Effect of Thymol oil on the growth performance and survival of African catfish (*Clarias gariepinus*) challenged with *Aeromonas hydrophilia*

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Abstract
Intensive aquaculture systems are associated with outbreak of diseases that cause economic losses. Over the years, diseases have been managed with antibiotics that are presently linked to environmental contamination and deposition of residual antibiotics in fish and people that consume such fish. Therefore, Thymol oil, a harmless product with antibacterial properties was used in the production of African catfish, to determine the effects on the growth performance, immune response and survival of the fish challenged with infestation of *Aeromonas hydrophilia.* A 40% protein basal diet was formulated, and Thymol oil (TMO) was added to it at (0, 0.8, 1.1, 1.4 and 1.7%) respectively making diets/treatments 1 to 5. The diets were fed in triplicate to African catfish juveniles to apparent satiation, twice daily for 70 days. Results showed that dietary Thymol oil significantly improved the fish growth performance in relation to fish fed diet without the oilcontrol diet). For instance, the mean weight gain increased consistently from 9.62 g in fish fed the control diet to 22.8g in fish fed diet with 1.70% thymol oil. Contrarily, the dietary thymol oil significantly reduced white blood cells and the carcass cholesterol. The WBC decreased from 8.70 10^3 mm^3 in fish fed the control diet to 4.80 10^3 mm^3 in fish fed diet with 1.70 % thymol oil. Also, the cholesterol decreased consistently from 85 mg/dl in fish fed the control diet to 65 mg/dl in the fish fed diet with 1.70% thymol oil. The biochemical profile of the fish revealed that dietary thymol oil significantly increased the glutathione peroxidase, serum albumin, globulin and serum protein with increasing levels of the oil in the diets. Also, dietary thymol oil improved the survival rate of the fish when challenged with the infestation of *Aeromonas hydrophilia*. In conclusion, dietary thymol oil improved the growth performance, immunity and survival of African catfish. However, more research is needed to determine the optimum level of the thymol oil for the best growth performance of the fish

Key words: African catfish, thymol oil, growth performance, immune system, challenge test**,** *Aeromonas hydrophilia*

Introduction

Expansion of aquaculture has been rapid and consistent over time, because aquaculture assumes a significant role as a viable and cost-effective alternative to the declining capture fisheries, for the provision of animal protein (Moyo and Rapatsa, 2021). Intensive aquaculture systems have resulted in rapid and wide spread of diseases within the facilities. Hence the sector faces a lot of challenges inform of disease outbreaks that causes substantial economic losses. Although a variety of antibiotics are currently employed for the treatment of fish diseases, their utilization presents significant concerns related to human health and the environment (Hafsan *et al.*, 2022). The discharge of antibiotics into natural aquatic environments has the potential to induce the development of antibiotic-resistant strains, alter the composition of indigenous flora and fauna, and undergo biomagnification within the food chain (Hafsan *et al* 2022). Additionally, the accumulation of antibiotics and their derivatives in the tissues of aquatic organisms poses a potential risk to human health when consumed (Cabello 2006; Manage 2018), Hence the use of non-harmful natural substances as substitutes for chemical agents is a potentially effective approach for bolstering fish growth, immune responses and management of ill-conditions (Dawood, 2021; Vijayaram *et al*, 2022). Similarly, Agarwal and Singh, (1999); Devasagayam and Sainis, (2002) reported that enhancing the defensive capabilities of fish by administering natural plant products as a preventive measure is a highly promising approach for managing illnesses in aquaculture.

In the field of fish culture, several active components have been identified, including *Allium sativum* (garlic), *Zingiber officinale* (ginger), Curcuma longa Linn (turmeric), *Trigonellfoenum graecum* (fenugreek), and others. These components have been documented to elicit various effects, such as promoting higher growth rates (Shalaby *et al*., 2006), stimulating appetite, reducing stress, and enhancing immune functions (Dorucu *et al*., 2009; Ergün *et al*, 2011). Thymol, also known as 2-isopropyl-5-ethylphenol (TYM), is the predominant phenolic component present in the essential oil derived from *Thymus vulgaris*. This monoterpene is commonly found in several herbal plants and essential oils (EOs). Thyme is thought to be the main source of thymol, a compound that has been used in medicine for its antifungal, antispasmodic, antiseptic, carminative, expectorant, sedative, antiviral, antihelminthic, antioxidative, diaphoretic, and antibacterial effects (Al-Shahrani *et al.*, 2017). Alagawany *et al.* (2021) conducted several studies to examine the impact of Thymol, when used as a food supplement, on the growth, hemology, and immunity of fish. Nevertheless, there exists a scarcity of data on the use of Thymol in African catfish culture. Therefore, this study investigated the potential of dietary thymol on the growth and survival of African catfish challenged with *Aeromonas hydrophilia* infestation.

