

Ameliorative Effects of Dietary *Ocimum gratissimum* Leafmeal on African Catfish, *Clarias gariepinus* Subjected to Pathogenic and Transportation-Induced Stress

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Authors' contributions

This work was carried out in collaboration among all authors. Author KGO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author OOS also help in completing the protocol, performed the statistical analysis. Authors AM and AIA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The primary aim of this project is the use of functional feed supplements to ameliorate or reduce the effects of stressors rather than using chemicals that could be harmful and expensive. Specifically, assessment of the ameliorative effect of locally available phyto-genic product, *Ocimum gratissimum* on the negative effects of stress in African catfish production was carried out in a study that lasted for 70 days. Five experimental diets were formulated, at different inclusion levels of *O. gratissimum*, as 0.00 g (control), 0.05 g, 0.10 g, 0.15 g, 0.20 g per 100 g of diet denoted as T1 (control), T2, T3, T4, and T5 respectively. At the end of the feeding trial, stress-induced treatments of experimental fish were done using pathogenic and transportation stressors. Zootechnical parameters in terms of

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growth and nutrient utilization were evaluated. Physiological stress assessment (Aspartate transaminase, AST and Alanine transaminase, ALT) and stress biomarkers (cortisol and glucose) were carried out using liver and blood samples from experimental fish. Results showed that zootechnical parameters were significantly ($P < 0.05$) enhanced with increasing supplementation levels of *O. gratissimum*. There were better performances in the growth and nutrient utilization indices like weight gain, specific growth rate, protein efficiency and feed conversion ratio as the level of *O. gratissimum* was increased to 0.15 mg/g in the diets. Curvilinear trends were recorded with a second degree polynomial regression model depicting a strong relationship between SGR and *O. gratissimum* supplementation in fish diet followed by adverse responses when increased to 0.20 mg/g in the diet. There were significant reduction in stress parameters with increasing supplementation levels of *O. gratissimum* leaf extract in the diets. The best supplementation level of *O. gratissimum* was 0.15 mg/g in T4. From the above deductions, this study confirmed the positive ameliorating effects of *O. gratissimum* on the African catfish during stressful episodes.

Keywords: Stressors; aquaculture; nutrition; phytogetic; supplementation.

1. INTRODUCTION

Over the decade, the African Catfish, *Clarias gariepinus* production has been recorded to be the fastest growing fish farming and local fish production industry in Nigeria [1]. Moreover, aquaculture, which involves rearing of fish at high densities, requires reducing or ameliorating the effects of stressors to maintain healthy growing fish [2]. Stressors in aquaculture are inevitable and results into deleterious episodes in fish rearing and management. Stratagems to reduce or ameliorate the effects of stressors should be paramount in aquaculture. Fish are unavoidably exposed to wide ranges of stress such as overcrowding, transport, handling, size grading, and poor water quality which tend to adversely affect the health of cultured fish. Therefore, the use of indigenous phytogetic products in fish nutrition should be considered to reduce or ameliorate the negative physiological responses in fish [3]. Growth performance and disease resistance conditions of fish are direct reflections of the metabolic and oxidative alterations in the biological system of the body [4]. In this respect, the need for specific nutrients may be increased during infection which could require feeding on diets formulated for optimal immune competence rather than growth and survival only [5,6]. Phytogetic extracts in aquaculture has been discovered to be useful for the prevention and treatment of diseases and also to avoid the indiscriminate use of antibiotics, to prevent the development of resistant strains of pathogenic microorganisms [7,8]. Many authors have studied the effects of different dietary plant extracts on fish to include *Magnifera indica* [9], *Garcinia mangostana* [10], *Chromolaena odorata* [11] and *M. oleifera* flower [4].

