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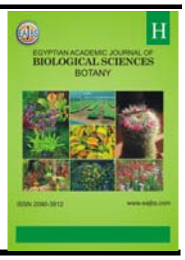

Gustation and Growth Performance of African Catfish, *Clarias gariepinus* Fed Varying Levels of Dietary African Basil, *Ocimum gratissimum* Leaf Supplementation

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ABSTRACT

The gustation and growth performance of African catfish, *Clarias gariepinus* fed African basil, *Ocimum gratissimum* leaf meal supplemented diets was assessed in the present study. *C. gariepinus* of the initial weight of 6.96 ± 0.01g was evaluated over a 56 days period. Five experimental diets were formulated at 0 mg/g (control), 10 mg/g, 20mg/g, 30 mg and 40 mg/g inclusion levels of *O. gratissimum*. The leaf of *O. gratissimum* was treated by soaking in water for 72 hours and sun-dried. All diets were isonitrogenous with each treatment having two replicates. Fish fed the 40 mg/g *O. gratissimum* leaf meal also recorded the best growth performance in body weight gain and specific growth rate (SGR). Statistically, there was significantly increased growth and nutritional performance of fish in this study with increasing inclusion levels of *O. gratissimum* ($P<0.05$). There was no adverse effect of *O. gratissimum* supplementation on the histometric index of fish in this study ($P>0.05$). There was also significant increase in the gustation of fish with increasing *O. gratissimum* supplementation ($P<0.05$). Therefore, the present study suggests that *O. gratisimum* leaf meal may be supplemented up to 40mg/g level to increase gustation and growth performance of *C. gariepinus*.

INTRODUCTION

The aquaculture sector is emerging as a significant production sector for high protein food. Aquaculture will continue to be relevant in the future as a source of protein. However, the growth in aquaculture has caused major challenges; one of these challenges is the production of practical feeds for the farming of fish (Globefish, 2014). Apart from the nutritionally balanced formulations, the most important factor determining the success of the feed manufacture is palatability and attractiveness of the feed that promotes ingestion leading to utilization of available nutrients (FAO, 2012).

Diet palatability and attractiveness would help to reduce the time that fish spend approaching the feed and reduce deterioration of rearing pond environments from overloaded nutrient input (Fournier, 2012). As with any animal production system, reducing feed loss - which is directly related to reducing costs - whilst maintaining a desirable level of output is the prime concern. Focusing on palatability in fish, many attractive molecules likely to be involved in olfaction and gustation should be identified.

African sweet basil (*Ocimum gratissimum*), which comes from a family known as Lamiaceae could be of a great value to aquaculture nutrition and health management (Charis, 2000). It is also known as clove basil or African basil and in Hawaii as wild basil. It is a species of *Ocimum* which is native to Africa, Madagascar, southern Asia and the Bismarck Archipelago, and naturalized in Polynesia, Hawaii, Mexico, Panama, West Indies, Brazil, and Bolivia. Most plants that belong to the family Lamiaceae are good feed attractants which can be used in practical diets for aquatic animals (Kanda *et al.*, 1971). The plant leaves are recommended for use in treating many human diseases such as digestive disorder, lowering glucose level in blood and of sedative effect in man (Almeida and Alviano, 2007). The use of this plant product is not popular in livestock production in many countries like Nigeria, probably because of the limited information about the nutritional properties which can have beneficial effects on many farm animals. *O. gratissimum* is a very good source of all of nutrients, minerals and vitamins such as omega-3 fatty acid, vitamin K, iron, calcium, vitamin A and vitamin C. Basil leaves come complete with an array of antioxidants and other wonder full phytonutrients (Almeida and Alviano, 2007). Harada (1992) found that spices were highly effective in attraction for fish and shellfish with special reference to strong effect for basil. Basils improve foods palatability through their aroma, increase digestibility and impact some medical function when consumed by man or animals as part of their food (Rutherford-Fortunati, 2013). In addition, natural herbs and spices such as basil, garlic and onions are incorporated in feeds only in small amounts, but they make important contributions towards the odour and flavour due to presence of volatile and fixed oil (Harada, 1992). The clariid catfish, *Clarias gariepinus* is the most important fish species cultured in Nigeria. The African catfish is preferred as cultured species due to several factors which also influenced the choice of the fish as a model for the current study. These include fast growth rate, high resistance to disease, tolerance to adverse environmental conditions, ability to feed on wide range of feed and capacity to withstand low pH and oxygen (Balogun and Dabrowski, 1992).