Aeromonas hydrophilia was used as a pathogenic organism, because of its capacity to survive in both aerobic and anaerobic conditions. It is recognized for its ability to cause infections in various organisms, including fish, reptiles, amphibians, and humans (Janda and Abbott, 2010).

Aeromonas hydrophila is responsible for inducing several diseases in fish, commonly referred to as Motile Aeromonas Septicemia (MAS), *hemorrhagic septicemia*, and red-sore disease (Parichat and Pongsak, 2020).

Materials and methods

Feed ingredients procurement and experimental diets

Thymol oil was purchased from Hashems Roastery and Markets, Permari Brand USA and feed ingredients were procured from Farm Support Service Limited, Akure Ondo State. Five (5) isonitrogenous (40% crude protein) diets were prepared containing fish meal, groundnut cake, soya bean meal, yellow maize, lysine, methionine, fish oil and carboxyl methyl cellulose (Table 1a). All the feed ingredients were blended into powdery form not more than 0.3mm in particle size, divided into five (5) parts according to the quantity of each ingredient in respective diets and supplemented with different levels of thymol except the control diet. Thymol was added in levels ranging from 0.0%, 0.8%, 1.1%, 1.4%, 1.7%, thereafter, warm water was added to each portion, homogenously mixed to form dough and pelleted to get uniform size pellet (2mm) to form diets with different inclusion levels of thymol. Each diet (D1, D2, D3, D4, and D5) was labelled and stored in different polythene bags at -4^{0}C before use.

Fish procurement and conditioning

Two hundred (200) juveniles of *Clarias gariepinus* were purchased from a reputable hatchery and were acclimatized to experimental condition at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology of the Federal University of Technology, Akure Ondo State for seven (7) days in a rectangular glass tank and were fed with a commercial feed 2mm pellet size.

Experimental design

The experiment was carried out at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology of the Federal University of Technology, Akure Ondo State. The experiment was designed as completely randomized. Fifteen (15) glass tanks were arranged in triplicates. Twelve (12) juveniles of approximately the same length and weight was weighed and randomly distributed into the glass tanks (70cm x 50cm x 50cm) containing 45 litres of water.

Feeding trials and data collection

The feeding trials lasted for 70 days, fish were fed with the diets to apparent satiation two times daily (08:00-10:00 hours and 16:00-18:00 hours). Fish were weighed bi-weekly with a METLAB balance (PB8001) to collect data for growth performance evaluation. The weight values were used to calculate growth performance indices, according to Nwanna and Tope-Jegede (2017).

Survival rate (SR, %) = 100 × Nf / Ni; where Nf and Ni are the number of catfish at the beginning and end of the experiment, respectively, in each treatment

i. Weight Gain (Wg) (g) = Wf - WI; where; Wf = Final weight; Wi= Initial weight
ii. Daily weight gain (DWG) (g) = Mean weight gain / days
iii. Specific growth rate (SGR) (g/day) = 100 (Ln Wf – Ln Wi) / T, where Wf and Wi are the final and initial weight, respectively, and T is the number of days in the feeding period
iv. Feed conversion ratio (FCR) = dry feed intake (g) / weight gain (g)
v. Protein efficiency ratio (PER) = weight gain (g) / protein intake (g)

Water quality parameters

Optimum water quality was maintained throughout the experiment by siphoning the uneaten feed and changing the water in the tanks twice in a week while the water quality parameters such as temperature, pH and dissolved oxygen were monitored weekly.

Proximate and mineral composition of the experimental feeds and fish

Three samples of the different feeds were analyzed for proximate composition, and three catfish were randomly collected from each tank and the whole-body was analyzed for proximate and minerals composition using standard methods of (AOAC (Association of Official Analytical Chemists International), 2006). Briefly, the ash digest of each sample, was subsequently transferred into a 100-ml volumetric flask using de-ionized distilled water and brought to the desired volume. The diluent was introduced into the atomic absorption spectrophotometer (AAS) via the aspiration tube. The mineral elements were analyzed individually at their specific wavelengths using the corresponding hollow cathode lamp, together with the right combination of fuel and oxidant.

