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Original Research

Mitigating Potential of Garlic and Turmeric in Aflatoxin-Contaminated Feeds of *Oreochromis niloticus*

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ABSTRACT

Fish feed contamination by mycotoxins presents serious challenges to farmers, as consuming aflatoxin-contaminated feed can result in toxin accumulation in fish, potentially posing risks to human health. This study assessed the detoxifying effects of garlic and turmeric powders on mycotoxin-contaminated feed and their impact on the growth and haematological parameters of juvenile *Oreochromis niloticus* (17.18±0.798g). Conducted in circular concrete tanks (0.5 m deep and 0.58 m in diameter), the experiment involved eight treatments (TDs) with three replicates each: TD1 (mold-free feed), TD2 (feed contaminated with *Aspergillus flavus* mould), TD3 (20 g garlic/kg contaminated feed), TD4 (40 g garlic/kg contaminated feed), TD5 (60 g garlic/kg contaminated feed), TD6 (20 g turmeric/kg contaminated feed), TD7 (40 g turmeric/kg contaminated feed), and TD8 (60 g turmeric/kg contaminated feed). Feeding trials spanned ten weeks to evaluate the effects of garlic and turmeric in mitigating aflatoxin impacts on fish growth and nutrients utilization. Some of the key findings are: Aflatoxin levels in analyzed feeds before (2.6448 µg/kg) and after (123.168 µg/kg) the inclusion of *Aspergillus flavus* varied. Feed processing methods, such as pelleting and drying, reduced aflatoxin concentrations. Significant reduction ($P<0.05$) in weight gain at TD2 (9.7 g) was observed compared to other treatments with inclusion of garlic and turmeric. TD6 (turmeric at 20 g/kg) showed the most pronounced improvement in fish growth parameters, with the highest final weight (16.07 g), weight gain (14.00 g), feed intake (10.98 g), and specific growth rate (2.95 %/day), while also achieving the lowest feed conversion ratio (0.78). Though pelleting and drying contributed to reducing aflatoxin levels, aflatoxins did not necessarily impact protein efficiency ratio (PER), nor did garlic and turmeric significantly enhance it. The

inclusion of garlic and turmeric showed an improved nutrients utilization in *Oreochromis niloticus* despite the presence of aflatoxins in the feed.

INTRODUCTION

Fish are essential and affordable source of protein in the diets of many consumers in Nigeria and other developing countries. However, mould growth in fish feed poses a significant challenge for farmers, especially in tropical regions where storage facilities are inadequate (Marijani et al. 2019). High moisture levels in fish feed promote the proliferation of moulds, which can produce toxic substances known as mycotoxins. These compounds, including aflatoxins, ochratoxins, and fumonisins, are harmful to both fish and humans who consume contaminated fish. Among them, aflatoxin is the most prevalent and is frequently found in locally produced animal feeds, including fish feed (Odoemelam & Osu 2009). Under favorable conditions for mould growth, aflatoxin contamination can occur in feed ingredients and finished feeds. Ogunbanwo (2005) reported that major feed ingredients are highly susceptible to aflatoxin contamination, whether in the field or during storage. This poses a serious challenge to farmers, as fish consuming aflatoxin-contaminated feed may accumulate toxins that negatively impact human health (Pietsch 2020).

Aflatoxins are toxic secondary metabolites primarily produced by *Aspergillus* species, such as *Aspergillus flavus* and *Aspergillus parasiticus* (Abila 2003). These toxins commonly contaminate oilseed crops such as cottonseed, peanut meal, and corn, as well as wheat, sunflower, soybean, and fish meal. Four major types – aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) – are direct contaminants of grains and finished feeds (Pitt 2000, Marijani et al. 2019). The presence of aflatoxins in fish feed raises serious concerns due to their adverse health effects, which are further exacerbated by climate change (Nešić 2018). Rodrigues et al. (2011) emphasized that mycotoxin contamination results from poor agricultural practices, inadequate storage, and improper handling by farmers, grain processors, and feed millers. Aflatoxins, which are classified as potential carcinogens, represent a major food safety risk (Bennett & Klich 2013, Ilesanmi et al. 2023).

Aflatoxin contamination in feed and food has led to significant economic losses and health risks for both animals and humans. Various strategies have been developed to mitigate mycotoxin contamination, including pre-harvest, harvest, and post-harvest measures aimed at controlling mould growth or detoxifying contaminated products. Among these strategies, the use of plant-based detoxifiers has gained attention due to their safety and environmental friendliness compared to chemical treatments. Research suggests that plant extracts from garlic, ginger, and turmeric, along with their essential oils, can inhibit *Aspergillus* growth and reduce aflatoxin production (Agbebi et al. 2013).

