Growth performance and nutrient utilisation of *Oreochromis niloticus* (Linnaeus, 1758) fed dietary *Ocimum americanum* L. essential oil from Nigeria

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Abstract

Ocimum americanum essential oil (OAEO) has shown growth-promoting properties in aquaculture. However, there is limited information on its optimal dietary inclusion level for enhancing growth and nutrient utilisation in Nile tilapia (*Oreochromis niloticus*). This study evaluated the effects of graded levels of OAEO on the growth performance of *O. niloticus* fingerlings. Three hundred Nile tilapia (initial weight 1.2±0.02g) were randomly assigned to five dietary treatments containing at 0, 0.25, 0.50, 0.75, and 1.00 ml OAEO/100g of feed, representing $OAEO_0$, $OAEO_1$, $OAEO_2$, $OAEO_3$, and $OAEO_4$, respectively and fed for 90 days. Growth metrics such as Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) were determined. Results showed that MWG, FCR and SGR were significantly influenced ($P < 0.05$) by OAEO inclusion. Highest and lowest MWG was obtained $OAEO_1$ (3.85±0.38) and OAEO4 (2.94±0.03) respectively. Least FCR and Highest SGR was obtained in $OAEO_2$ (1.58±0.14; 1.61±0.11) while highest FCR and least SGR was obtained in $OAEO_4$ (2.06±0.07; 1.40±0.02) respectively. Regression analysis identified 0.50ml ($OAEO_2$) as the dietary inclusion level for optimum growth rate.

Keywords: *Ocimum americanum*, Essential oil, *Oreochromis niloticus*, Specific Growth Rate, Weight Gain

Description of the problem

Aquaculture is critical for global food security, with *Oreochromis niloticus* (Nile tilapia) being a widely cultivated species due to its rapid growth, high nutritional value, and adaptability to various environments [1]. Among the major factors influencing its growth and reproductive potential, nutrition remains important [2]. Enhancing feed quality to boost growth performance is particularly beneficial for intensive aquaculture systems [3]. Studies have highlighted the promising effects of phytogenic compounds as feed additives, due to their bioactive properties [4,5]. *Ocimum americanum* (lemon basil), a member of the Lamiaceae family, is a widely distributed plant across tropical and subtropical regions [6]. Its essential oil is rich in eucalyptol, linalool, and eugenol, known for their antimicrobial, antioxidant, and anti-inflammatory activities [7]. These attributes make *O. americanum* essential oil (OAEO) a promising dietary supplement in aquaculture, with potential to enhance fish growth. However, information on its utilisation and appropriate dosage is limited. Therefore, this paper aims to investigate the effects of dietary *O. americanum* essential oil (EO) on the growth and nutrient utilisation of *O. niloticus.*

Materials and Methods

Essential oil extraction: *Ocimum americanum* leaves were sourced from Osoogun village, Iseyin Local Government Area of Oyo State, South-western Nigeria (longitude 7.71292° N, latitude 3.48145° E) and verified by the University of Ibadan's Botany Department. Essential oil was extracted by blending the leaves and distilled water (1:2) and hydro-distillation was performed using a modified Clevenger-type apparatus for 3 hours at 100ºC. The EO and water were collected and stored in a glass amber bottle at -4ºC until use.

Feed preparation: A basal diet containing 33% crude protein was formulated following FAO [8] guidelines using locally sourced feed ingredients. All ingredients were mixed and pelletised with manual pelletizer with a 2 mm die. Essential oil was sprayed on the feed at 0.25, 0.50, 0.75, and 1.00 ml/100g to produce $OAEO_1$, $OAEO_2$, $OAEO_3$ and $OAEO_4$ respectively. The control diet ($OAEO_0$) is the feed without OAEO inclusion (Table 1). The prepared feed was refrigerated until use. The feed is prepared weekly to ensure nutrient and essential oil retention and prevent deterioration. The feeds were analysed for proximate composition in duplicates [9].