The paradigm shift to phytogetic products is as result of the fact that they are a cheaper, non-toxic and renewable substitute to antibiotics. The anti-stress potential of plant extracts may be due to the presence of phenolic and polyphenolic compounds, and these bioactive compounds may render their effects through anti-oxidation of oxidative stress [10]. *O. gratissimum* belongs to the group of plants known as spices. It is of the family Labiatea, genus *Ocimum* and species *gratissimum* [12]. In Nigeria, it is popularly called "effinrin-nla" by the Yorubas, "Ahuji" by the Igbos and "Daidoya" by the Hausas [12]. It is also known by names such as tree basil and shrubby basil in English. Basil leaf has been used widely in the traditional system of medicine in many countries [12]. *O. gratissimum* leaves are rich in minerals and antioxidants. Extracts from the leaves are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α -tocopherol radicals, and inhibit oxidases [13]. Basil leaves are commonly available in the tropical areas and many researchers reported their inclusion in the diets of livestock but there is paucity of such research on aquaculture – related stress in African catfish. Therefore, a better understanding of the mechanism through which dietary *O. gratissimum* as a nutritional supplement influences the physiology of fish exposed to stress in aquaculture is necessary. Furthermore, it is important to investigate the efficacy of dietary *O. gratissimum* supplementation on some physiological responses induced by *A. hydrophila* infections and transportation - induced stress in African catfish, *C. gariepinus* as a means of providing an environmental friendly and sustainable solution that can reduce or ameliorate the negative effects of stressors in African catfish aquaculture.

2. MATERIALS AND METHODS

The leaves of *O. gratissimum* were collected from FUTA Botanical garden, Akure, Ondo State, Nigeria. It was identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure with the aid of Botanical Herbarium of medicinal plants in Africa [12]. Five hundred grams of the powdered leaf were soaked in 1.5 liters of warm water (60°C) for 12 hours. The solution was sieved with a muslin cloth and filtered using No 1 Whatman filter paper. The filtrate was collected in a beaker and concentrated with the aid of a rotary evaporator (Resona, Germany). The extracts were dried under shade for 48 hours and stored in a well labeled container at -4°centigrade in a refrigerator (LG®, Japan) before it was used in the preparation of experimental diets.

2.1 Fish Feed Preparation

The feed ingredients were purchased at Adebom Feedmill, Ondo road, Akure, Ondo State, Nigeria. Five isonitrogenous and isoenergetic diets was formulated to 40% crude protein for *C. gariepinus* (Table 1) fingerlings (National Research Council, 2010). All dietary ingredients were weighed with a weighing top balance (Metler Toledo, PB8001 London). The ingredients were ground to small particle size (approximately 20 µg). Ingredients including *O. gratissimum* leaf extract, vitamin and mineral premix were thoroughly mixed in a Hobart A-200T mixing machine (Hobart Ltd London England) to obtain a homogenous mass. *O. gratissimum* leaf extract, vitamin and mineral premix were thoroughly mixed in a Hobart A-200T mixing machine (Hobart Ltd London England) to obtain a homogenous mass. *O. gratissimum* was added at graded levels of 0.00 g (control), 0.05 g, 0.10 g, 0.15 g, and 0.20 g per 100 g of diet in treatments 1(Control), 2, 3, 4, and 5 and denoted as T1(control), T2, T3, T4 and T5 respectively. Cassava starch was added as a binder. The resultant mash was pressed without steam through a mincer using 2 mm diameter die attached to the Hobart pelleting machine. Diets were immediately air - dried, sieved, labeled and stored in air-tight transparent plastic containers at -4°centigrade until feeding.

2.2 Nutrient Composition of Feed Determination

The nutrient composition (ash, fat, carbohydrate and crude fibre) of the feed and fish carcass were determined using the standard [14] method

and the protein content was determined using the micro-Kjeldahl method [14].

2.3 Experimental Fish and Feeding Trial

Fingerlings of *C. gariepinus* were obtained from the Department of Fisheries and Aquaculture Research Farm, Federal University of Technology Akure, Ondo State, Nigeria before the feeding experiment. Fish were selected by size and groups of 15 fish of 30.00 ± 0.13 g per replicate and randomly stocked into cylindrical plastic tanks of 30 cm radius. Skretting® (40% crude protein), a commercial diet, was fed to all fish during a 2- week acclimation period. Each experimental diet was fed to five groups of fish in three replicates for 70 days. Fish were fed by 0900-1000 and 1500-1600 h GMT. All groups were fed their respective diets at the same fixed rate (5% of body weight per day). This rate was adjusted each week. Growth was monitored weekly by batch weighing of fish from each tank.

2.4 Proximate Analyses of Experimental Fish

Fish were sampled at the beginning and end of the trial. Before the analysis, MS-222 (tricaine methane sulphonate) at a concentration of 200 mg/l was used for terminal anesthesia of fish. A Sub-sample of fish paste from each tank was taken and stored for estimation of dry matter which was determined after drying in the oven (Gallenkamp, UK) at 105°C for 24 h. The remaining fish homogenate was dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace (Carbolite, USA) for 12 h at 550°C. A Parr bomb calorimeter was used to calculate gross energy content. The Kjeldahl technique was used to measure crude protein [14].