This study, therefore, investigates the detoxification effects of garlic and turmeric powder on mycotoxin-contaminated feed and its impact on the growth and nutrient utilization parameters of Nile tilapia (*Oreochromis niloticus*) – a widely cultured and economically important fish species in Nigeria.

2. MATERIALS AND METHODS

2.1. Experimental site

The experiment was carried out in the Department of Biology, Federal College of Education, Osiele Abeokuta, Ogun State. The experiment was conducted in a circular concrete tanks (0.5 m depth and 0.58 m diameter).

2.2. Inclusion of Aflatoxin in the formulated feed

The feed ingredient was purchased and pelleted at Agro-Allied Company mill at Odo Eran, Abeokuta. The experimental feed was prepared in the laboratory and formulated as in Table 1. The compounded ingredients was sprinkled with small amount of distilled water to make the feed moist and then mixed with cultured strain of *Aspergillus flavus* from the Microbiology Department, University of Lagos. The mixed feed was covered with a plastic sac for 72hrs to encourage mould growth in the feed.

Fresh garlic bulb and turmeric root were purchased, dried and ground at the Mile-1 Market, Kebbi State. They were rinsed with clean water, and grated before sun-dried. The dried form of each was then ground to powder with a locally fabricated hand grinder. The experimental feed was mixed appropriately according to treatment. There were 8 treatments (TD) in 3 replicates each adopted for the study (Table 2). These are TD1 (feed uncontaminated/mould-free), TD2 (contaminated feed of *A. flavus*), TD3 (20 g of garlic/kg of mouldy feed), TD4 (40 g of garlic/kg of mouldy feed), TD5 (60 g of garlic/kg of mouldy feed), TD6 (20 g of tumeric/kg of mouldy feed), TD7 (40 g of tumeric/kg of mouldy feed), TD8 (60 g of tumeric/kg of mouldy feed). The compounded feeds were pelletized with a pelleting machine and sun-dried immediately. Thereafter, the feed was kept in airtight container for further study.

The proximate analysis of all diets were determined before the start of the experiment. Briefly, a known weight of the feed sample was dried at 105°C until a constant weight was achieved, and the moisture content was calculated based on weight loss. The dried sample was incinerated at 550°C, and the remaining weight was used to calculate ash content. This was followed by the Kjeldahl method, where nitrogen compounds were converted to ammonia, distilled, and titrated to calculate crude protein, at an assumption of 16% nitrogen in proteins. The crude fat (lipids) was determined using the Soxhlet method, involving solvent extraction and weighing the fat residue. Crude fiber is measured by degreasing the sample, digesting it with sulfuric acid, drying, and then incinerating it to obtain the fiber content from the weight difference.

The carbohydrate content of the feed was calculated as follows:

CHO = 100 - (moisture + ash + crude protein + fibre + lipid) (AOAC 2006)

2.3. Determination of Aflatoxin

The mycotoxin analysis of each feed treatment was done at Nigerian Stored Product Research Institute, Ilorin Laboratory before the commencement of the experiment with HPLC method modified from modified from Barbas et al. (2005).

2.3.1 Sample Preparation and Extraction

The sample preparation began by accurately weighing 5.0 g of the homogenized test material using a calibrated analytical balance (±0.0001 g precision). The weighed sample was transferred into a 50 mL polypropylene centrifuge tube, and 20 mL of a methanol-water mixture (85:15, v/v) was added. The mixture was vortexed for 30 sec to ensure thorough wetting, followed by mechanical shaking at 250 rpm for 2 h at 4°C to minimize degradation. After extraction, the mixture was centrifuged at 10,000 rpm for 10 min (4°C), and the supernatant was carefully decanted into a clean glass vial.

2.3.2 Extract Concentration and Defatting

The collected supernatant was concentrated to near dryness under a gentle stream of nitrogen at 40°C using an evaporator. The residue was reconstituted in 5 mL of 10% NaCl (w/v) solution to precipitate interfering compounds. For defatting, 10 mL of redistilled n-hexane (HPLC-grade, ≥99.9% purity) was added, and the mixture was vigorously shaken for 1 min before phase separation. The upper hexane layer (containing lipids) was discarded, while the lower aqueous-methanol phase was retained for further cleanup.

2.3.3 Solid-Phase Extraction (SPE) Cleanup

A disposable silica gel SPE column (500 mg/6 mL, 55–105 µm particle size, 60 Å pore size) was preconditioned with 10 mL of methanol, followed by equilibration with 10 mL of deionized water. The defatted extract was loaded onto the column at a flow rate of 1 mL/min under vacuum. Sequential washing steps were performed to remove matrix interferences:

- First wash: 30 mL of n-hexane** (discarded).
- Second wash: 3 mL of ethyl acetate (discarded).
- Third wash: 3 mL of methylene chloride (discarded).

