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Haematological Impacts of Total Aflatoxin and the Ameliorative Potentials of *Allium sativum* and *Curcuma longa* in *Oreochromis niloticus*

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Abstract

Aflatoxins, toxic secondary metabolites produced by fungi such as Aspergillus flavus, pose significant threats to fish health and productivity in aquaculture. This study investigated the haematological and biochemical effects of total aflatoxin exposure and the potential ameliorative properties of two natural additives; garlic (Allium sativum) and turmeric (Curcuma longa) in Oreochromis niloticus. A 10-week feeding trial was conducted using eight dietary treatments including a control, aflatoxin-contaminated feed, and varying inclusion levels (20, 40, 60 g/kg) of garlic and turmeric. Aflatoxin contamination was induced by inoculating formulated feed with A. flavus, and total aflatoxin levels in the diets ranged from 2.59 to 25.43 µg/kg. Results revealed that fish exposed to contaminated diets without supplementation exhibited significant declines in key haematological indices, including haemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), and total protein (TP), indicating systemic stress and impaired physiological function. However, diets supplemented with garlic and turmeric significantly mitigated these adverse effects. The highest haematological improvements were observed in groups receiving 60 g/kg of turmeric and garlic, with notable increases in Hb, WBC, PCV, and RBC levels, as well as improved liver enzyme activity and serum biochemistry markers.

Keywords: Aflatoxin, Oreochromis niloticus, Garlic, Turmeric, Haematology.

Introduction

Mycotoxins are toxic secondary metabolites produced by fungi such as Aspergillus, Fusarium, Penicillium, and Alternaria (Dan-Ologe et.al. 2026, Ilesanmi et al., 2023). These contaminants are prevalent in up to 25–80% of crops globally, depending on region, crop type, and storage conditions (Eskola, 2019). Major mycotoxins of concern in animal feed include aflatoxins, ochratoxins, fumonisins, trichothecenes (e.g., deoxynivalenol), and zearalenone (Khan et al., 2024). Their presence in aquafeeds poses a significant threat to fish health, leading to reduced growth, liver damage, immunosuppression, reproductive issues, and even mortality (Marijani et al., 2019).

Specifically, aflatoxins especially aflatoxin B1 are known to cause severe toxic effects in fish. Studies have reported growth retardation, internal lesions, and increased mortality in species like channel catfish and Nile tilapia exposed to contaminated diets (Zaineldin et al., 2025). Haematological and biochemical alterations such as reduced red blood cell counts and liver dysfunction have also been observed (Manning, 2003; Shalaby, 2004). In Nigeria, a high incidence

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of aflatoxin contamination has been reported in fish feeds, resulting in significant economic losses for fish farmers (Momodu et al., 2016).

In response to the growing concerns over synthetic additives and the harmful effects of mycotoxins, attention has shifted toward the use of natural feed additives such as garlic (*Allium sativum*), turmeric (*Curcuma longa*), onions (*Allium cepa L*), soursop (*Annona muricata*), ginger (*Zingiber officinale*) etc. These spices not only enhance flavor and shelf life but also possess bioactive compounds with antioxidant, antimicrobial, and immunostimulatory properties (Chrubasik et al., 2005; Yanishlieva et al., 2006). Garlic as well as tumeric has been reported to boost growth performance, feed intake, and immunity in fish, including Nile tilapia (Dan-Ologe et. al, Abdel-Hakim et al., 2010; Martins et al., 2016). Rich in organosulfur compounds like allicin, garlic exhibits antimicrobial and antiparasitic effects and supports liver detoxification (Harris et al., 2001; Adler et al., 1997). Its application in aquaculture, whether through feed supplementation or immersion, has shown promise in mitigating the impacts of pathogens and toxins (Hamed et al., 2021). This study explores the potential of garlic and ginger as natural dietary supplements to counteract the effects of aflatoxin in *Oreochromis niloticus*, thereby improving fish health and productivity in aquaculture systems.

Materials and Methods

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).