Haematological profile and serum biochemical analysis

At the end of the experiment, 24 h after the last feeding, 6 randomly selected fish from each treatment group were anaesthetized using clove oil at 95mg/L (Adeshina *et al.* 2016) before the blood was taken to reduce handling stress. Blood samples were collected from the caudal vein with a 2-ml sterile hypodermic syringe and carefully transferred into 1.5mL sterile ethylene diamine tetra acetic acid (EDTA) heparinized tubes and after standing for 12 h at 4° C, centrifuged at 850 × g for 15 min at 4 °C. The supernatant, i.e. serum, was collected and stored at -8° C until enzyme activity assays were performed. Haemoglobin, pack cell volume, red blood cells and white blood cell counts were estimated by the Cyanmethemoglobin method according to the methods of (Rusia and Sood, 1992). Assay of glutathione peroxidase (GPx) activity was determined according to the method adopted by Rotruck *et al*. (2005). Total protein was determined using the enzymatic colorimetric method and measurement was done at 540nm. Serum cholesterol was evaluated using enzymatic-colorimetric methods (Naito 1984) with the aid of specific kits (BT29 4QY: RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom). Serum albumin and globulin were determined according to the methods of Wotton and Freeman (1982). And serum globulin = Total protein minus serum albumin (g/dl).

Challenge Test by infestation with *Aeromonas hydrophilia*

A specimen of *Aeromonas hydrophilia* was obtained from the laboratory of the Department of Microbiology at the Federal University of Technology, Akure. At the conclusion of the 70-day feeding trial, the total population of catfish in

each treatment group was standardized to 20 fish. The fish were subjected to a challenge by means of intraperitoneal injection of 1.5 ml (10^5 diluent) of *Aeromonas hydrophilia* into the muscle. Then physical changes and mortality were observed for ten days.

Statistical Analysis

Statistical analysis was performed using SPSS (version 21) software. Data were presented as mean ±SD. All the data were tested for normality (Kolmogorov-Smirnov test). Data were subjected to one way analysis of variance (ANOVA). The significant means was separated by Duncan's multiple range test at 95% confidence level ($P = 0.05$). (Duncan, 1955)

Results

Proximate composition of the experimental diets (Table 1b) shows closely related values confirming that the diets were well formulated and standard. Therefore, any changes in the fish growth performance and physiological properties could be attributed to the effects of the supplemental Thymol oil in the diets.

The growth and nutrient utilization indices of the experimental fish are presented in Table 2, which indicated that fish final weight, weight gain, specific growth rate, food conversion ratio and protein efficiency ratio improved significantly with increasing levels of the Thymol oil in the diets, in comparison with the values from the fish fed diet without the Thymol oil, the control diet.

In Table 3, is the blood profile of the experimental fish, which revealed that the supplemental Thymol oil did not affect the blood PCV, haemoglobin and red blood cells, but significantly reduced the white blood cells with increasing levels of the oil in the diets. This may mean that the thymol oil was able to boost the immune response of the fish.

The biochemical profile of the fish carcass (Table 4) showed that the Thymol oil significantly improved the glutathione peroxidase, serum albumin, globulin and serum protein with increasing levels of the oil in the diets. However, the dietaryThymol oil significantly reduced the fish cholesterol with increasing levels of the oil in the diets.

Minerals composition of the fish carcass (Table 5) indicated that the Thymol oil did not affect the carcass Ca, Mg and Zn deposition in the fish. Nevertheless, inclusion of the oil in the diets significantly increased the Mn content in the fish with increasing levels of the oil in the diets.

Results of the challenge test of the fish fed the dietary Thymol oil (Table 6) indicated that dietary thymol oil increased the survival rate of the fish when challenged with the infestation of pathogen, *Aeromonas hydrophilia.* Hence after the challenge test with the infestation with *A. hydrophilia* ,fish fed the control diet had 95% mortality, and mortality decreased with increasing levels of the oil such that fish fed diet 5 (1.70%) of the oil had no mortality.

Discussion

Proximate composition of experimental diets

Proximate composition of the experimental fish revealed that the values were closely related, regardless of the TMO concentrations. The parameters also did not show any recognizable trend attributable to the TMO. However, supplementation of the TMO resulted in slight increase in the fat content which also increased with increasing levels of the TMO in the diets Hence, the highest level was recorded for experimental diet 5, while the lowest was recorded for experimental diet 1. The moisture content value obtained was similar to the values indicated by Akinwole *et al.* (2020). The nitrogen free extract (NFE) values obtained was similar to the values obtained by Ochukwo *et al.* (2021).