The target analytes (aflatoxins B_1, B_2, G_1, and G_2) were eluted with 6 mL of chloroform-acetone (90:10, v/v) at a controlled flow rate of 0.5 mL/min. The eluate was collected in a salinized glass tube and evaporated to dryness under nitrogen. The residue was reconstituted in 200 µL of methanol and filtered through a 0.22 µm PTFE syringe filter prior to HPLC injection.

2.3.4 HPLC Analysis

Instrumentation and Chromatographic Conditions

- HPLC System: Agilent 1260 Infinity II (or equivalent), equipped with a fluorescence detector (FLD).
- Column: C18 reversed-phase column (150 mm × 4.6 mm, 3.5 µm particle size) maintained at 40°C.
- Mobile Phase:
- A: Water-methanol (60:40, v/v) with 0.1% formic acid.
- B: Methanol-acetonitrile (50:50, v/v).

- Gradient Program:
- 0–5 min: 20% B → 50% B (linear).
- 5–10 min: 50% B → 80% B (linear).
- 10–15 min: 80% B (isocratic).
- Post-run re-equilibration:5 min at initial conditions.
- Flow Rate: 1.0 mL/min.
- Injection Volume: 20 µL.
- Detection:
- FLD Settings:
- Excitation: 365 nm.
- Emission: 435 nm (for aflatoxins B_1 and G_1).
- Emission: 465 nm (for aflatoxins B_2 and G_2, via post-column photochemical derivatization).

Quality Control and Validation

- Calibration Standards: Aflatoxin working standards (0.5–50 µg/L) were prepared in methanol.
- Recovery Test: Spiked samples (5, 10, 20 µg/kg) were analyzed in triplicate, with recoveries between 85–110%.
- Limit of Detection (LOD): 0.05 µg/kg (S/N = 3).
- Limit of Quantification (LOQ):0.15 µg/kg (S/N = 10).

2.3.5 Data Analysis

Quantification was performed using Agilent OpenLab CDS ChemStation (v.2.4) or equivalent software. Peak identification was based on retention time (±2% tolerance) and spectral matching.

Table 1: The dietary composition of formulated feed used for the experiment

Ingredient	Feed (kg)
Maize	**22.5**
Groundnut cake	**30.50**
Fishmeal	**15.50**
Soya-bean meal	**30.50**
Mineral premix*	**0.50**
Methionine	**0.25**
Lysine	**0.25**
Total	**100**

[1] *Contains VitA 4000000IU; Vit D. 800000IU; Vit. E 40000 mg; Vit. K3 800 mg; Vit. B1 1000 mg; Vit. B2 6000 mg; Vit. B6 5000 m; Vit. B12 25 mg; Niacin 6000 mg; Patothenic acid 20000 mg; Folic acid 200 mg; Folic acid 200 mg; Biotin 8 mg; Manganese 300000 mg; Iron 80000 mg; Zinc 20000 mg; Cobalt 80 mg; Iodine 400 mg; Selenium 40 mg; Choline 800000 mg.

The compounded feeds were pelletized with a pelleting machine, dried immediately with an electric dryer at 45^{O}C for 24 hrs, and then kept in labeled airtight containers for further use. The proximate analysis of all diets was determined before the start of the experiment according to AOAC (2006).

2.4 Experimental procedure

12-week-old healthy juvenile fish (17.18±0.798 g) were selected and arranged in a group of 10 juvenile fish per tank. The 1.2 m3 circular concrete tanks used were 24 for the experiment for 10 weeks. The juvenile *Oreochromis niloticus* were obtained from Taiwo Farm, Ndele Ota Ogun State, acclimated to the experimental environment for 7 days, and maintained on Top® feed (45% CP) before the treatments.

Fish in every treatment group received 3% body weight of the prepared diet. The fish were fed the experimental diets twice a day at 9:00 am and 4:00 pm. The fish fed in each treatment group were monitored daily in the morning, at noon, and evening for swimming movement, breathing, possible bruises, and mortality. Weekly weight changes were noted using a weighing scale and the aquaria water changed every two days. After 10 weeks, the potential effects of garlic and tumeric on aflatoxin-induced feed were studied based on observed experimental growth and hematological characteristics.

2.5 Assessment of Growth Performance Parameters and Survival Rate

Average fish growth performance was assessed based on weight gain, average daily growth, specific growth rate, feed conversion ratio, feed efficiency ratio, and protein efficiency ratio were evaluated to determine the effects of the various treatments.