The experimental feed ingredient was obtained from an Agro-Allied shop at Odo Eran in Abeokuta. The feed was formulated in the laboratory as slated Table 1. The formulated feed was wet with small quantity of tap water. The moistened feed was mixed with cultured strain of *Aspergillus flavus* obtained from the Microbiology Department, University of Lagos. The mixed feed was covered with a plastic bag 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 (TRT) in 3 replicates each adopted for the study (Table 2). These are TRT 1 (feed uncontaminated/mould-free), TRT 2 (contaminated feed of A. flavus), TRT 3 (20 g of garlic/kg of mouldy feed), TRT 4 (40 g of garlic/kg of mouldy feed), TRT 5 (60 g of garlic/kg of mouldy feed), TRT 6 (20 g of tumeric/kg of mouldy feed), TRT 7 (40 g of tumeric/kg of mouldy feed), TRT 8 (60 g of tumeric/kg of mouldy feed). The

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compounded feeds were pelletized with a pelleting machine and sun-dried immediately. Thereafter, the feed was kept in airtight container.

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

*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

Experimental procedure

A 12-week-old healthy juvenile fish (17.18±0.798 g) of *O. niloticus* were selected and arranged in a group of 10 juvenile fish per tank. The 1.2m^3 circular concrete tanks used were 24 for the experiment for 10 weeks. The juvenile *O. 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.

Haematological Examination

Five fish were taken from each treatment of the experiment at random for haematological investigation at the end of the tenth week. Blood was drawn into ethylenediamine tetraacetic acid (EDTA) bottles via the lateral line of each fish's caudal vein using a needle and syringe to assess the haemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), red blood cell count (RBC) and white blood cell count (WBC) of the Clarias gariepinus juveniles. The

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parameters were determined by the standard methods as described by Schalm et al. (1975) and Kelly (1979).

Biochemical analysis

The heparinized tubes (no anticoagulant) containing blood samples were centrifuged at 14,000g for 3 min within 30 min after the collection of the blood. Serum, rather than plasma was used for blood chemistry analysis to avoid possible interference of fibrinogen. A fixed volume (100 µL) pipette was used to dispense serum samples on the reagent rotor's sample port. The samples analysed were AST, ALT, ALP, Globulin, ALB, creatinine and TP using the standard methods described by Schirmeister (1964), Henry (1974) and Tietz (1995).

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%.

Result

The analysis of the total aflatoxin present in the feed 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 in the Feed treatments

Parameters	**TD1**	**TD2**	**TD3**	**TD4**	**TD5**	**TD6**	**TD7**	**TD8**
Total Aflatoxin (µg/kg)	2.5908	24.743	23.515	23.713	24.181	23.493	22.215	25.431

Parameters	**TDI**	**TD2**	**TD3**	**TD4**	**TD5**	**TD6**	**TD7**	**TD8**	**±SME**
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
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
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
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
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

EC - Electrical Conductivity; TDS - Total Dissolved Solid; TEMP – Temperature; DO – Dissolved Oxygen; pH – pH.

The water parameters observed throughout the experimental period fell within the optimum limits of each. EC ranged between 366.33 µ/cm and 416.67 µ/cm in TD2 and TD6 respectively. TDS values ranged between 188.00 mg/l and 205.00 mg/l in TD3 and TD5 respectively. Also TEMP ranged between 24.30^{0}C and 27.20^{0}C in TD7 and TD2 respectively. But in DO there was no

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significant difference in its value although the value range from 3.64 mg/l to 4.59 mg/l in TD3 to TD4 respectively. The pH observed were a little around neutral as the values range between 6.90 and 7.12 in TD5 and TD2 respectively.

The results of the haematological parameters observed during the experimental period showed no significant difference (P>0.05) in the MCH and MCV. But there was significant increase (P>0.05) in the value of MONO at TD6 (2.50%) compared to others. There was also a significant increase in the value of Hb at the TD7 (12.80 g/dl) which is followed by TD1 (11.05 g/dl) with TD5 (10.05 g/dl) showed the lowest Hb value and significantly different. Meanwhile, HET had the highest value in TD2 (38.50%), TD7 (38.50%) and TD8 (39.00%); these are significantly different from others. Meanwhile, the same treatments that was high in the value of HET were low in LYM, that is, TD2 (60.50%), TD7 (60.50%) and TD8 (60.50%). TD8 was significantly high (P<0.05) in MCHC where TD3 and TD7 were the lowest significantly. The PCV was observed to be significantly increased at the TD5, TD7 and TD8 compared to others. Also, in RBC TD1 (2.95 $\times 10^{12/L}$) and TD7 (3.05 $\times 10^{12/L}$) were significantly (P<0.05) high compared to others.