Feeding trial: Nile tilapia (*O. niloticus*) fingerlings (1.2±0.02) were sourced from a reputable fish farm Abeokuta and transported in air-bag. They were acclimatised for 2 weeks in 1 m^3 plastic and fed 30% crude protein commercial diet. Thereafter, the fish were fasted for 24 hours and 300 fish and randomly allotted to 15 tanks (100-L capacity), in a completely randomised design. Feeding was done twice daily between 08:00–09:00 and 15:00–16:00 until apparent satiation. Feed fed were recorded, and uneaten feed was siphoned daily to calculate feed intake. Fish were weighed biweekly using a digital scale [5]. Water is changed by 50% every 2 days and 100% every week.

Water quality monitoring: Temperature, DO, pH, Nitrite and Ammonia were monitored daily using a mercury-in-glass thermometer, digital DO meter (Model AVI-660) and digital pH meter (Photoic 20) and nitrite kit (Aquasaol AE-207), respectively. Water temperature values ranged between 26.5 to 28.9^oC, DO ranged between 4.5 to 6.2 mg/L, pH between 6.7 to 7.8 and Ammonia varied between 0.03-0.04 mg/l.

Growth performance evaluation: On the 90th day of the experiment, the fish were subjected to a 24-hour fasting period, anesthetised using tricaine methanesulfonate (MS-222) at a concentration of 60 mg/L and weighed to conclude the trial. Growth performance was determined using the equations adapted by [5].

Table 1: Ingredients composition of experimental diet.

Ingredients	$OAEO_o$	$OAEO_1$	$OAEO_2$	$OAEO_3$	$OAEO_4$
Fish meal	14.13	14.13	14.13	14.13	14.13
Soy bean	25.00	25.00	25.00	25.00	25.00
GNC	15.00	15.00	15.00	15.00	15.00
Maize	36.13	36.13	36.13	36.13	36.13
vegetable oil	1.00	1.00	1.00	1.00	1.00
Corn starch	6.24	5.84	5.64	5.44	5.24
Premix	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.50	0.50	0.50	0.50	0.50
Premix*	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
DCP	0.50	0.50	0.50	0.50	0.50
Vitamin C	0.25	0.25	0.25	0.25	0.25
OAEO (µl/100g feed)	NIL	250	500	750	1000
Total	100.00	100.00	100.00	100.00	100.00
Proximate analysis (%)					
Dry matter	9.35	9.50	9.59	9.62	9.90
Crude protein	33.94	33.28	33.42	33.21	33.33
Ether extract	10.72	10.62	10.68	10.50	10.83
Total Ash	8.57	8.58	8.59	8.26	8.45
Crude fibre	4.42	4.51	4.58	4.50	4.82

$OAEO_0$ = no supplement, OAEO= *O. americanum* essential oil diet

* One kilogram contains: vitamin A, 4,000,000 IU; vitamin D3, 6,00,000 IU; vitamin E, 12,000 IU; vitamin K3, 15 mg; vitamin B1, 2,500 mg; vitamin B2, 1,750 mg; vitamin B6, 800 mg; vitamin B, 1,250 mg; nicotinic acid 3,750 mg; pantothenic acid, 5,000 mg; folic acid, 250 mg; biotin, 100.1 mg; choline chloride 1,20,000 mg; iron, 11,000 mg; copper, 1,800 mg; manganese, 6,000 mg; zinc, 20,000 mg; iodine, 400 mg; selenium, 40 mg; vitamin C (coated), 60,000 mg; inositol, 10,000 mg; cobalt, 150 mg.

Statistical analysis

All experimental data were analysed using descriptive statistics and one-way analysis of variance using GraphPad Prism software (version 8). Differences among individual treatment means were evaluated using Tukey's Multiple Range Test at a significance level of $P < 0.05$.