Average Weight Gain (AWG)

The average weight gain represents the variation between the starting weight and the weight after the trial. This is calculated according to (Sepahdar et al. 2009) using the formula:

Final mean weight (g) - Initial mean weight (g) = the average weight gain (g).

Average Daily Growth (AWG)

The average weight gained per day was calculated as:

$$ADG\ (mg) = \frac{\text{(Average Weight gain (mg))}}{\text{(Duration of the experiment (days))}}$$ (Hung et al. 1989)

Percentage Weight Gain (%WG)

Percentage weight gain is weight gained over time relative to the total weight and it is calculated as:

$$(\%WG) = \frac{\text{(Weight gain)}}{\text{(Initial Weight)}} \times 100$$ (Sepahdar et al. 2009)

Specific growth rate (SGR)

Specific growth rate (SGR) is the coefficient of the percentage increase in fish weight per day.

$$\text{Specific growth rate, [SGR (\%/day)]} = \frac{100\ (\log W2 - \log W1)}{(T2-T1)}$$ (Arnanson et al. 2009)

where W1 and W2 are the weights at time T1 and T2, respectively.

Feed conversion ratio (FCR)

Feed conversion ratio (FCR) represents the ratio or rate of efficiency with which the bodies of fish convert feed into the desired output and it is calculated as follows:

Feed Conversion Ratio (FCR) = (Total dry feed fed (g)) / (Total wet weight gain (g)) (Sepahdar et al. 2009)

Protein-efficiency-ratio (PER)

This is the connection between the amount of protein in feed and the moist weight increase of fish.

Protein Efficiency Ratio (PER) = (Wet weight gain (g)) / (Amount of Protein fed (g)) (Sepahdar et al. 2009).

2.6. Physico-chemical analysis of culture medium

A daily assessment of temperatures, conductivity, dissolved oxygen (DO), pH, and total dissolved solids (TDS) of aquaria is done using digital methods. The digital instruments used are as follows:

- Temperature meter with the range of 0.1-80.0°C and 32.0-176.0 ° F.
- A digital pH meter, (JUANJUAN®) with the range of 0.00-14.00pH.
- The EC meter made in Pakistan with the range of 0-9900 µs/cm
- The RCYAGO® Dissolved Oxygen Meter (range is 0.0-20mg/L) from United State
- The TDS meter made in Pakistan with the range of 0-9999 mg/l

2.7. Statistical Analysis

The growth and nutrients utilization data growth as well as water quality parameters were subjected to One-Way Analysis of Variance using statistical analysis software (SAS, 1999). Duncan's Multiple Range Test (DMRT) was used to evaluate significant averages between treatments at a probability level of 5%.

3. RESULTS

During the first 3 days of the trial, the fish showed little reaction to the experimental feed, but they had assumed full feeding by the 5th day. The experimental fish were active all through the experimental period and showed no stressful movement or sustained any external injuries across the Treatments.

The analysis of the total aflatoxin present in the initial feed (A) bought before the inclusion of the *Aspergillus flavus* species was observed to be 2.6448 µg/kg (Aflatoxin G2 - 0.0005 µg/kg, Aflatoxin G1 - 0.0054 µg/kg, Aflatoxin B2 - 0.1066 µg/kg and Aflatoxin B1 - 2.5302 µg/kg) but after the mixture with Aspergillus flavus (B) the concentration was higher at 123.168 µg/kg (Aflatoxin G2 - 0.072 µg/kg, Aflatoxin G1 - 0.84 µg/kg, Aflatoxin B2 - 22.678 µg/kg and Aflatoxin B1 - 99.589 µg/kg). Meanwhile, after the inclusion of the plant extracts (garlic and tumeric) according to the treatments and feed were pelleted, the total aflatoxin for TD1, TD2, TD3, TD4, TD5, TD6, TD7, and TD8 were 2.5908 µg/kg, 24.743 µg/kg, 23.515 µg/kg, 23.713 µg/kg, 24.181 µg/kg, 23.493 µg/kg, 22.215 µg/kg and 25.431 µg/kg respectively as stated in the Table 2.

