

# Effects of Blighia Sapida Seed Powder on Gene Expression of Juvenile Catfish *Clarias Gariepinus* (Burchell 1822).

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## ABSTRACT

The study investigates the effects of *Blighia sapida* (ackee) seed powder on the health and gene expression of juvenile African catfish, *Clarias gariepinus*. It aims to assess the toxicity of *Blighia sapida* (ackee) seeds which contains toxic bioactive compounds, notably hypoglycin A and hypoglycin B, which are known to disrupt metabolic processes in vertebrates. However, information on their molecular effects in aquatic organisms is limited. Juvenile *C. gariepinus* were exposed to varying concentrations (0.0, 30.0, 31.6, 33.3, 35.0, 36.6 mg/L) of the seed powder for 96 hours. The exposure of juvenile *C. gariepinus* to *Blighia sapida* seed powder induces significant stress behaviours such as erratic swimming, loss of reflex, air-gulping, and barbel deformation, which worsened with increasing concentrations. After exposure, Liver tissues were harvested for RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction (RT-qPCR) analysis of tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ). Results revealed significant upregulation of TNF- $\alpha$  and IL-1 $\beta$  gene expression in treated groups compared to the control, indicating dose-dependent inflammatory and stress responses. This aligns with previous findings that *Blighia sapida* phytochemicals can influence gene regulation, immunity, and metabolism which may compromise fish health and productivity in aquaculture systems. Hence, *Blighia sapida* seed should be avoided in close proximity to fish ponds and aquaculture environments.

**Keywords:** *Clarias gariepinus*, Gene expression, Phytochemicals, Behavioural changes, Toxicity.

## INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors globally. It plays an important role in food and nutrition security (FAO, 2022; Tacon and Metian, 2015). In Africa, the African catfish (*Clarias gariepinus*) is one of the most commonly farmed freshwater species. This is due to its fast growth rate, ability to withstand tough environmental conditions, and suitability for high-density farming (FAO, 2022). However, the increasing use of plant-based feed ingredients, agro-industrial by-products, and herbal additives raises concerns about their possible toxic effects on farmed fish species (Adeyemi *et al.*, 2012).

*Blighia sapida*, or Ackee, is a tropical fruit tree that originates from West Africa and can be found throughout Nigeria and other tropical areas (Morton, 1987). While the fruit's arils are safe to eat, the seeds contain toxic non-proteinogenic amino acids, including hypoglycin A and hypoglycin B. These substances disrupt fatty acid  $\beta$ -oxidation and gluconeogenesis, leading to severe metabolic disorders, liver damage, and low blood sugar in mammals (Kean and Hare, 2019). Despite its toxicity, *B. sapida* seed residues can enter water systems through agricultural runoff, improper disposal, or experimental feed use, which could harm aquatic life and farmed species (Adebayo *et al.*, 2015).

In fish, the liver acts as the main organ for breaking down harmful substances, detoxifying, and transforming compounds. This makes it especially vulnerable to toxic damage (Segner, 2007). Scientists widely use molecular immune markers, particularly cytokines, to assess sublethal toxic stress in aquatic organisms. Tumor necrosis

factor-alpha (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) are key players in inflammatory and immune reactions. They serve as sensitive indicators of immune system disruptions due to toxins in fish (Rebl and Goldammer 2018). Changes in the levels of these cytokines can provide early evidence of toxic stress before any physical or cellular abnormalities appear (Bols *et al.*, 2001).

While earlier studies have documented the toxic and histopathological effects of various plant-derived substances on *C. gariepinus* (Adeyemi *et al.*, 2012), little information is available on how this species responds at the molecular and gene expression levels to *B. sapida* seed exposure. Therefore, this study aimed to evaluate how *B. sapida* seed powder affects the expression of TNF- $\alpha$  and IL-1 $\beta$  genes in juvenile *C. gariepinus* thereby providing insights into its potential genotoxic and immunotoxic impacts on aquaculture species.