MATERIALS AND METHODS

Experimental diets

Five isonitrogenous diets containing 40% crude protein wee formulated for fingerlings catfish, *C. gariepinus* in an eight-week trial experiment (Table 1). African basil leaf, *Ocimum gratissimum* was used as supplement at 0mg/g 10mg/g, 20mg/g, 30mg/g and 40mg/g in diet 1,2,3,4 and 5 respectively. All dietary ingredients were first milled to small particle size. The dry ingredients were thoroughly mixed by adding hot water until a consistent dough resulted. The dough was then pelleted using Hobart A- 200 pelleting machine with a 2.0mm die. After pelleting, the diets were immediately sun dried (27^0C) for a week and later broken mechanically into small sizes and packed in dry, air tight small containers (labeled) prior to use. CMC (carboxyl methyl cellulose) was used as binder.

Table 1: Composition of the experimental diet in g/100g dry matter containing various inclusion level of basil leaves for *Clarias gariepinus*

INGREDIENTS	T1 (control)	T2	T3	T4	T5
Fishmeal (72%)	24.0	24.0	24.0	24.0	24.0
Soybean meal	42.0	42.0	42.0	42.0	42.0
Maize	23.0	23.0	23.0	23.0	23.0
Vegetable oil	7.0	7.0	7.0	7.0	7.0
Vitamin	2.00	2.00	2.00	2.00	2.00
CMC	2.00	2.00	2.00	2.00	2.00
African basil mg/g	0.00	10.00	20.00	30.00	40.00

Vitamins supplied mg/100g diet supplied by Vitafeed: thiamine (B1) 2.5mg: riboflavin (B2), 2.5mg pyridoxine 2.0mg: pantothenic acid, 5.0mg: inositol, 3mg: biotin, 0.3mg: folic acid, 0.75mg para-amino benzonic, 2.5mg: chlorine, 200mg; niacin, 10.0mg cynobalamin (B_{12}), 10.0mg: menadoxine (k), 2.0mg. CMC (carboxyl methyl cellulose)

Experimental fish and management

C. gariepinus fingerlings with average weight of 6.96±0.01 g were obtained from the hatchery of Fisheries and Aquaculture Teaching and Research Farm, FUTA, Akure, Ondo state and were randomly distributed into plastic tanks (40cm x 30cm x 35cm) at ten fish per tank. Each treatment was in replicate groups of fish. The fish were then acclimated in the plastic tanks for 7 days while being fed on commercial diets. The fingerlings were not fed for 24 hours before they were started on the experimental diet to maintain a uniform stomach condition of the fish and to induce their appetite for the commencement of the feeding trial. The feeding trials runs for a period of 56 days and the fish were fed to satiation with their respective diets at 5% body weight twice daily between 9:00 am and 5:00pm. The weight of the fish in each tank was taken and recorded.

Proximate composition

Proximate compositions of diets and fish carcasses before and after experiment were performed according to AOAC (1990) for moisture content, fat, fibre and ash. The result from the proximate analysis of experimental diet is shown in Table 2.