Table 2: Total Aflatoxin analysis of the experimental feed

Parameters	A	B	TD1	TD2	TD3	TD4	TD5	TD6	TD7	TD8
Aflatoxin G2 (µg/kg)	0.0005	0.072	0.0005	0.008	0.005	0.006	0.008	0.004	0.004	0.008
Aflatoxin G1 (µg/kg)	0.0054	0.840	0.0075	0.075	0.058	0.066	0.069	0.056	0.042	0.06
Aflatoxin B2 (µg/kg)	0.1066	22.678	0.0974	0.591	0.785	0.832	0.426	0.838	0.855	1.621
Aflatoxin B1 (µg/kg)	2.5302	99.589	2.4854	24.069	22.667	22.809	23.678	22.596	21.314	23.742
Total (µg/kg)	2.6448	123.168	2.5908	24.743	23.515	23.713	24.181	23.493	22.215	25.431

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Table 3: Proximate analysis of the experimental feed samples

Samples	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	CHO (%)	Fibre (%)
TD1	3.15	18.02	38.81	6.1	8.36	25.56
TD2	6.17	15.67	39.30	6.1	8.92	23.84
TD3	2.67	16.80	39.75	6.5	9.80	24.48
TD4	3.82	15.27	39.13	6.3	10.27	25.21
TD5	4.47	14.11	38.34	6.2	12.20	24.68
TD6	4.87	9.68	39.25	6.0	12.29	27.91
TD7	3.07	18.88	39.13	8.2	15.28	15.44
TD8	5.61	12.01	39.25	8.4	13.36	21.37

The proximate analysis of the experimental feed was analyzed as stated in Table 3. The results recorded for moisture, ash, protein, lipid, CHO and fibre dry matter and energy range from 40.12 – 39.62, 8.51 – 8.451, 7.03 – 6.88, 6.30 – 5.83, 94.35 – 93.60 and 17.81 – 17.58 (kJ/g) respectively.

There was variations in the value of aflatoxin of the analyzed feeds. It was observed that the processing activities (pelleting and drying) had a reducing effect on the concentration of the total aflatoxin.

There was no significant difference ($P>0.05$) in the initial weight of the experimental fish, they were evenly distributed into the tanks. Meanwhile, after the experimental period, there was a significant difference ($P>0.05$) in the final weight and weight gain of the experimental fish across the treatment. TD6 had the highest final weight and weight gain which was significantly different ($P>0.05$) from others, TD2 was significantly low ($P>0.05$) both in the final weight (11.62 g) and weight gain (9.74 g). In the amount of the feed intake recorded, the experimental fish fed with TD1 (10.90 g) and TD6 (10.98 g) had the best response significantly ($P<0.05$) to the feed and feed intake of the experimental fish was significantly low ($P<0.05$) in the TD2 (9.91 g) and TD8 (9.89 g) treatments.

The records during the experimental set-up are presented in Table 5. The culture medium temperature was within 24.300C and 27.200C. The pH was within the range of 7 that is 6.90 and 7.03. Also, electrical conductivity also was within the cultured system range lowest is 366.33 µ/cm and highest is 416.67 µ/cm. The oxygen (DO) level was observed to be the optimum value for cultured fish ranges between 3.64 mg/l and 4.59 mg/l. In addition, total dissolved solids (TDS) were also within the cultured system range (114.67 mg/l and 205.00 mg/l).

Table 4: Growth parameters and survival rate of *O. niloticus* fed experimental diets

Parameters	TDI	TD2	TD3	TD4	TD5	TD6	TD7	TD8	±SME
Initial wgt (g)	2.02^{a}	1.88^{a}	1.87^{a}	2.07^{a}	2.23^{a}	2.07^{a}	2.00^{a}	1.92^{a}	0.0715
Final wgt(g)	14.48^{b}	11.62^{d}	12.06^{dc}	12.20^{c}	10.83^{e}	16.07^{a}	14.23^{b}	13.66^{d}	0.3460
Wgt Gain (g)	12.46^{b}	9.74^{g}	10.19^{e}	10.13^{e}	10.60^{f}	14.00^{a}	12.23^{c}	11.74^{d}	0.3458
F. I (g)	10.90^{a}	9.91^{d}	9.89^{d}	10.19^{bc}	10.13^{c}	10.98^{a}	10.87^{a}	10.75^{ab}	0.1158
P. I (g/kg)	4.25^{ab}	3.86^{d}	3.96^{c}	3.97^{c}	3.85^{d}	4.28^{a}	4.24^{ab}	4.19^{b}	0.0521
PER	2.03^{a}	1.70^{a}	1.66^{b}	1.70^{a}	1.70^{a}	1.58^{c}	1.58^{c}	1.58^{c}	0.0109
FCR	0.87^{d}	0.93^{cd}	0.97^{b}	1.01^{b}	1.16^{a}	0.78^{e}	0.88^{cd}	1.10^{a}	0.0254
SGR (%/day)	2.80^{ab}	2.68^{c}	2.56^{c}	2.46^{c}	2.16^{d}	2.95^{a}	2.78^{ab}	2.47^{c}	0.0550
Survival (%)	100	100	100	100	100	100	100	100	0.0000

[1] Initial wgt – Initial weight; Final wgt – Final weight; Wgt Gain – weight gain - F. I – Feed Intake; P. I – Protein intake; PER – Protein Efficiency ratio; FCR – Feed Conversion Ratio; SGR – Specific Growth Rate.