Growth and nutrient utilization of African catfish fed experimental diets

The positive influence of the dietary Thymol oil on the growth and nutrient utilization of the fish is a good development and supports the work of other researchers. For instance, Khalil *et al*. (2020) documented that Thymol oil increased digestive enzymes activities, nutrients bioavailability regulation of the intestinal microbiota, resulting in decrease of harmful bacteria and improved growth performance and nutrient utilization in *Oreochromis niloticus*. According to Diao *et al*. (2015), incorporation of TMO into animal diets yielded significant enhancements in feed efficiency, nutrient absorption, digestion, and growth trajectory. Similarly, Effiong and Yaro (2019) and Shehzad *et al*. (2019) also demonstrated that dietary Thymol oil significantly improved the growth performance of African catfish, as measured by specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). From another study, Ahmadifar *et al.* (2011) reported that TMO improved carcass fibre, lipids and whole-body protein in juvenile rainbow trout. Also, Zheng *et al*. (2009) asserted that TMO enhanced the growth performance of Channel catfish (*Ictalurus panctatus*). In addition, Amer *et a*l. (2018) reported that incorporation of TMO into the diet of Nile tilapia significantly enhanced the growth performance, while Sónmez *et al*. (2015) described that inclusion of 1.5 g/kg of Thymol oil into the diet significantly enhanced the weight gain and feed conversion ratio of the rainbow trout. Again, results of the present study corroborate those of Zargar *et al*. (2019) which showed that (0.5 mg kg^{-1}) of thymol oil significantly enhanced weight gain, length increase, and specific growth rate of rainbow trout, (*Oncorhynchus mykiss*). However, Hoseini and Yousefi (2018) reported that thymol oil had no significant effect on the final weight, weight gain, and feed efficiency in rainbow trout.

Haematological parameters of the experimental fish

Haematological parameters are important health biomarkers that are used in assessment and monitoring of the health and immune system profiles of fish (Ogueji *et al*., 2018). Ochukwo *et al*. (2021) discussed that the fundamental way to ascertain the physiological health profile of fish is through the blood quality. The result from the present study which showed significant decreasing trend in the WBC with increasing levels of thymol oil agrees with the work of Ochukwo *et al*. (2021) which showed similar effect when African catfish was fed with dietary *T. occidentalis.* Also, the non-significant differences recorded in PCV, Hb and RBC are in consonance with the reports of GENC *et al.* (2020) who fed dietary prebiotics to African catfish.

Biochemical profile of the experimental fish

Components of blood profile are crucial in determining the overall health status of fish tissue (Fazio, 2019). Therefore, fish biochemical parameters measured revealed that dietary thymol oil significantly improved glutathione peroxidase, serum albumin, globulin and total protein. This increment in

Table 1a. Proximate composition of experimental diets

Ingredients	Diet 1 0% TMO	Diet 2 0.8% TMO	Diet 3 1.1% TMO	Diet 4 1.4%TMO	Diet 5 1.7% TMO
Fish meal (65% CP)	25.8	25.8	25.8	25.8	25.8
Soybean meal (45% CP)	25.0	25.0	25.0	25.0	25.0
Groundnut cake (48% CP)	25.0	25.0	25.0	25.0	25.0
Yellow maize	16.6	15.8	15.5	15.2	14.9
Fish oil	6.00	6.00	6.00	6.00	6.00
Methionine	0.40	0.40	0.40	0.40	0.40
Lysine	0.20	0.20	0.20	0.20	0.20
Cellulose	1.00	1.00	1.00	1.00	1.00
Thymol oil	0.00	0.80	1.10	1.40	1.70

Vitamin and minerals supplied by Vitamix fish premix: 50,000,000 I.U, Vitamin D3, 1,600,000 I.U, Vitamin E 15,000, thiamine, 2000 mg, riboflavin, 7500 mg, vitamin B6, 3000 mg, vitamin B12, 20 mg, vitamin K, 2000mg, vitamin C, 100,000mg, nicotinic acid, 10,000mg, folic acid, 600 mg, biotin, 0.5 mg, BHT, 125,000 mg, manganese, 100,000 mg, iron, 100,000 mg, zinc, 40,000 mg, copper, 5000 mg, iodine, 500 mg, cobalt, 250 mg, selenate, 125 mg, zinc bacitracin, 15,000 mg, chloride, 20, 000 mg.