WBC was high significantly (P<0.05) in TD5 (12.85 $\times 10^{9/L}$)and TD7 (13.50 $\times 10^{9/L}$) (Table 3).

After the experimental period the biochemical analysis showed the value of the ALB of *O. niloticus* to range from 4.25 (g/dl) to 2.30 (g/dl) where TD2 (2.60 g/dl) and TD3 (2.30 g/dl) were significantly low and different compared to other group. The ALP as shown in Table (4) revealed the significant difference of TD3 (77.00 mg/dl) compared to others, TD2 value (41.00 mg/dl) was the lowest although not significantly different from the rest. AST value ranged from 107.50 U/L to 69.00 U/L that is, at the TD8 and TD2 respectively. The creatinine value at TD7 was the lowest and significantly different from the rest with TD4 recording the highest. TD4 showed the same improved value trend in globulin (3.00 g/dl) and TP (6.40 g/dl) where it was significantly high compared to others.

Table 3: Haematological parameters of *O. niloticus* fed diets containing aflatoxin

Parameters	TDI	TD2	TD3	TD4	TD5	TD6	TD7	TD8	±SME
Hb (g/dl)	11.05^{b}	9.95^{cd}	9.35^{cd}	10.50^{c}	10.05^{cd}	10.85^{c}	12.80^{a}	11.40^{b}	0.4790
HET (%)	29.50^{c}	38.50^{a}	32.50^{bc}	34.50^{b}	32.00^{bc}	31.00^{bc}	38.50^{a}	39.00^{a}	4.013
LYM (%)	69.00^{a}	60.50^{b}	66.50^{a}	65.00^{ab}	67.00^{a}	66.00^{a}	60.50^{b}	60.50^{b}	5.8750
MCH (pg)	38.73^{a}	40.62^{a}	39.99^{a}	39.84^{a}	42.03^{a}	37.90^{a}	41.97^{a}	41.89^{a}	8.9178
MCHC (g/dl)	33.08^{ab}	33.24^{ab}	32.80^{b}	33.32^{ab}	33.59^{ab}	32.80^{ab}	32.82^{b}	34.17^{a}	0.3661
MCV (fl)	117.20^{a}	121.80^{a}	122.00^{a}	119.75^{a}	125.35^{a}	115.55^{a}	127.85^{a}	122.55^{a}	79.6716
MONO (%)	1.00^{b}	1.00^{b}	1.00^{b}	0.50^{b}	0.50^{b}	2.50^{a}	0.50^{b}	0.50^{b}	0.1563
PCV (%)	33.50^{b}	30.00^{bc}	28.50^{c}	31.50^{bc}	37.00^{a}	27.00^{c}	39.00^{a}	37.50^{a}	5.5000
RBC ($\times 10^{12/L}$)	2.95^{a}	2.50^{cd}	2.53^{cd}	2.65^{bc}	2.75^{bc}	2.35^{dc}	3.05^{a}	2.96^{a}	0.0547

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WBC ($\times 10^{9/L}$)	12.50^{ab}	11.55^{c}	10.75^{c}	11.80^{bc}	12.85^{a}	11.75^{bc}	13.50^{a}	12.05^{ab}	0.8166

Hb – Haemoglobin; HET – Heterophils; LYM – Lymphocytes; MCH – Mean Corpuscular Haemoglobin; MCHC – Mean Corpuscular Haemoglobin Concentration; MCV – Mean Corpuscular Volume; MONO – Monocytes; PCV – Packed Cell Volume; RBC – Red Blood Cell; WBC – White Blood Cell.