2.5 Water Quality Parameters

Water quality parameters such as dissolved oxygen were measured using HANNA 98103SE (HANNA instruments, Rhode Island). Other physicochemical parameters such as temperature and pH were measured using YSI-IODO 700 Digital probe (IFI Olsztyn, Poland).

2.6 Zootechnical Performance of Experimental Fish

Zootechnical performance was determined using growth performance and nutrient utilisation parameters [13]: Daily weight gain (g/fish) = (final

body weight (BW) (g) – initial body weight (BW) (g) / days

Feed conversion ratio (FCR) = feed consumed (g) / (final BW (g) - initial BW (g))

Specific growth rate (%/day) = $100 \times (\ln [\text{final body weight}] - \ln [\text{initial body weight}]) / \text{experimental period (days)}$

Protein efficiency ratio (PER) = fish weight gain (g) / protein fed (g)

Survival (%) = Number of fish at the end of the experiment / Number of fish stocked at the onset of the experiment X 100.

2.7 Stress Induced Treatments of Fish Fed Leaf *O. gratissimum* Supplemented Diets

2.7.1 *Aeromonas hydrophila* challenge

After six weeks of the feeding trial, fish previously fed with each experimental diet was exposed to a pathogenic strain of *Aeromonas hydrophila* (MPSTR 2143) according to the modified process of [1]. This isolate was grown in brain-heart infusion broth (EM Science, Darmstadt, Germany) in a shaking bath at 27°C overnight at the Department of Microbiology, FUTA. The concentration of bacterial suspension was determined by the serial plate count method and diluted to 9.3×10^5 CFU (colony forming unit)/ml

in fresh well water as described by [6]. Fish from each dietary treatment was immersed in the bacterial suspension for 5 hours. After bath exposure, fish from each dietary treatment was placed back into the plastic tanks and mortality monitored during the feeding trial. Fish were fed with experimental diets throughout the feeding trial and mortality was monitored for the remaining four weeks of the experiment.

2.8 Transportation Induced Stress

At the end of the feeding trial, fish previously fed each experimental diet from each treatment were kept in plastic tanks for a 2-hour journey from FUTA Teaching and Research farm Obakekere. The mortality rate of fish was recorded during transportation. Blood samples were collected immediately after transportation of fish for further analyses.

2.9 Fish Blood Collection and Analyses

At the end of the feeding trial, two fish specimens from each tank were removed for blood analyses. Blood was collected by puncture of the caudal blood vessels. This was done with the aid of 2 ml disposable syringe. Serum glucose concentration was measured according to [15] using Bio-La-Test oxochrome GLUCOSA (Glu 250E) based on the oxidation of glucose catalyzed by glucose oxidase to hydrogen peroxide and gluconate.

Table 1. Feed composition for African catfish, *Clarias gariepinus* fingerlings fed the experimental diet (g/100 g) containing dietary *O. gratissimum*

| Ingredients | T1(Control) | T2 | T3 | T4 | T5 |
|----------------------------|-------------|-------|--------------------|-------|-------|
| Fishmeal (66%) | 26.00 | 26.00 | 26.00 | 26.00 | 26.00 |
| Soyabean meal (45%) | 27.10 | 27.10 | 27.10 | 27.10 | 27.10 |
| Groundnut cake (48%) | 24.40 | 24.40 | 24.40 | 24.40 | 24.40 |
| Maize | 7.50 | 7.50 | 7.50 | 7.50 | 7.50 |
| Rice bran | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |
| Vitamin/mineral premix | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Vegetable oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Starch | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| <i>O. gratissimum</i> mg/g | 0.00 | 0.05 | 0.10 | 0.15 | 0.20 |
| Proximate composition | T1 | T2 | T3 | T4 | T5 |
| Moisture | 10.33 | 10.16 | 10.53 | 10.12 | 9.68 |
| Ash | 10.81 | 11.01 | 10.71 ^b | 10.08 | 10.08 |
| Lipid | 20.73 | 20.00 | 21.00 | 20.85 | 20.40 |
| Protein | 39.77 | 39.17 | 39.45 | 39.65 | 39.60 |
| Fibre | 4.43 | 4.32 | 4.35 | 4.16 | 4.35 |
| NFE | 13.95 | 15.30 | 13.97 | 15.15 | 14.85 |

2.10 Cortisol Level

Cortisol concentrations was determined in the plasma samples using enzyme linked immunosorbent assay ELISA (ELX-800) with a Coat Count Kit Diagnostic Products Corporation (ISELAB DRAKE LA) analysis as described by [15]. The standards used in the ELISA immunoassay were prepared from plasma stripped of endogenous steroids with activated charcoal.

