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Effects of Varying Levels of *Ocimum gratissimum* Leaf Powder on Growth Performance and Feed Utilization of *Heterobranchus bidorsalis* Fingerlings

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Abstract

Growth performance and feed utilization of *Heterobranchus bidorsalis* fingerlings fed varying inclusion levels of diets supplemented with *Ocimum gratissimum* leaf powder were assessed in the study. Apparently healthy H. bidorsalis fingerlings (12.05g ± 0.01) were randomly divided into five treatment groups representing iso-nitrogenous diets (about 40% crude protein) containing varying inclusion levels 0g/100g (control), 0.5g/100g, 1.0g/100g, 1.5g/100g, and 2.0g/100g of *O. gratissimum* leaves denoted as CTR, OG1, OG2, OG3 and OG4 respectively. The fish were fed these diets for 70 days in triplicate. The experimental fish were randomly distributed into fifteen plastic tanks of dimension 40 ×30×35cm3 at a stocking density of ten fish per tank. *Heterobranchus bidorsalis* fingerlings fed 1.5g/100g diet of *O. gratissimum* recorded the best growth performance in terms of body weight gain, feed conversion ratio (FCR) and specific growth rate (SGR). There was a significant increase in growth and nutritional performance of *H. bidorsalis* fingerlings with increasing inclusion of *O. gratissimum* leaves ($P<0.05$). A differential equation ($y = -15.227x^4 + 53.533x^3 - 54.453x^2 + 17.887x + 22.07$; $R^2 = 1$) showed that highest weight gain occurred at *O. gratissimum* level of approximately 1.65 g/100g. The study therefore showed that *O. gratissimum* leaves as an additive significantly improved growth and feed utilization parameters in the treated groups.

Keywords: *Heterobranchus bidorsalis*, *Ocimum gratissimum*, growth performance, nutrient utilization, specific growth rate.

Introduction

The use of feed additives in diets of cultured fish is aimed at improving fish performance, immunity and carcass quality. Continuous search for new feed additives is quite important for researchers in aquaculture (Cho and Lee, 2012). Some of these additives used in feed mill are medicinal herbs and plants that can enhance efficiency of feed utilization and animal productive performance (Mohamed *et al.,* 2003).

There are a large number of feed additives available to improve fish growth performance. Some synthetic additives used in feed mill especially hormones and antibiotics may cause negative effects on fishes. Therefore, to resolve these problems, increasing attention is being given to the use of natural alternative feed additives (Shubha, 2015). Plant extracts have also been found to be cheaper, safer, biodegradable and biocompatible (Sudagar and Hajibeglou, 2010). Citarasu - (2010) reported that various extracts from

herbs and spices have improved animal performance either by stimulating action on gut secretions or by having a direct bactericidal effect on gut microflora. The herbal ingredients fish diets reportedly induce the secretion of the digestive enzyme while the growth promoters in herbs induce high protein synthesis.

H. bidorsalis is a freshwater fish that belongs to the family *Clariidae*. The genus *Heterobranchus* is similar in many respects to Clarias but can be readily differentiated from *Clarias* by the fact that it has the rayed dorsal fin followed by an adipose fin. The flesh contains lesser oil in comparison with that of Clarias. It has good taste, fast growth rate and is rarely parasitized making it well prized (Holden and Reed, 1972). *H. bidorsalis* has the capacity of utilizing a remarkably wide range of food organisms suggesting a euryphagous diet (Fagbenro *et al*., 1991).

Ocimum gratissimum belongs to the group of plants known as spices (Vierra and Simon, 2000). It is of the family Labiatea, genus *Ocimum* and species *gratissimum* (Iwu, 1993). In Nigeria, it is popularly called "effinrin-nla" by the Yorubas, "Ahuji" by the Igbos and "Daidoya" by the Hausas (Effraim *et al.,* 2003). It is also known by names such as tree basil and shrubby basil in English. It has been used extensively in the traditional system of medicine in many countries (Rabelo *et al.,* 2003). The infusion of *O. gratissimum* leaves is used as pulmonary antisepticum, antitussivum and antispasmodicum (Ngassoum *et al*., 2003).