Result and Discussion

The physicochemical parameters recorded in this study were within the standard range for optimal *O. niloticus* culture [10]. The growth performance of *O. niloticus* fingerlings fed dietary *O. americanum* essential oil during the 90-day experiment is summarised in Table 2. The initial body weights of both species were statistically similar across all groups ($P > 0.05$). The final body weight ranged from 4.11 g to 5.05g and the fish exhibited a growth increase of 3.52 to 4.30 times their initial stocking weight. Growth metrics were significantly influenced by dietary OAEO levels ($P < 0.05$). Final weight and MWG was higher in $OAEO_1$ (250 µl) but decreased in $OAEO_4$ (1000 µl/100 g). The lowest FCR (1.66 ± 0.07) and highest PER (1.81 ± 0.08) were recorded in $OAEO_2$ (250µl) with no significant difference from $OAEO_1$ (500µl) indicating more efficient feed and protein utilisation at inclusion levels between 250µl - 500µl. The SGR followed a similar trend, peaking in $OAEO_1$ and declining at higher inclusion levels. Since FI did not differ significantly among treatments, the observed improvement in growth performance can be attributed to enhanced feed utilisation efficiency rather than increased feed consumption. This result agrees with previous studies [5,11] that phytogenic feed additive may reduce performance when used excessively. Survival ranged from 90.20% to 96.1%, with no significant differences among treatments, demonstrating that OAEO supplementation did not adversely affect fish survivability. Regression analysis from this study indicates that optimal growth performance may be achieved at OAEO inclusion levels of 250µl (0.25%).

Table 2: Growth performance and nutrient utilisation of *O. niloticus* fed dietary *O. americanum* essential oil

	$OAEO_0$	**$OAEO_1$**	**$OAEO_2$**	**$OAEO_3$**	**$OAEO_4$**
Initial weight (g)	1.18±0.02	1.20±0.02	1.15±0.02	1.16±0.02	1.17±0.03
Final Weight (g)	$4.67±0.06^{ab}$	$5.05±0.20^{a}$	$4.70±0.26^{ab}$	$4.18±0.15^{b}$	$4.11±0.03^{b}$
MWG (g)	$3.49±0.07^{ab}$	$3.85±0.22^{a}$	$3.56±0.27^{ab}$	$3.02±0.14^{b}$	$2.94±0.02^{b}$
FI (g)	6.23±0.15	6.42±0.30	5.85±0.22	5.58±0.29	6.07±1.88
FCR	$1.79±0.01^{ab}$	$1.67±0.02^{a}$	$1.66±0.07^{a}$	$1.86±0.12^{ab}$	$2.07±0.06^{b}$
Survival (%)	96.10±1.96	92.20±1.96	92.20±1.96	90.20±3.92	96.10±1.96
SGR (%)	1.53±0.03	1.60±0.06	1.57±0.07	1.42±0.04	1.40±0.02
PER (%)	$1.67±0.01^{ab}$	$1.79±0.02^{a}$	$1.81±0.08^{a}$	$1.63±0.11^{ab}$	$1.45±0.06^{b}$

$OAEO_0$ = no supplement, OAEO= *O. americanum* essential oil diet, Values are the mean ± SE of triplicate samples.
$^{a, b, c}$ Values in the same row with different superscripts differ significantly ($P < 0.05$), FCR: Feed Conversion Ratio; SGR: Specific Growth Rate; FI: Feed Intake; PER: Protein Efficiency Ratio; MWG: Mean Weight Gain

The relationship between weight gain and OAEO levels; SGR and OAEO is represented by the equation below

$Y=3.486+0.0037-1.017+5.920$ ($R^2 = 0.9977$)

$Y=1.526+0.0008-2.183+1.227$ ($R^2 = 0.9676$)