Table 2: Proximate composition of experimental diets used in feeding *Clarias gariepinus*

INGREDIENTS	T1 (control)	T2	T3	T4	T5
Moisture content	9.28	7.99	11.04	11.21	12.62
Ash	8.27	7.39	10.10	11.15	7.15
Crude protein	39.63	40.14	39.88	40.08	39.65
Fat	10.53	13.03	12.55	12.50	13.04
Fibre	0.81	1.32	1.25	1.11	1.84
NFE	27.46	28.11	23.16	21.54	23.89
African basil mg/g	0.00	10.00	20.00	30.00	40.00

[1]NFE, Nitrogren Free Extract

Performance evaluation

Fish performances during the experiment were based on productivity indices on growth performance and nutrient utilization efficiencies as described by Fasakin *et al*. (1997) as follows;

Daily weight gain (g/fish/day) = (final weight (FW) - Initial weight (IW)

Feed Intake (FI) = feed consumed (g fish^{-1}day^{-1})

Specific growth rate (SGR) = ($\log_e Wt - \log_e W_i$)/ T x 100

Where Wt = Final weight (g), W_i = Initial weight (g) and T = rearing periods (days),

Feed conversion ratio (FCR) = dry weight of feed (g)/ fish weight gain (g),

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

Hepatosomatic index

At the end of feeding trials, fish were removed from each treatment for hepatosomatic index according to Drury and Wellington (1980). Fish were randomly picked from each tank and dissected using a dissecting kit: each organ was carefully traced and cut out. The organ was placed on a petri dish and weighed (considering the weight of the petri dish). 2 fishes from each treatment were separated for liver samples. The percentage relative organ weight was calculated as follows:

$$\text{HSI \%} = \frac{\text{Liver weight}}{\text{Somatic weight}} \times 100$$

Intestinosomatic index

At the end of experimental period, fish were also removed for the intestinosomatic index. Fish were randomly picked from each tank and dissected using a dissecting kit: each organ was carefully traced and cut out. The intestine was placed on a petri dish and weighed (considering the weight of the petri dish). 2 fishes from each treatment were separated for intestine samples. The percentage relative organ weight was calculated as follows:

$$\text{ISI \%} = \frac{\text{Intestine weight}}{\text{Somatic weight}} \times 100$$

Gustation

Gustation was determined following the procedure of the bioassay described by Sveinsson and Hara (1990). Fish were transported to the research farm and kept in rectangular-shaped experimental tanks containing up to 30 litres level of water. The fish were fed *ad libitum* twice a day. Pellets were dropped into the tanks one by one with an interval of 10-15 minutes. In each treatment, several parameters were registered, which are; the treatment tank, number of grasps at the pellet, retention time of the pellet after the first grasp and the total retention time of all grasps; whether the pellet was swallowed or finally rejected by the fish. If the fish does not grasp the pellet within two minutes, the trial was stopped and not registered. The pellets containing different treatments were offered to the fish at random sequence. Pellets rejected or not swallowed were removed from the aquarium immediately after the end of each time.

Data analysis

Biological data obtained were subjected to one-way analysis of variance (ANOVA). Where means were significantly different, they were compared with Duncan’s multiple range test (Zar, 1984). Surface plot of the gustation was done with the aid of mini tab version 17.0.

RESULTS AND DISCUSSION

The results as shown in Table 3 showed that the productivity rates of the fish (SGR, WG, FCR, PER, HSI and ISI) were significantly different ($P<0.05$) from other groups of fish fed *O. gratissimum* supplemented diets. Fish fed 40 mg/g African basil leaf showed the highest percentage weight gain and specific growth rate of 8.41±0.76 and 1.41±0.08 respectively. The feed conversion ratio among in the treatments showed significance difference ($P<0.05$). However, the best feed conversion ratio (FCR) was recorded in fish fed 10mg/g *O. gratissimum* leaf meal, which was significantly different from other dietary treatments ($P<0.05$). Statistically, there was significantly increased growth and nutritional performance of fish in this study with increasing inclusion levels of *O. gratissimum* ($P<0.05$). Feed related mortality was not observed during the feeding trials. The present study suggests that *O. gratisimum* leaf meal may be included in the diets of *Clarias gariepinus* at an inclusion level of 40mg/g.