## MATERIALS AND METHODS

### Fish collection and acclimatization

Three hundred (300) healthy *C. gariepinus* were collected from Research Farm of Aquatem Ado Ekiti, Ekiti state, Nigeria; and transported with an open plastic keg of 50litres filled with pond water to the central laboratory of Fisheries and Aquaculture department laboratory, Adekunle Ajasin University Akungba Akoko, Ondo State. The health status of selected fish was assessed based on the presence or absence of physical injuries and other morphological deformations symptom. The fish were certified healthy by assessment before the commencement of the study. The juvenile catfish were acclimatized for two weeks (14 days) under laboratory conditions. During acclimatization, fish were fed with commercial diet (35% crude protein) twice daily (8:00am and 4:00pm). Water quality parameters were maintained within acceptable limits for catfish culture (temperature 26–28°C, pH 6.5–7.5, dissolved oxygen  $\geq 5$  mg/L). Feeding was discontinued 24 hours before the commencement of the experiment. Fish showing any signs of disease, malformation, or behavioural abnormality were excluded from the study.

### Preparation of *Blighia sapida* seed powder

Mature seeds of *B. sapida* were collected from healthy tress at the Ibaka market in Akungba Akoko. The seeds were thoroughly washed to remove debris, air dried at room temperature (25°C). Dried seeds were grinded into fine powder using a laboratory blender. The seed powder was stored in an airtight container until use.

### Experimental Design and toxicant concentration

A completely randomized design (CRD) was used in this study. Fish were randomly distributed into six treatments groups of 18 treatment tanks (75x45x45 cm) filled with 30 litres unchlorinated water with 10 fish per treatment. Each treatment groups contains appropriate concentration of each toxicant, T1 (0 mg), T2 (30mg/L), T3 (31.6 mg/L), T4 (33.3 mg/L), T5 (35.0 mg/L), and T6 (36.6 mg/L). These concentrations were selected based on preliminary range-finding tests. Fish were exposed for 96 hours and monitored for behavioural changes and mortality.

### Sample Collection and RNA Extraction

At the end of the exposure period, liver tissues were excised and preserved in RNA stabilization solution. Total RNA was extracted using a commercial RNA extraction kit. RNA purity and concentration were assessed using spectrophotometry.

### cDNA Synthesis and Quantitative RT-PCR

Complementary DNA (cDNA) was synthesized using a commercial reverse transcription kit. Gene expression of TNF- $\alpha$  and IL-1 $\beta$  was quantified using RT-qPCR. GAPDH was used as the housekeeping gene for normalization.

## Statistical Analysis

Data were expressed as mean  $\pm$  standard error (SE). Differences among groups were analysed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Statistical significance was accepted at  $p < 0.05$ .

## RESULTS

### Behavioural responses *C. gariepinus* exposed to *Blighia sapida*

The behavioural responses *C. gariepinus* exposed to *Blighia sapida* are presented in Table 1. Fish exposed to different concentrations of *Blighia sapida* displayed behavioural responses such which as motionless state, barbell deformation, loss of reflex, air gulping, and erratic swimming increase as the concentration increases. As the experiment progressed, the test fish loss balance characterized by fish swimming backwards and highly reduced activity during which they remained in vertical position, this period was followed by death.