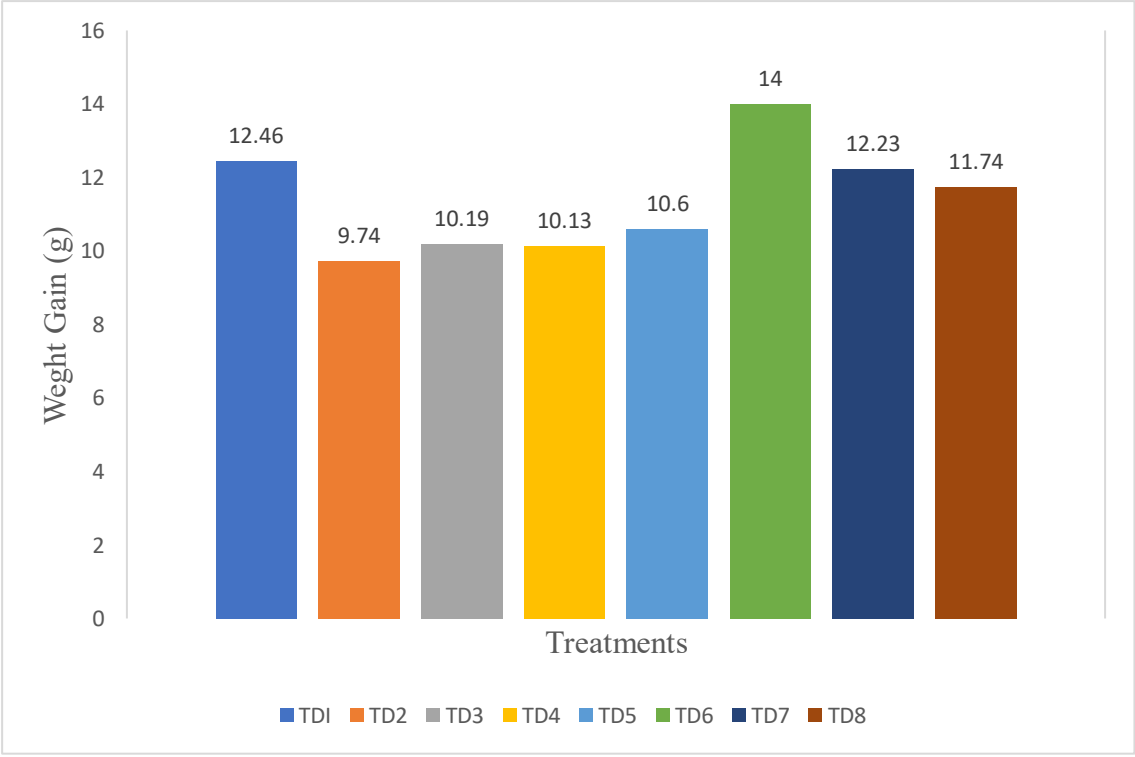

Figure 1: Weight Gain of *O. niloticus* fed experimental diets

Table 5: Average water quality parameters in the culture medium

Parameters	TDI	TD2	TD3	TD4	TD5	TD6	TD7	TD8	±SME	Aquaculture Mean Value
EC (µ/cm)	389.00^{b}	366.33^{c}	376.67^{bc}	384.00^{b}	410.67^{a}	416.67^{a}	381.67^{bc}	391.33^{b}	80.25	20-1500 (Boyd, 2003)
TDS (mg/l)	195.00^{b}	181.67^{d}	188.00^{c}	192.50^{bc}	205.00^{a}	203.00^{a}	193.33^{bc}	114.67^{e}	12.25	<400 (WHO, 2004)
TEMP (°C)	25.43^{cd}	27.20^{a}	25.70^{bc}	26.07^{b}	25.07^{de}	24.70^{ef}	24.30^{f}	25.17^{cde}	0.11	25–30 (FAO, 2006)
DO (mg/l)	3.93^{a}	3.83^{a}	3.64^{a}	4.59^{a}	4.10^{a}	4.23^{a}	3.70^{a}	4.50^{a}	0.24	3 – 20 (Boyd, 2003)
pH	6.96^{bcd}	7.12^{a}	6.98^{bcd}	7.04^{ab}	6.90^{d}	7.03^{abc}	6.95^{bcd}	6.92^{cd}	0.004	6.0–9.0 (Davis, 1993)

[1] EC – Electrical Conductivity; TDS – Total Dissolved Solids; TEMP – Temperature; DO – Dissolved Oxygen.