Table 1b. Proximate composition of experimental diets

	Diet 1 0% ThyO	Diet 2 0.8% ThyO	Diet 3 1.1% ThyO	Diet 4 1.4% ThyO	Diet 5 1.7% ThyO
Protein	42.3	42.1	42.5	42.2	42.2
Fat	10.7	11.1	11.2	12.0	12.8
Fibre	5.84	5.37	6.29	5.15	5.01
Ash	9.45	9.00	9.16	9.98	9.58
Moisture	10.2	10.6	10.5	10.4	10.2
NFE	21.5	21.8	20.4	20.3	20.2

Means of 3 values on the same row are not significantly different (P>0.05)

NFE means Nitrogen free extract

Table 2. Growth and nutrient utilization of African catfish fed experimental diets

	Diet 1 0% TMO	Diet 2 0.8% TMO	Diet 3 1.1% TMO	Diet 4 1.4% TMO	Diet 5 1.7% TMO
Initial Wt (g)	6.28±0.05	6.32±0.08	6.28±0.09	6.24±0.01	6.33±0.18
Final Wt (g)	15.9^{a} ±0.12	24.4^{b}±0.82	24.8^{b}±0.10	26.9^{c}±0.49	29.1^{d}±0.24
Wt. gain (g)	9.62^{a}±0.14	18.1^{b}±0.90	18.5^{b}±0.21	20.7^{c}±0.34	22.8^{d}±0.24
SGR (g/day)	1.66^{a}±0.01	2.41^{b}±0.03	2.45^{b}±0.02	2.61^{c}±0.00	2.72^{d}±0.02
FCR	4.18^{e}±0.02	3.94^{d}±0.01	3.87^{c}±0.00	3.54^{b}±0.01	3.25^{a}±0.02
PER	4.32^{a}±0.03	4.66^{b}±0.02	4.70^{c}±0.01	4.72^{c}±0.01	4.73^{c}±0.02

Means values on the same row with similar superscripts are not significantly different (P>0.05)

Table 3. Blood profile of fish fed the experimental diets

	Diet 1 0% TMO	Diet 2 0.8% TMO	Diet 3 1.1% TMO	Diet 4 1.4% TMO	Diet 5 1.7% TMO
PCV	32.0±0.04	33.0±0.05	34.0±0.06	33.0±0.08	35.0±0.01
Hb	10.7±0.05	11.1±0.07	10.70±0.08	10.9±0.05	11.7±0.02
RBC (10^3 mm^3)	3.50±0.03	3.60±0.05	3.45±0.06	3.20±0.07	3.90±0.07
WBC (10^3 mm^3)	8.70^{e}±0.05	6.10^{d}±0.07	5.80^{c}±0.08	5.40^{b}±0.05	4.80^{a}±0.03
Neutrophil	58.0±0.04	60.0±0.05	61.0±0.07	61.0±0.08	60.0±0.03
Lymphocyte	40.0±0.05	39.6±0.06	38.9±0.03	39.0±0.07	40.0±0.01

Means values on the same row with no superscripts are not significantly different (P>0.05)

Table 4. Biochemical profile of the experimental fish

	Diet 1 0% TMO	Diet 2 0.8% TMO	Diet 3 1.1% TMO	Diet 4 1.4% TMO	Diet 5 1.7% TMO
Glutathione peroxidase (mg/dl)	51.0^{a}±0.01	52.0^{a}±0.05	54.0^{ab}±0.04	56.0^{bc}±0.03	58.0^{cd}±0.01
Serium Albumin (g/dl)	3.80^{a}±0.02	3.90^{a}±0.03	4.00^{ab}±0.05	4.30^{c}±0.01	5.00^{d}±0.02
Cholesterol (mg/dl)	85.0^{d}±0.05	81.0^{c}±0.03	72.0^{b}±0.05	70.0^{b}±0.04	65.0^{a}±0.06
Serum globulin (g/dl)	3.30^{a}±0.06	3.50^{a}±0.03	3.60^{b}±0.01	4.20^{b}±0.02	4.30^{bc}±0.05
Serum proteing/dl)	7.30^{a}±0.04	7.32^{a}±0.03	7.50^{b}±0.01	7.50^{b}±0.04	7.70^{c}±0.05