**Table 1: Behavioural response of juvenile *C. gariepinus* exposed to *Blighia sapida* seed powder**

Behavior Exposure	24hours (mg/L)						48 hours (mg/L)						72hours (mg/L)						96hours (mg/L)					
	0	30	31.6	33.3	35.0	36.6	0	30	31.6	33.3	35.0	36.6	0	30	31.6	33.3	35.0	36.6	0	30	31.6	33.3	35.0	36.6
Hyperactive	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
Air gulping	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
Erratic swimming	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Barbell deformation	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Loss of reflex	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+

Key: + represent present, - represent absent

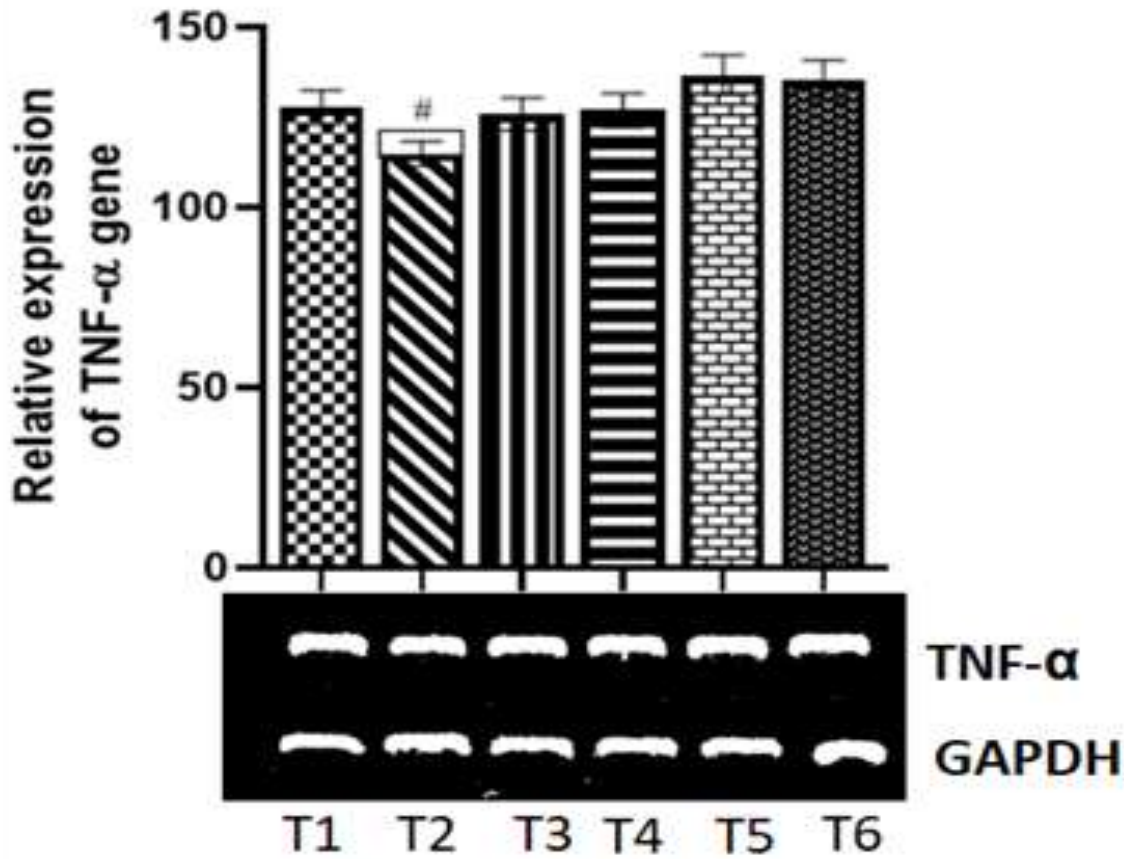
### Expression of TNF- $\alpha$ and IL-1 $\beta$ Genes in the Liver of Juvenile *Clarias gariepinus*

Relative expression of TNF- $\alpha$  and IL-1 $\beta$  genes in the liver of juvenile *Clarias gariepinus* exposed to varying concentrations of *Blighia sapida* seed powder for 96 h is presented in Table 2 and visually represented in Figures 1 and 2.