Table 3: Growth performance and histometrics of *C. gariepinus* (mean ± se)

PARAMETERS	D1(control)	D2	D3	D4	D5
IW (g $fish^{-1}$ $tank^{-1}$)	$6.94±0.01^{a}$	$6.89±0.01^{a}$	$6.92±0.00^{a}$	$6.96±0.01^{a}$	$6.95±0.01^{a}$
FW (g $fish^{-1}$ $tank^{-1}$)	$14.3±0.31^{ab}$	$4.36±0.17^{ab}$	$13.75±0.21^{a}$	$3.62±0.08^{a}$	$15.36±0.77^{b}$
Weight gain (g)	$7.08±0.31^{ab}$	$.46±0.81^{ab}$	$6.81±0.17^{a}$	$6.65±0.06^{a}$	$8.41±0.76^{b}$
FI (g $fish^{1}day^{1}$)	$0.67±0.02^{a}$	$0.71±0.10^{a}$	$0.68±0.01^{a}$	$0.68±0.01^{a}$	$0.74±0.50^{a}$
SGR	$1.25±0.04^{ab}$	$1.30±0.02^{ab}$	$1.22±0.01^{a}$	$1.19±0.01^{a}$	$1.41±0.08^{b}$
FCR	$0.17±0.01^{ab}$	$0.17±0.00^{a}$	$0.18±0.00^{ab}$	$0.18±0.00^{ab}$	$0.18±0.00^{b}$
Survival (%)	$96.66±3.33^{a}$	$89.99±3.33^{a}$	$96.66±3.33^{a}$	$100.00±0.00^{a}$	$93.33±6.67^{a}$
HSI	$4.05±0.95^{a}$	$3.50±1.30^{a}$	$4.65±0.35^{a}$	$2.30±1.00^{a}$	$2.15±0.55^{a}$
ISI	$2.95±1.55^{a}$	$2.85±1.25^{a}$	$2.20±0.60^{a}$	$2.10±1.20^{a}$	$1.80±0.20^{a}$

Figures in each row having the same superscript are significantly different (P<0.05)

HS - Hepatosomatic Index

ISI – Intestinosomatic Index

The result of the proximate analysis of catfish carcasses at the beginning and the end of the experimental period was shown in Table 4. There was significant difference (P < 0.05) between the initial and the final body composition of fish used during the experiment with respect to moisture, crude protein, crude lipid and ash content. For crude protein, the highest was recorded for fish fed 40mg/g of basil leaf supplementation.

Table 4: Proximate content of African catfish fed with experimental diets

	INITIAL	T1	T2	T3	T4	T5
MC	$2.94±0.00^{a}$	$8.21±0.05^{e}$	$7.08±0.06^{d}$	$5.47±0.26^{c}$	$4.41±0.18^{b}$	$3.02±0.02^{a}$
Ash	$11.20±0.02^{a}$	$11.98±0.11^{b}$	$13.16±0.08^{c}$	$14.23±0.07^{d}$	$15.38±0.22^{e}$	$17.14±0.20^{f}$
Fat	$24.92±0.03^{a}$	$17.42±0.07^{c}$	$17.41±0.53^{c}$	$15.28±0.15^{b}$	$14.73±0.32^{b}$	$13.40±0.21^{a}$
CP	$52.12±0.08^{b}$	$49.34±0.78^{a}$	$52.92±0.00^{b}$	$58.14±0.01^{c}$	$58.55±0.00^{c}$	$60.69±1.22^{d}$
NFE	$2.94±0.02^{a}$	$13.04±0.88^{d}$	$9.43±0.67^{c}$	$6.87±0.16^{b}$	$6.91±0.27^{b}$	$5.74±0.82^{b}$

Figures in each row having the same superscripts are not significantly different (P> 0.05)