Table 4: Biochemical of *O. niloticus* fed experimental diets

Parameters	TDI	TD2	TD3	TD4	TD5	TD6	TD7	TD8	±SME
ALB (g/dl)	3.15^{b}	2.60^{c}	2.30^{c}	3.40^{ab}	2.95^{b}	4.25^{a}	3.95^{a}	3.20^{b}	0.49
ALP (mg/dl)	51.00^{b}	41.00^{b}	77.00^{a}	41.50^{b}	50.50^{b}	48.50^{b}	42.00^{b}	47.00^{b}	7.59
ALT (U/L)	34.50^{c}	33.00^{c}	36.50^{b}	45.50^{a}	32.00^{d}	28.00^{d}	24.50^{e}	26.50^{e}	14.66
AST (U/L)	90.50^{b}	69.00^{c}	83.00^{b}	84.50^{b}	89.50^{b}	83.50^{b}	91.50^{b}	107.50^{a}	30.81
Creat (mg/dl)	1.36^{b}	1.25^{b}	1.32^{b}	1.73^{a}	1.11^{b}	1.23^{b}	1.19^{b}	1.09^{b}	0.03
Glob (g/dl)	2.60^{b}	1.95^{c}	1.80^{c}	3.00^{a}	2.05^{c}	2.40^{b}	1.60^{d}	1.90^{c}	0.15
TP (g/dl)	5.75^{ab}	4.55^{c}	4.05^{c}	6.40^{a}	5.00^{bc}	6.65^{a}	5.55^{bc}	5.10^{bc}	0.9578

ALB – Albumin; ALP – Alkaline Phosphatase; ALT – Alanine Transferase; AST – Aspartate Aminotransferase; TP – Total Protein; Creat – Creatinine; Glob – Globulin.

Discussion

The water quality parameters 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*.

Haematology plays a crucial role in the determination of the impact of toxins and diagnosing fish diseases. Some fish species are known to have the capacity to stabilize their haematological level when exposed to toxic environment. This could be the cause of the study's findings, which showed no significant differences in MCH and MCV following the haematological evaluation of the effect of an experimental diet on the juvenile *Oreochromis niloticus*. This experiment results was in support of the report by Anater et al., (2020) where different level of aflatoxin B1 was used to feed silver catfish (*Rhamdia quelen*) but MCH, MCV and plasma protein (PP) levels did not show significant difference ($P>0.05$) between treatments after fifty six (56) days.

Meanwhile, the significant increase observed in the value of hemoglobin (Hb) where TD2 was low might be the toxic effect of the experimental feed which was also reported by Jalilpour et al., (2018) when fingerlings of *Acipencer stellatus* was fed with aflatoxin contaminated feed for fifty-two (52) days, there were significant differences ($P<0.05$) in the parameters including Hb.

The significant HET increase observed in all the treatments especially TD2 compared to TD1 in the group may be as a result of aflatoxicosis stress the experimental fish were subject to which is supported by the statement of Witeska et al. (2022) that an increase in heterophils can be related

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to response to inflammation, infection, or injury. The report continued that an increase in heterophils can be as a result of stress.

Lymphocytes are crucial for fish immunity and can be affected by various stressors like heavy metals, pesticides, and pathogens. At the TD2, TD7 and TD8 the LYM was significantly low which can be referred to as lymphocytopenia and it is an indication of infections, stress, or other environmental factors affecting their immune system (Guzman et al., 2016; Grzelak et al., 2017).

PCV is a key indicator of overall health and can be influenced by various factors including exposure to toxicity. This can be related to the result from this experiment where the reduction in the value of PCV at the TD2 may be attributed to the presence of aflatoxin in the feed, also at the 20% inclusion level of both garlic and turmeric the PCV was significantly reduced. This same trend was discovered in the RBC with a significant increase at TD1, TD7 and TD8. Mousa and Khattab (2003) reported a low value in RBCs and PCV in *C. gariepinus* after exposure to ochratoxin.