The protein intake TD1 (4.91) and TD6 (4.94) were the highest and significantly different ($P<0.05$) from others and TD2 (4.46 g) and TD8 (4.45 g) were significantly low ($P<0.05$). Meanwhile, the protein efficiency ratio was significantly low in TD2 (2.23) and TD4 (2.21).

Based on the record from the period of the experiment, the experimental fish with the highest value of feed conversion ratio was TD2 (1.05) and significantly difference ($P<0.05$) from others while at TD6 it was significantly low from the others. The specific growth rate at the TD6 was significantly high ($P<0.05$) than others but low at TD2 and TD3 significantly ($P<0.05$) from others. All through the 10 weeks experimental period there was no mortality, therefore, the survival rate was 100% all through.

4. DISCUSSION

The experimental feed ingredients bought directly from the feed mill contained some concentration of aflatoxin which is an indication that feed got contaminated by mycotoxins before it gets to the farm. The report of Biomin survey (2017) supported this that about 74% of the ingredients used in compounding aquaculture feed are contaminated with mycotoxins which can have a substantial negative economic impact on the aquaculture industry. Likewise, the variation in the total concentration of the aflatoxin present in the feed might be because of the differences in the handling of the feed during the pelleting and drying. The aflatoxin content of the experimental diet is higher than the standard limit level by CECC (2003) in animal feeds which is 20 µg/kg and 5 µg/kg to 10 µg/kg in compounded feeds for dairy animals and lambs respectively. The processing effect of pelleting feed can contribute to the reduction in aflatoxin concentration in the feed. This is noticed in the TD2 (mould feed) before (123.168 µg/kg) and after (24.743 µg/kg) the pelleting of the feed this supported the observation made by Neme and Mohammed (2017) and Ilesanmi et al. (2024), that the concentration of mycotoxin can be reduced by processing techniques. Also, the extrusion technique reduces AFs by 50% to 80% depending on the processing temperature and granule moisture content reduction (Shanakhat, et al. 2018, Kabak 2006).

The growth rate parameters were recorded to track the development of *Oreochromis niloticus*. The growth observations recorded might be because of aflatoxin concentrations inside the experimental feed. The reduced weight gain observation at the TD2 of this experiment confirmed the report of low or reduced weight gain stated by Deng et. al. (2010) and Anh Tuan et. al. (2002) where fingerlings of Nile tilapia were given aflatoxin-B1 contaminated feed for 20 days, 25 days, and 56 days respectively. Although the concentration of aflatoxin in TD2 (24.743 µg/kg) was not the highest, TD8 had higher concentrations but the improved weight gain at TD8 may be as a result of the mitigating effect of turmeric over aflatoxin and ability to improve weight gain (Teich et al., 2017; Shawky et al., 2022). The improved AWG recorded in TD6, TD7, TD8 and other treatment may be due to the addition of garlic and turmeric in them. Therefore, the presence of garlic and turmeric may be the reason for the improved weight gain recorded in the experiment despite the concentration of total aflatoxin present in the feed, this was also observed by Saber et al., (2010). The inclusion of garlic extract to the feed of Juvenile *Acipenser ruthenus* demonstrated a notable improvement in feed efficiency and weight gain over a 10-week feeding period (Lee et al. 2012). Bello et.al. (2012) corroborated this importance of plant extracts when they observed a comparable rise in weight growth in *Clarias gariepinus* given meals supplemented with leftover walnut leaves and onion bulbs.

The feed intake at TD2 was significantly low ($P<0.05$) compared to others which may be as a result of the feed contamination with *Aspegillus flavus* because there was a better feed intake in the feed without the fungi. Meanwhile, TD8 had similar concentration level of aflatoxin (25.431 µg/kg) with TD2 (24.743 µg/kg) but the increase in the feed intake in TD8 and other treatments may be as a result of the pleasant odour from these plant extracts (Hasegawa et al., 2015). In agreement with this, Agbon et al. (2013) stated that there is a low response to feed by juvenile *Clarias gariepinus* fed aflatoxin B1 contaminated feed for 12 weeks. Meanwhile, other treatments with the inclusion of garlic and turmeric responded well to the feed and may be assumed that the spices taste of these extract is responsible for it. This is also observed Agbebi et al. (2013), that there is an increase in the feed intake of *Clarias gariepinus* fed diet mixed with ginger compared to the one without ginger for 56 days. Comparing the feed intake with the treatment with the turmeric inclusion were better than that of garlic, this may be because turmeric has a pleasant smell than garlic and brighter colour. According to this research garlic or turmeric may have effects on the feed acceptability which will directly influence the weight gain of the fish.