2.11 Assessment of Alanine Transferase (ALT) and Aspartate Transferase (AST)

Physiological stress activities were determined by AST and ALT tests according to the modified procedures of [15,1]. The livers of 3 fish from each treatment were removed by dissection and weighed. The tissue was homogenized with a chilled 0.25 M sucrose solution in a glass tube using a mechanical tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was then centrifuged (5000x g for 10 minutes at 40°C) in a cooling centrifuge machine and stored at -20°C till use. AST and ALT were measured by the estimation of oxaloacetate and pyruvate released in a spectrophotometer at 540nm and the results were read on the calibrated graph respectively.

2.12 Data Analysis

All data collected during the trial were tested for normality using the Kolmogorov–Smirnov test and homogeneity of variance using Levene's test. Completely randomized design (CRD) was used to test for significant differences in the mean of treatments. Differences between mean of treatments were considered significant at $P \leq 0.05$ by one way analysis of variance (ANOVA) using Statistica® software. Follow-up procedures were performed where significant differences occurred in the means with the aid of Tukey test.

3. RESULTS AND DISCUSSION

The zootechnical performance in terms of growth parameters and nutrient utilization of *C. gariepinus* fed with experimental diets with different inclusion level of *O. gratissimum* is shown in Table 2. Significant differences ($P < 0.05$) were recorded in the daily weight gain, specific growth rate, feed conversion ratio and

percentage survival of African catfish, *C. gariepinus* fed with the experimental diet. Fish fed with 0.15 mg/g *O. gratissimum* showed the best zootechnical performance in terms of the highest percentage weight gain and specific growth. Statistically, there was increased growth and nutritional performance of fish in this study with increasing levels of *O. gratissimum* ($P < 0.05$) with optimum performance at 0.15 mg/g. A second degree polynomial regression model depicted that a strong relationship existed between the SGR and *O. gratissimum* supplementation levels in the experimental fish. The differential equation (Fig. 1) shows that the optimum SGR could be achieved at the approximate level of 0.15 mg/g *O. gratissimum* supplementation. The lowest daily weight gain from the current study was observed in the control and the highest was found in treatment four (T4). This is in agreement with the growth performance and nutrient utilization of African catfish, *Clarias gariepinus* obtained by Gbadamosi and Salako (2016), where *O. gratissimum* was used as supplements. It was reported that diets with *O. gratissimum* had better growth performance and nutrient utilization than the control which had no *O. gratissimum* supplementation. The positive influence of *O. gratissimum* could be credited to the role of the antioxidants in the plant which helped in the suppression of the reactive oxygen species (ROS) formation involved in free radical generation. Phytochemical constituents of *O. gratissimum* such as nitriles and glycosides are believed to be responsible for enhancing immunity against oxidative stress and microbial diseases. The enhanced protein efficiency ratio in all the fish fed diets with *O. gratissimum* suggests that the supplementation of *O. gratissimum* had beneficial effects on the nutrition and health of a host by improving its feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, growth promoting factors and an increased immune response [16]. The physicochemical parameters of water observed in this study were temperature, dissolved oxygen, and PH and the values were within the acceptable ranges recommended for the farming of African catfish [17].

Physiological stress biomarkers like glucose and cortisol significantly reduced with increasing *O. gratissimum* supplementation (Table 3). Results from this study revealed that alanine transferase and aspartate transferase ($P < 0.05$) were significantly higher in fish fed the control diet