Aside its several uses in humans, *Ocimum gratissimum* reportedly improved the growth of *Clarias gariepinus* (Adewole and Faturoti, 2017; Abdel-Tawwab *et al.,* 2018), exerted anesthetic effect on *Oreochromis niloticus* juveniles (Deshina and Yusuf, 2017) and improved heamatological profile of *C. gariepinus* (Sogbesan and Ahmed, 2018). There is a dearth of information on the use of *Ocimum gratissimum* in the diet of *H. bidorsalis*. This necessitated the present study which evaluated the effect of *Ocimum gratissimum* leaf powder on growth and nutrient utilization in the diet of *Heterobranchus bidorsalis* fingerlings.

Materials and Methods

This study was carried out at Research Laboratory of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Nigeria.

Fresh *Ocimum gratissimum* leaves were acquired from Odopetu Market in Akure, Ondo State, Nigeria. The leaves from *O. gratissimum* were identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The leaves were destalked, washed and air dried for seven days after which the dried leaves were reduced to powdery form using Kenwood electric blender BL440 (UK). The resultant powder obtained was stored in air-tight container and kept in the freezer till required in the preparation of experimental diets.

Five iso-nitrogenous diets (40% crude protein) were formulated as control (CTR) along with four other diets containing four graded levels of *O. gratissimum*; 0.5% (OG1), 1.0% (OG2), 1.5% (OG3) and 2.0% (OG4). Diet formulation were according to Fagbenro *et al.,* (1993) as shown in Table 1. All dietary ingredients were milled into a small particle size. The ingredients including protein sources, oil and vitamin premix were thoroughly mixed in a Hobart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogeneous mass and cassava starch was added as a binder. The resultant mash was pressed without steam through a mixer with 2mm diameter die attached to the Hobart pelleting machine. The pellets produced were dried, broken up, sieved and kept in a cool and dry place until the start of experiment at a temperature of (27-30^{0}C) and stored at - 20^{0}C until the start of the feeding experiment. Proximate analysis of *O. gratissimum* leaves and experimental diets was as described by AOAC (2005).

Groups of 10 apparently healthy *H. bidorsalis* fingerlings (12.05g ± 0.01 body weight) were stocked into 15 plastic tanks of dimension 40 ×30×35cm^3 filled with water. Each experimental diet was fed to five groups of fish in three replicates for 70 days. Fish were fed between 08:00-09:00h and 18:00-19:00h GMT. All groups were fed their respective diets daily at 5% body weight. Each group of fish were batch weighed fortnightly to monitor growth and adjust feeding rates accordingly. The physical assessment of culture water was carried out daily according to APHA (1987) and the parameters assessed include

temperature (°C), hydrogen-ion concentration (pH) and dissolved oxygen (DO). Proximate Analysis of *O. gratissimum* leaves and experimental diets was as described by AOAC (2005). Growth and diet utilization indices were evaluated as:

Weight gain = final weight of fish - the initial weight of fish

Percentage weight gain= $\frac{\text{Final mean weight of fish}}{\text{Initial mean weight of fish}} \times 100$

Specific Growth Rate = $\frac{100\ (\ln W_2 - \ln W_1)}{t}$

Where; W_1 and W_2 are the initial and final fish weight, respectively, and t represents the duration of the feeding trial.

Percentage survival = $N_1 / N_0 \times 100$

Where: N_1 = Total number of fish survival in pond at end of experiments.

N_0 = Total number of fish in tank at the beginning of experiments.

Feed Conversion Ratio (FCR) = $\frac{\text{dry weight of feed intake (g)}}{\text{Wet weight gain by fish (g)}}$

Protein Efficiency Ratio (PER) = $\frac{\text{Weight gain (g)}}{\text{Protein fed (g)}}$

Protein Productive Value (PPV) = 100[protein retained in tissue (g)/protein fed (g)]

Energy utilization (EU) = 100 x (energy gain (g)/energy intake (g)).

Feed efficiency ratio (FER) = weight gain/ feed intake.

Data generated were appropriately subjected to one-way ANOVA test ($P<0.05$) while statistical differences of treatment were determined using New Duncan's Multiple Range Ad-hoc Test (Dytham, 1999) using Statistical Package for Social Science (SPSS), version 22.0. Fourth degree polynomial regression analysis was used to determine *H. bidorsalis* fingerlings responses to the level of *O. gratissimum* in the formulated diet.

