O. aureus (Hurrell 1997; Lieu et al., 2001; Prentice et al., 2016) and Makwinja and Geremewa (2020) reported that Iron helps in increase growth and prevents anemia in tilapia.

Conclusion

Nanoparticles of Hematite and Zinc oxide can successfully relieve aflatoxin B₁ noxious effects in Nile tilapia.
Table 1. Growth performance and Survival rate of *Oreochromis niloticus* as affected by the toxicity of aflatoxin and additives of Nanoparticles and their interaction 1,2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight (g fish$^{-1}$)</th>
<th>Final body weight (g fish$^{-1}$)</th>
<th>Weight gain (g fish$^{-1}$)</th>
<th>Daily gain (g day$^{-1}$)</th>
<th>Specific growth rate (% day$^{-1}$)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>16.671a</td>
<td>52.784bc</td>
<td>36.113bc</td>
<td>0.393bc</td>
<td>1.254bc</td>
<td>86.667abc</td>
</tr>
<tr>
<td>G2</td>
<td>16.603a</td>
<td>55.857b</td>
<td>39.255b</td>
<td>0.427b</td>
<td>1.319b</td>
<td>86.667abc</td>
</tr>
<tr>
<td>G3</td>
<td>16.571a</td>
<td>61.500a</td>
<td>44.929a</td>
<td>0.488a</td>
<td>1.425a</td>
<td>88.889ab</td>
</tr>
<tr>
<td>G4</td>
<td>16.467a</td>
<td>63.874a</td>
<td>47.407a</td>
<td>0.515a</td>
<td>1.473a</td>
<td>95.556a</td>
</tr>
<tr>
<td>G5</td>
<td>17.005a</td>
<td>37.856e</td>
<td>20.851f</td>
<td>0.227f</td>
<td>0.868f</td>
<td>57.778e</td>
</tr>
<tr>
<td>G6</td>
<td>16.761a</td>
<td>45.507d</td>
<td>28.746e</td>
<td>0.313e</td>
<td>1.086d</td>
<td>75.556d</td>
</tr>
<tr>
<td>G7</td>
<td>16.819a</td>
<td>47.007d</td>
<td>30.188de</td>
<td>0.328de</td>
<td>1.117d</td>
<td>77.777d</td>
</tr>
<tr>
<td>G8</td>
<td>16.680a</td>
<td>49.762cd</td>
<td>33.082cd</td>
<td>0.360cd</td>
<td>1.189cd</td>
<td>84.444bcd</td>
</tr>
<tr>
<td></td>
<td>± 0.451</td>
<td>± 1.418</td>
<td>± 1.345</td>
<td>± 0.015</td>
<td>± 0.034</td>
<td>± 3.043</td>
</tr>
</tbody>
</table>

|        | Probability                         |                                  |                             |                          | < 0.0001                           | < 0.0001         |

1Values are means of three replicate groups of fish (n=3).
2Values in a column that do not have the same superscript are significantly different according to Duncan's test (P <0.05).


Table 2. Feed utilization of *Oreochromis niloticus* as affected by the toxicity of aflatoxin and additives of Nanoparticles and their interaction 1,2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Feed intake (g/fish)</th>
<th>Feed conversion ratio</th>
<th>Protein efficiency ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>52.631ab</td>
<td>1.462b</td>
<td>2.275b</td>
</tr>
<tr>
<td>G2</td>
<td>49.190bc</td>
<td>1.253bc</td>
<td>2.649a</td>
</tr>
<tr>
<td>G3</td>
<td>52.344ab</td>
<td>1.170c</td>
<td>2.852a</td>
</tr>
<tr>
<td>G4</td>
<td>55.942a</td>
<td>1.187c</td>
<td>2.812a</td>
</tr>
<tr>
<td>G5</td>
<td>40.278e</td>
<td>1.950a</td>
<td>1.712c</td>
</tr>
<tr>
<td>G6</td>
<td>44.291d</td>
<td>1.541b</td>
<td>2.151b</td>
</tr>
<tr>
<td>G7</td>
<td>46.015cd</td>
<td>1.525b</td>
<td>2.175b</td>
</tr>
<tr>
<td>G8</td>
<td>49.688b</td>
<td>1.502b</td>
<td>2.210b</td>
</tr>
<tr>
<td></td>
<td>± 1.168</td>
<td>± 0.063</td>
<td>± 0.105</td>
</tr>
<tr>
<td>Probability</td>
<td>0.0026</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

1Values are means of three replicate groups of fish (n=3).
2Values in a column that do not have the same superscript are significantly different according to Duncan's test (P <0.05).

References


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