Means values on the same row with similar or no superscripts are not significantly different (P>0.05)

Table 5. Mineral composition of the experimental fish (carcass)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0% TMO	0.8% TMO	1.1% TMO	1.4% TMO	1.7% TMO
Calcium (mg/g)	29.7±0.04	30.0±0.02	29.8±0.05	30.3±0.06	30.4±0.03
Magnesium (mg/g)	11.7±0.05	11.8±0.07	11.5±0.03	11.3±0.02	11.8±0.07
Zinc (µg /g)	2.22±0.01	2.23±0.03	1.99±0.05	2.03±0.08	2.00±0.03
Mn (µg /g)	1.05^{a}±0.05	1.05^{a}±0.06	1.17^{b}±0.01	1.42^{c}±0.04	1.46^{d}±0.01

Means values on the same row with similar or no superscripts are the same (P> 0.05)

Table 6. Challenge test of with *A. hydrophilia* infestation of *C. gariepinus* fed dietary thymol oil

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	0% TMO	0.8% TMO	1.1% TMO	1.4% TMO	1.7% TMO
Number of fish	20	20	20	20	20
Type of inoculate					
(1.5ml/L of water)	*A.hydrophilia*	*A.hydrophilia*	*A. hydrophilia*	*A. hydrophilia*	*A. hydrophilia*
Period of test	10 days	10 days	10 days	10 days	10 days
Mortality (%)	95	50	15	15	0

the biochemical parameters obtained from the present study supports the work of Yousefi *et al*. (2018) which reported similar increase in the biochemical profile of common carp fed dietary thymol oil. Similarly, the results from the present study are consistent with those reported by (Gabriel *et al*., 2021; Nindum *et al*., 2022). In another development, reduction of carcass cholesterol by dietary thymol oil is consistent with the works of Nwanna *et al.* (2014) which showed reduced cholesterol levels in African catfish fed dietary plantain (*Musa paradisiaca)* peels. Similarly, Nwanna and Tope-Jegede (2017) reported the same trend in African catfish fed dietary *Lactobacilus plantarum.* However, studies by (Abou-Zeid *et al.,* 2023) showed increase in cholesterol contents of Nile tilapia fed dietary thymol oil. This may suggest that dietary influence of Thymol oil may differ in different fish species.

Mineral composition of the fish carcass

Carcass minerals deposition in the fish (Ca, Mg and Zn) did not differ significantly, except in Mn which significantly increased with increasing levels of the dietary Thymol oil. The incremental values of Mn observed from the present study is consistent with the report of Ma *et al.* (2015) and Nie *et al*. (2016) that expressed higher Mn deposition in Turbot (*Scophthalmus maximum*) and Cobia (*Rachycentron canadum)* juveniles respectively. Similarly, (Ahmadifar et al., 2014; Kong *et al*., 2021) reported increase in Mn deposition in Great sturgeon (*Huso huso*) fed dietary thymol oil. Also, Kong et al (2021) described increase in Mn content of *Channa argus* fed dietary thymol. However, the report from the present study that dietary thymol increased the Mn deposition is in disagreement with the report by Nwanna and Oni (2018) which discussed no significant changes in manganese deposition in African catfish fed diet with different ratios of Calcium and Phosphorus.

Response of fish fed dietary thymol oil and challenged with *Aeromonas hydrophilia.*

Intensive aquaculture is associated with stress that could cause depression, compromise fish immunity and predisposes them to infections, disease conditions and economic losses (Abdel *et al.*, 2019). Immunodeficiency may also arise due to indiscriminate use of antibiotics, failure of vaccinations, and infections. Therefore, the use Thymol oil improved fish immunity, resistance and survival of African catfish challenged with *A. hydrophila.* From another study Abdelhamid *et al.* (2021) reported that thymol extract improved resistance of fish to *A. hydrophilia*. In Hafsan *et al.* (2022), Thymal extract enhanced the immune response in rainbow trout and its resistance against *Streptococcus iniae* infection. Furthermore, Morselli *et al.* (2020), demonstrated that dietary thymol increased the survival rate of grass carp exposed to *Aeromonas hydrophilia* by up to 62.5%.

Conclusion

Supplementation of thymol oil in diets enhanced the growth performance, carcass quality, immune system and survival rate of African catfish, *C. gariepinus*. This makes thymol oil (TMO) a suitable feed additive in aquaculture.

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