Table 1: Composition of the basal experimental diet (g/100g)

Ingredients	CTR	OG1	OG2	OG3	OG4
Fish meal (68% CP)	23.0	23.0	23.0	23.0	23.0
Soybean Meal (42%CP)	26.0	26.0	26.0	26.0	26.0
Groundnut Cake (45% CP)	28.0	28.0	28.0	28.0	28.0
Yellow Maize (10% CP)	11.0	11.0	11.0	11.0	11.0
Vegetable Oil	4.0	4.0	4.0	4.0	4.0
Rice Bran	2.0	2.0	2.0	2.0	2.0
Bone meal	2.0	2.0	2.0	2.0	2.0
Vit/Min Premix*	2.0	2.0	2.0	2.0	2.0
Binder	2.0	2.0	2.0	2.0	2.0
O. grattisimum leaf powder	0	0.5	1.0	1.5	2.0
Proximate composition (%)					
Moisture					
Ash	8.51	10.16	10.02	10.51	9.94
Crude lipid	10.96	13.05	13.22	13.33	13.39
Crude fibre	1.98	2.09	2.29	2.50	2.64
Crude protein	40.29	40.26	40.30	40.32	40.31
Nitrogen free extract	29.72	25.49	25.52	24.63	24.73
Gross energy(Kcal/g)	4.53	4.50	4.56	4.54	4.55

*Vitamin premix- A Pfizer livestock product containing the following per kg of feed: A = 4500 I, U, D = 11252 I.U, E = 71I.U, K3=2mg, B12=0.015mg, pantothenic acid = 5mg, nicotinic acid = 14 mg, folic acid = 0.4mg, biotin = 0.04 mg. choline = 150mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg.**GE= Gross Energy: Gross energy was calculated as 5.64, 9.44 and 4.11Kcal per gram of protein, lipid and carbohydrate respectively according to NRC (2011).

Results

There were no adverse effects of different inclusion levels of *O. gratissimum* in the experimental diets on the water quality parameters of the culture water. The pH value ranged between 7.05 and 7.23, temperature of 24.7-24.8°C and dissolved oxygen concentrations 5.25-5.90ppm.

The proximate analysis of *O. gratissimum* leaf used in this study shows that it has moisture content of 10.57±0.01, ash content of 4.82±0.01, Crude Lipid content of 5.58±0.00, crude fibre content of 8.93±0.01, protein content of 13.29±0.01 and carbohydrate content of 56.81±0.01. The proximate composition of *H. bidorsalis* fingerlings prior to feeding with experimental diets is shown in Table 2. *Heterobranchus bidorsalis* fingerlings used in this study had moisture content of 7.31%±0.01, ash content of 5.56%±0.01, crude lipid content of 16.76%±0.00, protein content of 46.44±0.00 and Nitrogen free extract content of 23.9%±0.01. The results of the proximate composition of *H. bidorsalis* fed varying inclusion level of *O. gratissimum* (Table 2) showed significant variations in crude protein, crude lipid and ash contents of the fish with increasing level of *O. gratissimum* in the diet. NFE is highest in control and lowest in OG_4 while moisture content is lowest in the control diet and highest in Og_1.

Growth and nutrient utilization indices of *H. bidorsalis* fingerlings fed *O. gratissimum leaves powder* at varying inclusion levels (Table 3) showed that significant variations occurred in mean weight gain, % weight gain, protein productive value, feed conversion rate and feed intake among the treated fish groups. However, the fish showed no significant differences (P>0.05) in feed efficiency ratio, protein efficiency ratio and survival. The

optimum dietary *O. gratissimum* level of *H. bidorsalis* fingerlings is presented in Figure 1. The 4th order polynomial regression model depicted that a significant and moderately strong relationship existed between the specific growth rate and *O. gratissimum* concentration (g/100g) in the fish diets. A differential equation ($y = -15.227x^4 + 53.533x^3 - 54.453x^2 + 17.887x + 22.07$; $R^2 = 1$) showed that highest weight gain occurred at *O. gratissimum* level of approximately 1.65 g/100g (Figure 1).