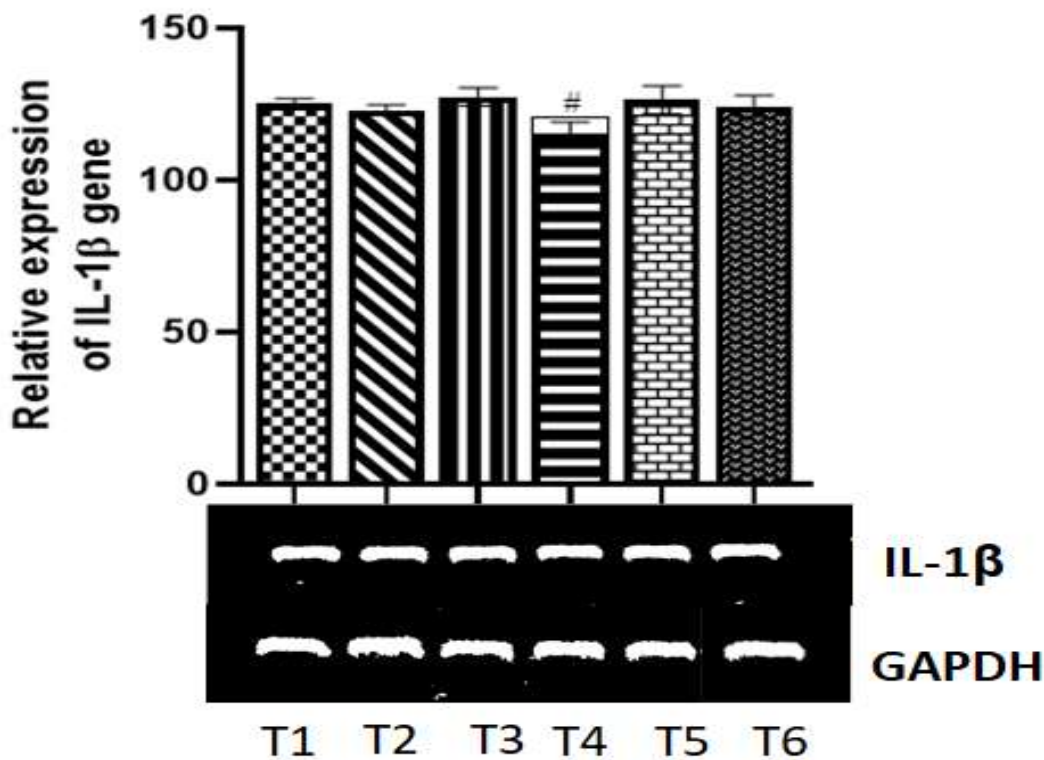
**Table 2: Relative mRNA Expression of TNF- $\alpha$  and IL-1 $\beta$  in the Liver of Juvenile *Clarias gariepinus* Exposed to *Blighia sapida* Seed Powder for 96 hours**

Treatment	Concentration (mg/L)	TNF- $\alpha$ Expression (Mean $\pm$ SE)	IL-1 $\beta$ Expression (Mean $\pm$ SE)
T1	0.0	127.65 $\pm$ 3.35 <sup>a</sup>	125.25 $\pm$ 1.07 <sup>a</sup>
T2	30.0	114.86 $\pm$ 2.42 <sup>b</sup>	122.98 $\pm$ 1.21 <sup>a</sup>
T3	31.6	125.85 $\pm$ 3.25 <sup>a</sup>	127.43 $\pm$ 2.14 <sup>a</sup>
T4	33.3	126.88 $\pm$ 3.31 <sup>a</sup>	115.08 $\pm$ 2.94 <sup>b</sup>
T5	35.5	136.76 $\pm$ 3.91 <sup>c</sup>	126.45 $\pm$ 3.27 <sup>a</sup>
T6	36.6	135.30 $\pm$ 3.86 <sup>c</sup>	124.30 $\pm$ 2.48 <sup>a</sup>

Values are expressed as Mean  $\pm$  Standard Error (SE). Means with different superscript letters within the column differ significantly at  $P < 0.05$ .