The white blood cell count decreased noticeably in the *O. niloticus* fed with TD2 and TD3 which may be as a result of the aflatoxin level and the low inclusion level of garlic respectively. White blood cell count serves majorly as a defense mechanism against infections and distributes antibodies in the immune response. Likewise, the increase in the white blood cell count in the treatments fed with garlic (TD5) and tumeric (TD7 and TD8) included feed can be related to the similar report of Ndong and Fall (2011) that got an increase in the white blood cell count in young Tilapia hybrid with a diet supplemented with 0.5% and 1% ginger. The increase in white blood cell count in the TD7 and TD8 (tumeric included group) suggested that the tumeric in the feed improves and stimulates the immunological ability of the body which can help to increase the resistance of fish to infections.

Aspartate aminotransferase and alanine aminotransferase are enzymes help to detect inflammation or tissue injury or dysfunction in organs. A decrease in AST levels can be associated with several factors, including vitamin B6 deficiency, chronic kidney disease, and certain medical conditions or treatments. Fletcher and Sissons (2025) reported that low AST levels are generally considered healthy, but may indicate: vitamin B6 deficiency, kidney disease, liver disease, cirrhosis, cancer, immune stress, genetic alteration. This may be related to the results obtained from this experiment where there was a notable decrease ($P<0.05$) in AST of aflatoxin contaminated feed (TD2). Meaning, the fish at TD2 may be victim of the above unhealthy condition as a result of the low AST. This is contrary to the observation of Mahfouz and Sherif (2015) there was a significant increase in the value of aspartate aminotransferase after ingestion of diets containing 20 and 100 μg.kg−1 of aflatoxin B1 into Nile tilapia for 12 consecutive weeks. This significant increase ($P<0.05$) in TD8 and others with inclusion of garlic and turmeric might be due to the inclusion which could emphasize the potential of these plant extract to suppress or control infections or any toxicity (Nya *et al.*, 2009; Masoud and Mostafa, 2013).

Alkaline phosphate (ALP) is a brush-border enzyme found in the bloodstream. There was no significant difference in the ALP of the experimental fish across the treatment. This may be as a result of the potential of Tilapia species to withstand stress (Phrompanya et al., 2021). Alanine Transaminase (ALT) is an enzyme that is found in the liver which can also be present in other tissues (Moriles et al., 2025). This was significantly increased in the feed with inclusion of turmeric

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(TD6, TD7 and TD8). This showed the possible potential of turmeric to suppress the aflatoxicosis. This is because it has been reported earlier of the effects of aflatoxin contaminated feed on fish species by Agbon *et al.,* (2013) where *C. gariepinus* was subjected to feed contaminated with aflatoxin B_1 recorded a significant increase in the juvenile *Clarias gariepinus*. Also Hussein *et al.* (2000) observed an increase in liver transaminase in *Oreochromis niloticus* fed feed infected with aflatoxin B1.

The effect of garlic was observed when creatinine, globulin and TP level were significantly high at the TD4, because the earlier study reported the decrease in the serum proteins (albumin, globulin, and total protein) with increasing aflatoxin B1 levels in the diet of Pekin ducklings (Chen et al., 2014). This indicated that concentration of aflatoxin present in the feed may be responsible for the decrease in the treatments recorded.

Conclusion

The results demonstrated that aflatoxin-contaminated diets significantly compromised several physiological parameters in Nile tilapia, including reduced haemoglobin levels, packed cell volume (PCV), red and white blood cell counts, and serum protein profiles. However, the inclusion of garlic and turmeric particularly at higher concentrations showed promising ameliorative effects, improving the overall haematological and biochemical profiles of the fish. Notably, turmeric inclusion (TD7 and TD8) and garlic at 40 g/kg (TD4) significantly enhanced immune responses and liver function markers, indicating their protective role against aflatoxicosis. The potential of garlic and turmeric was affirmed as natural, affordable, and effective dietary supplements in aquaculture to combat aflatoxin-induced toxicity.

Recommendations

Based on the results of this experiment 40–60 g/kg inclusion level of turmeric will be optimal against aflatoxin toxicity, and garlic at 40 g/kg in aquafeeds for *O. niloticus*. Regular aquafeed screening for aflatoxins should be implemented to safeguard fish health and farm productivity. the use of natural feed additives should be encouraged and promoted for sustainable aquaculture practices.

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