compared with other dietary treatments. Glucose and cortisol are important endogenous stress indicators, and their activities are key indicators of the immunity of the fish [8]. Supplementation of *O. gratissimum* leaf decreased the level of glucose in fish fed the experimental diets in this study. However, the highest levels of cellular enzymes, cortisol and glucose were found in the control group. This result is in agreement with other studies that reported the role of plant extracts in modulating the immune system and ameliorating the effects of antioxidative stress enzymes in fish species [13]. For example, ameliorative effects of *Cynodon dactylon* (L.) on the non-specific immunity and disease resistance of Indian major carp, *Catla catla* [8]. Phytochemical components of *O. gratissimum* such as glycosides was reported to be responsible for enhancing immunity against oxidative stress and microbial diseases [13]. This research showed that the existence of antioxidants in *O. gratissimum* supplemented diets helped in ameliorating the harmful effects of stressors in African catfish. In the current study, exposure of fish to stressors increased the stress biomarkers as evidenced by high glucose and cortisol concentration in the fish blood therefore rendering the fish immune-compromised. However, the activities of these stress indicators

were significantly reduced with supplementation of *O. gratissimum* leaf in African catfish. The elevated levels of the AST and ALT in the liver tissue of experimental fish induced by *A. hydrophila* infections and transportation-induced stress were significantly reduced ($P < 0.05$) by the inclusion of *O. gratissimum* in experimental fish diets (Table 3). Amino-transferase concentrations in the liver of fish in the current study were found highest in the control group compared to the other dietary groups. The higher activity of AST and ALT shows that there was a mobilization of aspartate and alanine via gluconeogenesis for glucose production to mitigate the effects of the stressors in the present study [4] which also reflected in higher glucose level observed in the control group in this study. Increase in the activity and mobilization of cellular enzymes (AST and ALT) has been reported to be an indicator of cellular damage in stressed fish [4]. *O. gratissimum* leaf supplementation protected the membrane integrity of the cells against stressors by significantly mitigating against the activities of AST and ALT in experimental fish. Hence, the addition of *O. gratissimum* plant extracts ameliorate the effects of stress and improve the growth and health of fish in the current study.

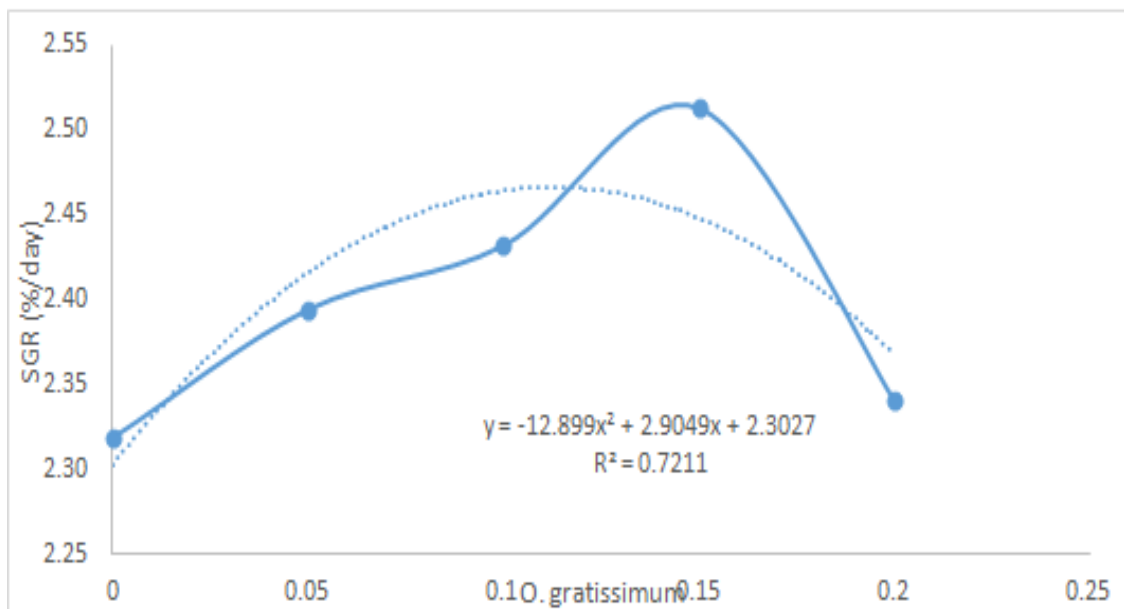


Fig. 1. Second degree polynomial regression analysis of SGR of fish fed experimental supplemented diets