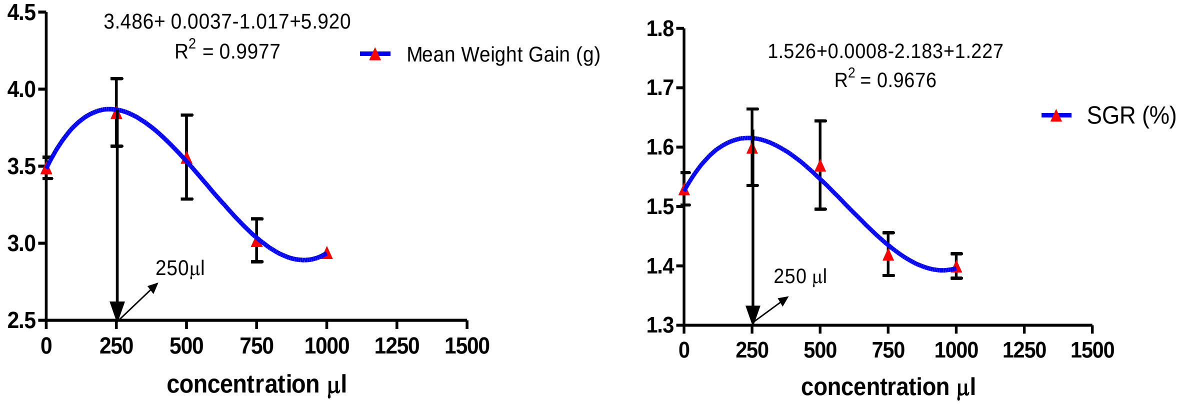

Fig. 1a, b. The relationship between weight gain (g), specific growth rate (SGR; %g/day) *O. niloticus,* fed diets containing different levels of *O. niloticus* essential oil for 90 days

Conclusion and Application

Dietary inclusion of *Ocimum americanum* essential oil at 250 µl/100 g feed enhances growth performance and nutrient utilisation in *Oreochromis niloticus*. Higher doses (e.g., 1000 µl/100 g) may reduce performance, possibly due to reduced feed utilisation efficiency warranting further investigation.

References

1. El-Sayed, A-F. M. 2006. Tilapia Culture. Oxfordshire, CABI Publishing. 277 pp.
2. Assan, D., Huang, Y., Mustapha, U. F., Addah, M. N., Li, G. and Chen, H. (2021). Fish Feed Intake, Feeding Behavior, and the Physiological Response of Apelin to Fasting and Refeeding. *Frontiers in endocrinology*, *12*, 798903. https://doi.org/10.3389/fendo.2021.798903
3 Adeoye, A.A., Yomla, R., Jaramillo-Torres, A., Rodiles, A., Merrifield, D.L. and Davies, S.J. 2016. Combined effects of exogenous enzymes and probiotic on Nile tilapia (*Oreochromis niloticus*) growth, intestinal morphology and microbiome. *Aquaculture,* 463: 61-70.
4. Abdel-Tawwab, M., Adeshina, I., Emikpe, B.O., Jenyo-Oni, A., Ajani, E.K. and Tiamiyu L.O. 2019. Effect of dietary clove basil, *Ocimum gratissimum,* leaves extract on healing of artificially wounded African catfish, *Clarias gariepinus* (B.) juveniles. *Journal of Applied Aquaculture***,** 31(4):289-300
5. Omitoyin, B. O., Ajani, E. K., Orisasona, O., Bassey, H. E., Kareem, K. O. and Osho, F. E. (2019). Effect of guava (*Psidium guajava* L.) aqueous extract diet on growth performance, intestinal morphology, immune response and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Aquaculture Research*, *50*(6), 1–11. https://doi.org/10.1111/are.14068
6. Hosseini, M. S., Farajpour, M., Boroomand, N. and Solaimani-Sardou, F. (2017). Ethnopharmacological studies of indigenous medicinal plants in the south of Kerman, Iran. *Journal of Ethnopharmacology*, 199, 194–204. https://doi.org/10.1016/j.jep.2017.01.045
7. Sharifi-Rad, J., Adetunji, C. O., Olaniyan, O. T., Ojo, S. K., Samuel, M. O., Temitayo, B. T. and del Mar Contreras, M. (2023). Antimicrobial, antioxidant and other pharmacological activities of *Ocimum* species: Potential to be used as food preservatives and

functional ingredients. *Food Reviews International*, *39*(3), 1547–1577.

8. FAO (2019). Cultured Aquatic Species Information Programme. Oreochromis niloticus. Cultured Aquatic Species Information Programme. Text by Rakocy, J. E. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 18 February 2005.

9. AOAC (2000). Association of Official Analytical Chemists Official Methods of Analysis. 18th edition, Arlington, Virginia, USA

10. Ibrahim K.L. (2017). Physicochemical parameters of water and their effects on fish production. *Journal of Agriculture and Veterinary Sciences,* 9 (2): 20-31

11. Adeyemo A. and Faturoti, E.O. (2017). Effects of Basil Leaf (*Ocimum gratissimum*) as Dietary Additives on Growth Performance and Production Economics of *Clarias gariepinus. International Journal of Aquaculture*.7(6), 42-50. DOI:10.5376/ija.2017.07.0006