**Fig. 1:** Relative expression of TNF- $\alpha$  gene in the liver of juvenile *Clarias gariepinus* exposed to *Blighia sapida* seed powder for 96 h.



**Fig. 2:** Relative expression of IL-1 $\beta$  gene in the liver of juvenile *Clarias gariepinus* exposed to *Blighia sapida* seed powder for 96 h.

Both genes exhibited significant modulation in response to exposure, indicating molecular stress and immune activation. Error bars represent standard error of the mean, and different superscripts indicate significant differences among treatments ( $p < 0.05$ ).

### TNF- $\alpha$ Gene Expression

TNF- $\alpha$  expression showed a gradual increase with increasing concentrations of the toxicant, with the highest value observed at 35.5 mg/L (T5). The lowest expression was recorded at 30 mg/L (T2). Statistical analysis indicated significant differences among treatment groups ( $P < 0.05$ ). The results indicate a dose-dependent increase in TNF- $\alpha$  transcript levels, suggesting activation of pro-inflammatory pathways in response to *B. sapida* exposure.

### IL-1 $\beta$ Gene Expression

Similarly, IL-1 $\beta$  gene expression was significantly upregulated in treated groups compared to the control. The expression pattern showed a gradual increase across treatments, with higher expression levels recorded in T3, T5, and T6 groups. Although IL-1 $\beta$  expression increased with exposure, the magnitude of induction was comparatively lower than that observed for TNF- $\alpha$ .

## DISCUSSION

Behavioural changes were observed in *C. gariepinus* exposed to different concentrations of *Blighia sapida* seed powder. These behaviours included loss of reflex, gulping for air, erratic swimming, and barbel deformation. These effects may result from interference with neurotransmitters in the nervous system, which caused the fish to experience breathing difficulties. Stressed fish were seen lying at the bottom of the tank and eventually died. Toxic exposure affects swimming behaviour, which serves as a sign of stress experienced due to the presence of *Blighia sapida* seed powder in the water where *C. gariepinus* was exposed for 96hr. The behavioural responses seen in this study were similar to those reported by Alkahemal-Balawi *et al.*, 2011 when *Cyprinus carpio* was exposed to fenthion. Likewise, Adewumi *et al.*, 2018 observed similar behaviours in *C. gariepinus* juveniles exposed to *chlorpyrifos* and DD force and Adene *et al.*, 2023 on exposure of *C. gariepinus* to pretiolachor pyribenzoxim.

This research investigated how *Blighia sapida* seed powder affects the hepatic expression of pro-inflammatory cytokine genes specifically tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) in young *Clarias gariepinus*. The liver serves as a key organ for metabolism, detoxification, immune monitoring, processing of foreign compounds, and managing oxidative stress in fish (Segner, 2007). Therefore, changes in liver cytokine gene activity serve as important molecular markers for immune and genetic damage induced by harmful substances (Bols *et al.*, 2001).

Results demonstrated notable changes in TNF- $\alpha$  and IL-1 $\beta$  gene activity in fish treated with *B. sapida* seed powder compared to untreated controls ( $P < 0.05$ ). TNF- $\alpha$  expression increased progressively as toxicant concentrations rose, indicating an inflammatory response dependent on dose levels and triggered by harmful components in the seeds. Likewise, IL-1 $\beta$  expression was substantially increased in exposed groups, reflecting activation of innate immune defenses likely linked to cellular damage and stress reactions (Secombes and Wang, 2012; Whyte, 2007).

Tumor necrosis factor-alpha (TNF- $\alpha$ ) exhibited higher expression levels and greater sensitivity to *Blighia sapida* exposure compared to interleukin-1 beta (IL-1 $\beta$ ), suggesting that TNF- $\alpha$  is a more responsive biomarker of hepatic inflammation in juvenile *Clarias gariepinus*. TNF- $\alpha$  is recognized as an early-response cytokine involved in the initiation of inflammatory cascades and apoptosis, whereas IL-1 $\beta$  primarily functions in amplifying inflammatory signaling pathways (Secombes and Wang, 2012).