Table 2: Proximate composition of *Heterobranchus bidorsalis* fed varying inclusion levels of *Ocimum gratissimum* leaf powder

PARAMETERS	INITIAL	CONTROL	OG1	OG2	OG3	OG4
Moisture	7.31±0.01	7.91±0.00^a	8.87±0.00^e	8.59±0.00^c	8.77±0.00^d	8.25±0.00^b
Ash	5.56±0.01	3.33±0.00^a	4.20±0.00^b	4.35±0.00^c	4.50±0.00^d	4.77±0.00^e
Crude Lipid	16.76±0.00	19.07±0.00^a	21.11±0.00^b	21.18±0.00^c	21.23±0.00^d	21.36±0.00^e
Crude Protein	46.44±0.01	48.10±0.00^a	54.68±0.00^b	54.92±0.00^c	55.14±0.00^d	55.61±0.00^e
NFE	23.92±0.01	21.59±0.00^e	11.14±0.00^d	10.96±0.00^c	10.36±0.00^b	10.01±0.00^a

NFE = Nitrogen free extract
Figures in each row having the same superscripts are not significantly different (P>0.05)

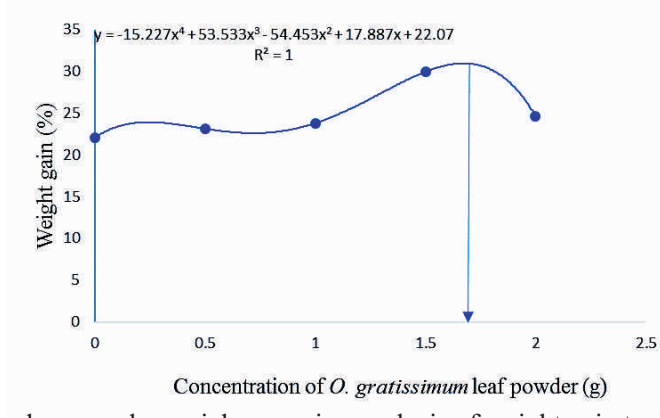

Figure 1: Fourth order degree polynomial regression analysis of weight gain to dietary *O. gratissimum*

Table 3: Growth performance and nutrient utilization of *H. bidorsalis* fed dietary supplementation of *Ocimum gratissimum* powder

PARAMETERS	CONTROL	OG1	OG2	OG3	OG4
Initial mean weight (g)	12.06±0.02^a	12.04±0.02^a	12.07±0.03^a	12.06±0.03^a	12.05±0.04^a
Final mean weight (g)	34.12±1.33^a	35.18±0.41^a	35.88±0.77^a	42.03±2.20^b	36.72±1.89^a
Mean weight gain (g)	22.07±1.32^a	23.14±0.47^a	23.81±0.75^a	29.97±2.18^b	24.67±1.86^a
PWG (%)	183.01±10.80^a	192.19±3.20^a	180.79±5.99^a	248.48±17.64^b	204.64±15.06^a
Survival (%)	73.33±16.67^a	83.33±3.33^a	86.67±13.33^a	93.33±8.82^a	90.00±11.55^a
SGR (%/day)	1.48±0.05^a	1.53±0.02^a	1.56±0.03^a	1.78±0.07^b	1.59±0.07^a
Feed intake	43.26±0.01^b	40.96±0.06^a	41.91±0.02^a	51.25±0.02^c	43.17±0.07^b
FCR	1.96±0.07^b	1.77±0.17^a	1.76±0.04^a	1.71±0.15^a	1.75±0.15^a
PER	1.48±0.14^a	1.34±0.03^a	1.24±0.04^a	1.39±0.12^a	1.39±0.13^a
PPV	41.10±0.38^a	47.57±0.47^c	47.86±1.24^c	49.27±0.87^b	48.88±2.10^c
EU	93.63±0.20^a	95.80±0.12^b	96.43±0.39^b	99.20±0.43^d	97.93±0.09^c
FER	0.51±0.06^a	0.56±0.01^a	0.57±0.02^a	0.58±0.05^a	0.57±0.05^a

Figures in each row having the same superscripts are not significantly different (P>0.05)
PWG: percentage weight gain, SR: survival rate, SGR: specific growth rate, FCR: food conversion ratio, PER: protein efficiency value, PPV: protein productive value, EU: energy utilization, FER: feed efficiency ratio.