However, there was no significant difference (P > 0.05) in the protein content of fish fed diet T3 and T4 (20mg/g and 30mg/g respectively) and fish fed with the control diet and T2 (10mg/g). There was significant difference (P< 0.05) in the crude protein of control diet and T1, T3, T4 and T5. The highest crude lipid was recorded in the final body composition of the fish used for experiment which was significantly different (P< 0.05) from that of the initial body composition. There was no significant difference (P>0.05) in lipid content of fish fed with control diet and T2 (10mg/g). Also, there was no significant difference (P>0.05) between fish fed with 20mg/g and 30mg/g of basil leaf meal. However, there was significant difference (P< 0.05) in the lipid content fed with control diet and the treatment with highest basil leaf meal supplementation. The highest ash content was recorded in fish fed with 40mg/g of basil. There was significant difference (P<0.05) in ash content among the fish fed with different inclusion level of basil leaf meal supplementation.

The present study showed a significant improvement of fish growth, feed utilization and gustation activities by the administration of basil leaf to diet compared to the control. The beneficial effects of the inclusion of basil leaf meal on fish growth is in agreement with higher protein retention and improved digestive enzymes activities reported by El Dhakar *et al* (2015) in Gilthead sea bream, *Sparus aurata* diet. The results indicated that basil has some ability to increase the growth performance of African catfish fed *O. gratissimum.* African basil contains active compounds such as planteose, mucilage, polysaccharides and fixed oil that consists of linoleic acid (50%), linolenic acid (22%), oleic acid (15%) as well as 8% unsaturated fatty acids (List and Hörhammer, 1977) was higher than those fed with the control. The apparent gustation coefficients represented by the values of number of grasps, time of grasps and retention time in *Clarias gariepinus* fed basil leaf meal was higher than those fed with the control diets (Fig. 1). The values of number of grasps, time of grasps and retention time of fish fed with the highest inclusion level of basil leaf meal supplementation (40mg/g) recorded in this study were in agreement with the values reported in the dietary supplementation of CT (Condensed tannins) to enhance gustation through LMM (Leaf Meal Mixture) which significantly improved the retention, and inhibited the different developmental stages of *Haemonchus contours* in experimental sheep (Pathak, 2013).

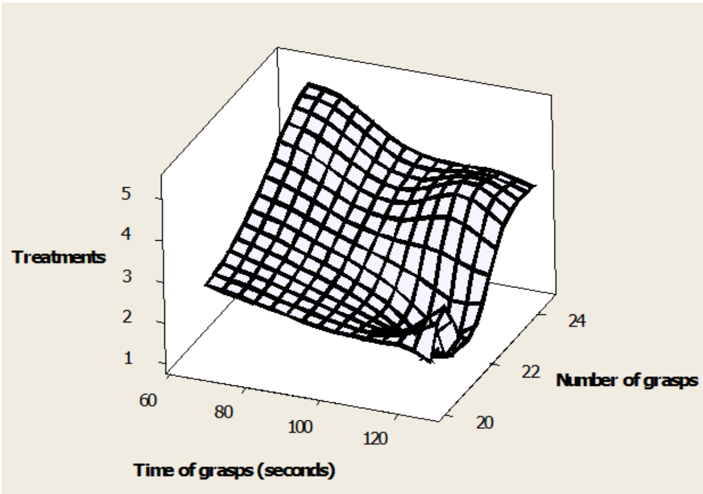

Fig. 1: Surface plot of the gustation of *C. gariepinus* fed experimental diets showing the number and time of grasps.

There were no apparent histometric alterations observed in the liver and intestines of fish fed the African basil leaf supplemented diets compared with the control diet. Generally, this study showed that dietary *O. gratissimum* can be incorporated up to 40mg/g into the feed of *C. gariepinus*, without adverse effect on the growth performance and health of the fish compared to the control. However, further research is necessary to evaluate the effect of dietary basil supplementation on the olfaction of African catfish, *C. gariepinus*. African basil appeal to the taste of fish by improving their gustation and it has no negative effect on growth performance of fish fed with inclusion level ranging from 10mg/g to 40mg/g during the experimental period.

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