The pronounced induction of TNF- $\alpha$  suggests that *B. sapida* seed constituents may activate primary inflammatory signaling pathways in the liver, potentially leading to hepatocellular injury, immune dysregulation, and impaired physiological performance in exposed fish. The upregulation of IL-1 $\beta$  further confirms the

activation of innate immune responses, although its comparatively lower expression indicates a secondary or downstream response relative to TNF- $\alpha$  (Rebl and Goldammer, 2018).

The upregulation of these cytokines is consistent with earlier research showing elevated inflammatory gene activity in fish exposed to toxic plant materials and environmental pollutants. For instance, Zhang *et al.* (2019) found increased hepatic TNF- $\alpha$  and IL-1 $\beta$  levels in zebrafish exposed to pesticide residues, and Adeyemi *et al.* (2020) observed comparable gene expression changes in *C. gariepinus* exposed to plant-derived toxic compounds. Pro-inflammatory cytokines like TNF- $\alpha$  and IL-1 $\beta$  are recognized as early indicators of inflammatory and genetic stress, with prolonged elevation often linked to liver cell damage, programmed cell death, and tissue breakdown (Secombes and Wang, 2012).

The observed changes in gene expression may result from hypoglycin A and hypoglycin B toxic amino acid compounds found in *B. sapida* seeds which interfere with fatty acid breakdown and disrupt mitochondrial energy production, causing oxidative stress and cellular malfunction (Bowen-Forbes and Minott, 2011; Jang *et al.*, 2015). These metabolic disruptions trigger inflammatory signaling pathways, including nuclear factor kappa B (NF- $\kappa$ B), which increases cytokine gene transcription such as TNF- $\alpha$  and IL-1 $\beta$  (Lawrence, 2009).

Additionally, the liver gene expression profiles in this study indicate that *B. sapida* seed powder has immunotoxic and potentially genotoxic effects on juvenile catfish. Similar outcomes have been documented in fish exposed to heavy metals and pesticides, where increased cytokine expression occurred alongside DNA damage, oxidative stress, and structural abnormalities in liver tissue (Kumar *et al.*, 2018; Li *et al.*, 2016). These results explain the potential hazards of *B. sapida* toxicity to aquatic species.

From an aquaculture management standpoint, exposure to *B. sapida* seeds via contaminated water or unintentional incorporation into fish feed could negatively impact fish health, development, and survival. Chronic inflammatory responses may reduce metabolic performance, weaken immune function, and heighten susceptibility to infections, ultimately jeopardizing sustainable aquaculture operations (FAO, 2016; Ashley, 2007).

## CONCLUSION

The study reveals that exposure to *B. sapida* (ackee) seed powder significantly affects the behaviour and gene expression of juvenile *C. gariepinus* (African catfish). The toxic effects include observable behavioural changes such as air gulping, erratic swimming, and barbel deformation, which intensify with higher concentrations. This study also demonstrates that *B. sapida* seed powder significantly alters hepatic gene expression of TNF- $\alpha$  and IL-1 $\beta$  in juvenile *Clarias gariepinus*. The upregulation of these pro-inflammatory cytokines indicates that *B. sapida* seed powder induces inflammatory and potential genotoxic stress in the liver. These findings highlight the toxicological risks associated with the use or environmental presence of *B. sapida* seeds in aquaculture systems. Further studies integrating histopathology, oxidative stress biomarkers, and DNA damage assays are recommended to fully elucidate the mechanisms of toxicity.