Therefore this research showed that the reduced or no concentration of aflatoxin in the feed can improve the weight gain of *Oreochromis niloticus*. On the other hand, the acceptability of the feed with the inclusion of garlic and tumeric may be responsible for the improved weight gain experienced in this research. Meanwhile, TD3 had the lowest feed intake value but with a better weight gain compared to the TD2, meaning that the plant extracts also have effects on the weight gain apart from the feed acceptability.

According to the proximate analysis of the feed and the subsequent protein intake by each treatment, TD6 had the highest protein intake compare to others but in protein efficiency ratio TD1 had the highest, this maybe

as a result of the absence of plant extracts (garlic and tumeric) and low level of aflatoxin concentration in the feed that is responsible for the effective utilization of the quantity of PI available for the treatment. There was no difference in the PER of feed with turmeric inclusion (TD6, TD7 and TD8). This result was similar to what was discovered by Onyeniyoma et al. (2024) when broilers fed with turmeric inclusion feed had low PER despite being fed with high protein intake. This may probably be as a result of the presence of anti-nutritional factors in plant extracts like flavonoids, alkanoids, tannin, phytate and saponin that are associated with nutrient impairment (Ari et al., 2012). It is noted from this research that presence of aflatoxin in feed may not necessarily affect the PER and plant garlic and turmeric may not as well improve the PER.

The feed conversion ratio which can relate to how economical the feed is or the utilization of the feed by the fish has its highest value in the TD2 which was significantly difference from others in the group. This results may be attributed to the deteriorating effects of aflatoxin on the food conversion of the fish which was in confirmation of the report of Ghafarifarsani et al. (2021). TD6 after the experimental period had the best feed conversion ratio which is significantly different from others in the group.

The specific growth rate at TD2 and TD3 during the experimental period were significantly low ($P<0.05$) compared to others in the group. The specific growth rate value recorded in the TD2 and TD3 might be due to the aflatoxin contamination and the low quantity of the garlic inclusion level respectively. This was also supported by Ghafarifarsani et al. (2021) when rainbow trout was fed with aflatoxin B1 and zearalenone contaminated feed.

The experimental fish used appeared to be in good condition during the experimental period while fish survival was 100%. Fish respond differently to aflatoxin depending on the concentration and length of exposure (Zhang et al. 2021). Ten (10) weeks of exposing the fingerlings of *Oreochromis niloticus* to 50 ppb of aflatoxin contaminated feed showed little or no effect on the performance of the fish (El-Banna et al. 1992). On the contrary, Effiong and Alatise (2009) in their research observed a low survival rate of catfish (*Heterobranchus longifilis*) when they were given mould-contaminated feed for 6 weeks. In fish, it has been commonly reported that aflatoxicosis comes with serious health challenges, poor growth rates, and reduced appetites (Sotolu et. al. 2014, Cagauan et. al. 2004). This research may not be able to ascertain the impact of the aflatoxin contaminated feed on the experimental fish but report of Agbon et al. (2013) and Agbebi et al. (2013) established it that aflatoxicosis has a negative effect on the biochemical and histology of *Clarias gariepinus* respectively.

The water quality parameters (temperature, pH, conductivity, dissolved oxygen and total dissolved solid) recorded weekly during the experiment were within the recommendation by the legislation (Boyd 2003, Davis 1993, FAO 2006) so the experimental feed did not have an influence on zootechnical parameters of juvenile *O. niloticus*. Fish survive and grow best in water within the recommended water quality parameters.

5. CONCLUSIONS

In conclusion, the addition of garlic and turmeric improved the feed intake, weight increase, and feed conversion ratios of Nile tilapia (*Oreochromis niloticus*) in spite of aflatoxins, indicating their potential to lessen the negative impacts of mycotoxins. The results highlight how important it is to implement natural detoxifying techniques to improve food safety and sustainability in aquaculture, especially in areas where mycotoxin contamination is a problem. However, turmeric at 20 g/kg produced the most advantageous results among the treatments, achieving the highest weight gain, specific growth rate and feed conversion ratio, while ensuring a 100% survival rate across all treatments.

The research was just for 10-week feeding trial and did not examine the long-term physiological or histological impacts of aflatoxin exposure or the incorporation of plant extracts. Also, only two plant-based additives (garlic and turmeric) were tested, and their bioactive compound concentrations were neither standardized nor quantified. These can be for future research alongside with the residual aflatoxin levels in fish tissues.

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