Table 2. Zootechnical performance of African catfish fed *O. gratissimum* supplemented diets

| Parameters | T1 | T2 | T3 | T4 | T5 |
|--------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| IW(g) | 30.82±0.89 ^c | 30.54±0.16 ^c | 31.63±0.35 ^{bc} | 31.18±1.07 ^{bc} | 31.86±0.67 ^b |
| FW(g) | 81.62±2.15 ^b | 83.48±1.72 ^b | 87.84±1.62 ^b | 89.58±2.07 ^{ab} | 82.62±1.53 ^a |
| MWG(g) | 50.80±8.05 ^b | 52.94±4.56 ^{ab} | 56.21±5.97 ^{ab} | 58.4±0.99 ^{ab} | 51.72±3.19 ^a |
| SGR | 2.23±0.60 ^{ab} | 2.39±0.27 ^{ab} | 2.43±0.15 ^{ab} | 2.51±0.10 ^{ab} | 2.34±0.22 ^a |
| FI (g) | 65.71±0.95 ^a | 65.40±1.49 ^a | 68.96±0.18 ^b | 66.34±2.04 ^a | 66.61±1.02 ^a |
| FCR | 1.29±0.21 ^b | 1.24±0.23 ^b | 1.23±0.12 ^b | 1.14±0.06 ^a | 1.20±0.50 ^a |
| PER | 1.28 ±0.20 ^b | 1.35±0.11 ^{ab} | 1.42±1.15 ^{ab} | 1.47±0.20 ^{ab} | 1.33±0.08 ^a |
| SURVIVAL (%) | 96.33±3.33 ^a | 96.00±0.00 ^a | 96.67±3.33 ^b | 96.67±1.67 ^a | 96.00±0.00 ^a |

Values on the same row, having different superscripts are significantly different ($P < 0.05$); Note: IW- Initial Weight; FW- Final Weight; MWG-Mean Weight Gained; FI- Feed Intake; FCR-Feed Conversion Ratio; PER-Protein Efficiency Ratio

Table 3. Physiological stress biomarkers of *C. gariepinus* fed the experimental diets

| Stress biomarkers | T1 | T2 | T3 | T4 | T5 |
|-------------------|----------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|
| Glucose (mg/dl) | 120.53 ± 1.32 ^e | 103.86 ± 2.81 ^d | 91.7 ± 5.13 ^c | 65.86 ± 2.15 ^a | 86.09 ± 2.14 ^a |
| Cortisol (ng/ml) | 194.01 ± 4.83 ^d | 149.60 ± 2.81 ^c | 136.84 ± 2.66 ^b | 129.43 ± 9.00 ^{ab} | 142.17 ± 2.01 ^a |
| Lysozyme (µg/mol) | 11.67 ± 1.53 ^c | 13.33 ± 1.53 ^b | 14.30 ± 0.88 ^a | 13.97 ± 2.00 ^a | 14.24 ± 2.51 ^a |
| AST (µM) | 49.80 ± 1.91 ^c | 43.93 ± 2.13 ^{bc} | 29.52 ± 1.19 ^a | 26.63 ± 2.13 ^a | 31.29 ± 2.91 ^a |
| ALT (µM) | 37.65 ± 5.01 ^c | 25.36 ± 6.32 ^b | 15.27 ± 2.13 ^a | 14.59 ± 1.58 ^a | 19.71 ± 2.05 ^{ab} |

Note: ALT- Alanine transferase; AST- Aspartate transferase ^{a,b,c,d,e}, values in each row with different superscripts are significantly different ($P < 0.05$)

4. CONCLUSION AND RECOMMENDATION

In the present study, results showed improvement in the growth parameters and feed utilization of African catfish fed with feed containing *O. gratissimum* at different supplementation levels during stressful periods. The best supplementation level in terms of FCR was found in T4. Furthermore, the highest inclusion level of *O. gratissimum* did not prove to be the best according to the results obtained from the experiment. Furthermore, this study revealed that the supplementation of basil leaves had ameliorative effects on pathogenic and transportation- induced stress in African catfish. The stress ameliorative action of *O. gratissimum* leaf can be attributed to its potentials to eliminate free radicals, therefore inhibiting ROS damage in fish. The result of the present study regarding zootechnical parameters of fish confirms *O. gratissimum* as a phytogetic product which can promote zootechnical performance and ameliorate the effects of stress in *C. gariepinus*. From the above deductions *O. gratissimum* can be used as an important phytogetic product in aquaculture at a recommended supplementation level of 0.15 mg/g.

ETHICAL APPROVAL

Animals were handled according to standard ethical protocols and consideration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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