## REFERENCES

1. Adebayo, E. A., Olanrewaju, J. D., and Bello, A. (2015). Environmental impact of agricultural waste on aquatic ecosystems in Nigeria. *Environmental Monitoring and Assessment*, 187(3), 1–12.
2. Adeyemi, J. A., Ajani, E. K., and Omitoyin, B. O. (2012). Toxicological effects of plant extracts on African catfish (*Clarias gariepinus*). *Journal of Applied Sciences Research*, 8(2), 1234–1240.
3. Adeyemi, J. A., Ajani, E. K., and Omitoyin, B. O. (2020). Toxicological effects of plant-derived extracts on African catfish (*Clarias gariepinus*). *Aquaculture Research*, 51(4), 1587–1596.
4. Alkahemal-Balawi, H. F. (2011). Toxicity bioassay of lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in *Clarias gariepinus*. *African Journal of Biotechnology*, 10(53), 11039–11047.
5. Ashley, P. J. (2007). Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science*, 104(3–4), 199–235.

6. Bols, N. C., Brubacher, J. L., Ganassin, R. C., and Lee, L. E. J. (2001). Ecotoxicology and innate immunity in fish. *Developmental and Comparative Immunology*, 25(8–9), 853–873.
7. Bowen-Forbes, C. S., and Minott, D. A. (2011). Tracking hypoglycin A and B in ackee (*Blighia sapida*) fruit and products. *Journal of Agricultural and Food Chemistry*, 59(8), 386–394.
8. FAO. (2016). The state of world fisheries and aquaculture. Food and Agriculture Organization of the United Nations.
9. FAO. (2022). The state of world fisheries and aquaculture 2022. Food and Agriculture Organization of the United Nations.
10. Jang, H., Kim, J., and Kim, Y. (2015). Hypoglycin A-induced inhibition of fatty acid oxidation and mitochondrial dysfunction. *Toxicology Letters*, 232(2), 267–274.
11. Kean, S., and Hare, K. (2019). Hypoglycin toxicity and ackee poisoning. *Clinical Toxicology*, 57(3), 151–158.
12. Kumar, N., Srivastava, S., and Singh, D. (2018). Toxicant-induced oxidative stress and inflammatory responses in fish. *Environmental Toxicology and Pharmacology*, 62, 1–9.
13. Kumar, V., Abbas, A. K., and Aster, J. C. (2018). Robbins basic pathology (10th ed.). Elsevier.
14. Lawrence, T. (2009). The nuclear factor NF- $\kappa$ B pathway in inflammation. *Cold Spring Harbor Perspectives in Biology*, 1(6), a001651.
15. Li, Z., Xu, J., and Yang, Y. (2016). Heavy metal-induced immunotoxicity in fish: Cytokine expression and DNA damage. *Environmental Toxicology and Chemistry*, 35(8), 2001–2010.
16. Makkar, H. P. S., Francis, G., and Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects on fish. *Aquaculture*, 271(1–4), 1–20.
17. Morton, J. (1987). Ackee. In *Fruits of warm climates* (pp. 269–271). Creative Resource Systems.
18. Rebl, A., and Goldammer, T. (2018). Under control: The innate immunity of fish from a regulatory perspective. *Fish and Shellfish Immunology*, 77, 1–6.
19. Secombes, C. J., and Wang, T. (2012). The innate and adaptive immune system of fish. In *Fish Physiology* (Vol. 30, pp. 3–68). Academic Press.
20. Segner, H. (2007). Fish cell lines as tools in aquatic toxicology. *Biochemical and Biophysical Research Communications*, 356(1), 1–6.
21. Tacon, A. G. J., and Metian, M. (2015). Feed matters: Satisfying the feed demand of aquaculture. *Reviews in Fisheries Science and Aquaculture*, 23(1), 1–10.
22. Whyte, S. K. (2007). The innate immune response of finfish—a review of current knowledge. *Fish and Shellfish Immunology*, 23(6), 1127–1151.
23. Zhang, Q., Li, J., and Wang, Z. (2019). Pesticide-induced inflammatory responses in zebrafish liver. *Environmental Science and Pollution Research*, 26(4), 3451–3462.