Discussion

In the present study, *H. bidorsalis* fed *O grattisimum* supplemented diets showed improved growth performance indices over the control with an optimum inclusion level of 1.65g/100g. Improvements in growth performance and nutrient utilization indices could be due to the quality of the protein content of the experimental diets. The present findings could also be attributed to the presence of antioxidants in *O. gratissimum* leaf powder. Abdel-Tawwab *et al.*, (2018) also suggested that dietary supplementation of *O. gratissimum* may control and distort the growth and colonization of pathogenic bacteria in fish guts, which could lead to a greater feed utilization efficiency resulting to improved fish growth.

Results in the present study aligned with the findings of Abdel-Tawwab et al., (2018) who fed *Clarias gariepinus* fingerlings with diets containing extracts of *O. gratissinum* leaves (0,5,10 and 15g/kg) for 12 weeks. They reported increase in fish growth with increasing inclusion of extracts of *O. gratissinum* in the diets and that fish growth was maximized at an inclusion level of 12g/kg inclusion level of *O. gratissinum* leaf extract. Adewole and Faturoti (2017) reported that the optimum growth of *C. gariepinus* was observed between 0.125 and 0.25% inclusion levels of *O. gratissimum* when they evaluated the effect of dietary *O. gratissimum* leaf meal at levels of 0.0, 0.125, 0.25, 0.5, 1.0, and 2.0% on *C. gariepinus* fingerlings (10.94 ± 0.02g) for 12 weeks. They also reported that inclusion of *O. gratissimum* between 0.5 and 2.0% in the fish diets resulted into retarded growth and high fish mortality. The findings in the present study also agreed with the reports of Dada and Abiodun (2014) when Nile tilapia (*Oreochromis niloticus*) fingerlings fed dietary fluted pumpkin (*Telfaria occidentalis*) extract showed significantly improved growth performance and feed utilization indices over the control group. The fish fed experimental diets in this present study showed better growth performance and nutrient utilization indices when compared with the reports of Afe and Omosowone (2019) who fed *Clarias gariepinus* fingerlings with *Acacia auriculiformis* leaf supplemented diets. The gradual increase in weight gain observed in the present study agreed with the reports of Saleh *et al*., (2015) who observed gradual improvement in weight gain of sea bass (*Dicentrarcus labrax*) fed either onion or garlic supplemented diets. The specific growth rates observed in fish fed *O. gratissimum* across all treatment groups are higher when compared with those reported by Gbadamosi and Salako (2015) in *C. gariepinus* fed *O. gratissimum* supplemented diets. However, contradicting results were reported by Sogbesan *et al*., (2017) when fermented *O. gratissimum* leaf meal was included (at 0%, 1%, 2%, 3% and 4%) in the diet of *Clarias gariepinus* fingerlings where fish growth reduced with increasing inlusion of *O. gratissimum* leaf meal. The result obtained for FCR in this study agreed with the reports of Soltan and El-Laithy (2008) who fed *Oreochromis niloticus* fry with diets containing probiotics (*Bacillus subtilis* and Biogen®) and two spices (garlic and fennel). This is an indication that fish diets supplemented with *O. gratissimum* optimized protein use for growth which can reduce the quantity of feed required for fish growth thereby reducing production costs.

The whole body crude protein of fish in the present study significantly increased with increasing level of *O. gratissimum* in the diets. This may be attributed to the presence of *O. gratissimum* in the diets that could be responsible for a rise in muscle free amino acid and hence improvement in protein synthesis. Saleh *et al.,* **(**2015) observed significant increase in the whole body protein content of *Dicentrarcus labrax* when fed garlic and onion powder supplemented diets. The contrasting result between the present study and some previous studies on the effects of dietary *O. gratissimum* on fish growth and feed utilization, chemical composition and survival is attributable to variations in fish species, fish physiology, dietary inclusion level of the phyto-addictive and rearing conditions.

The values of water quality parameters monitored during the feeding experiment remained within acceptable limits as recommended for African catfish (Viveen *et al*., 1985) which is an indication of adequate water management practices.

Conclusion

The inclusion of *O. gratissimum* in the diets of *H. bidorsalis* in the present study showed improved growth performance and nutrient utilization in the treated group over the control group. This suggests that the recommended level of *O. gratissimum* in the diet of *H. bid orsalis* should between 1.5 and 1.65g/100g.

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