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EFFECT OF AFRICAN BASIL (*Ocimum gratissimum*) ON OREXIGENIC FACTORS AND ZOOTECHNICAL PERFORMANCE OF AFRICAN CATFISH (*Clarias gariepinus*)

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Abstract: **Appetite and food intake in fish is not only affected by external factors but also endogenous factors such as the hypothalamus which is the hub controlling appetite, energy balance and integration of peripheral signals related to appetite, feed intake, digestion and metabolism. African basil, *Ocimmum gratissimum* leaf is proven to improve food palatability and stimulate appetite through its characteristic aroma. In this study, the effects of African basil *Ocimmum gratissimum* leafmeal on orexigenic factors and zootechnical performance in African catfish *Clarias gariepinus* diet was assessed for a period of 56 days. Six isonitrogenous diets were formulated at different inclusion levels of *Ocimmum gratissimum* leaves at 0 mg/g, 10 mg/g, 20 mg/g, 30mg/g, 40 mg/g, 50 mg/g denoted as T1, T2, T3, T4, T5 and T6 respectively. *C. gariepinus* fingerlings (2.57±0.37g) were randomly divided into the replicates of six treatments group. The test fish were randomly distributed into 18 plastic tanks with the dimension of 40×30×35 (cm)^3 at the stocking density of 10 fish per tank. After the feeding trials, histology of orexigenic factors, growth performance and nutrient utilisation parameters were assessed. *C. gariepinus* fingerlings fed 30mg/g diet of *O. gratissimum* recorded the best growth performance in terms of feed intake, body weight gain, feed conversion ratio (FCR) and specific growth rate (SGR). There was a significant increase ($p<0.05$) in growth and nutritional performance of *C. gariepinus* fingerlings with increasing inclusion of *O. gratissimum* leaves ($P<0.05$) peaking at the inclusion level of 30mg/g in treatment 4 (T4). The brain histology of fish in this study revealed that there was positive enhancement of glial, ghrelin and olfaction cells which improved the appetite, feed intake and zootechnical performance in *C. gariepinus*. This study therefore confirmed the endogenous and physiological benefits of *O. gratissimum* on the the appetite and zootechnical performance of *C. gariepinus*.**

Keywords: **Appetite, aquafeed, digestion, fish farming, growth, physiology.**

1. INTRODUCTION

World's human population is increasing daily and so is the demand for food. A growing source of protein is fish. The amount of fish caught in the wild has become stable over the past years; the farming of aquatic animals has been growing rapidly and its expected to continue to grow till at least 2025 (Diana, 2009). Globally, the production of African catfish is increasing rapidly, with an estimated global production of 194,000 tonnes in 2011 (FAO, 2012). The control of food intake and energy metabolism is vital for the development and survival of an organism (fish). These processes ensure optimal allocation of energy resources to cover the basic maintenance of metabolism and immune system, the cost of foraging and other daily activities, somatic growth, reproductive investment and sufficient energy stores to survive periods of low food availability. Food intake is affected by external factors such as temperature and photoperiodism, stress, predators and food

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availability as well as by internal factors such as genetics, life age, gut feeling and stored energy. The hypothalamus is the hub that controls appetite and energy balance and integrate peripheral signals related to food intake and digestion, metabolism and energy storage. These include not only the endocrine signals (gut peptides) but also other signals such as nutrient levels through central nutrient sensing systems and presence/absence of food in the gastrointestinal (GI) tract through vagal afferents projecting to the brain. Fishes represent over 30,000 species with an enormous variation in their ecological niches and habitats as well as life history adaptations, transition between life stages and feeding behaviours (Ronnestadetal., 2017). In the context of food intake and appetite control, common adaptation to extend periods of starvation or periods of abundance food availability are of particular interest. Also, the large variation appetite between species and within a specie (individual variation) are intriguing). A large fraction of fish species has indeterminate growth, i.e these species continue to grow during their own life span (Mommsen, 2001). This contracts with growth in mammals and other model animals including zebra fish (Danio rerio) which reach a maximum length size as adults. Thus, while control of appetite and food intake is often viewed as a behavioural component of maintaining an energy balance, the general concept of energy homeostasis needs to be used with caution.

The rapid growth of aquaculture activities causes public concern about the sustainability in animal welfare. Although animal welfare has been defined in different ways (Ohl and Putman, 2014). Key to all definition is that poor welfare is associated with overtaxing the adaptive capacity of animals (allostatic overload; McEwen and Wingfield, 2003) which may result in chronic stress-related physiology and behaviour, pathology and increased mortality. Successive or cumulative exposure to stressors may compromise the adaptive capacity of an animal and lead to allostatic overload and poor welfare (Korte et, al.,2007; Ohl and van der Staay 2012). To minimise the stress, chemicals such as tricaine methane sulphate is been used but the uses of chemicals are being discourage because of its residual effect in the tissue of the fish, expensive nature of the drugs and increasing advocate for organic aquaculture. However, previous studies has reported the use of clove oil as anaesthetic material in fish (Fujimoto et al., 2018). Fish handlers usually sustain injuries during handling of *C. gariepinus* because it has several fins making it difficult to handle. Activities such as blood taking and transportation are common practices in today aquaculture research therefore, expose fish to trauma, pester anxiety leading to loss of mobility and loss of balancing among others. *O. gratissimum* (Lamiacea), commonly known as ”alfavaca” also known as “efinrin” in Yoruba language is a native to Africa. In Nigeria, the plant grows virtually in all regions. It could be found in many farms, residential and industrial areas. It grows and survive well in south-west of Nigeria and could be found at backyard where it is not intentionally planted.

2. MATERIALS AND METHODS

Experimental site

The study was carried out at the Teaching and Research farm of the Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, Ondo State for a period of 56days.

Experimental System and Fish

Clarias gariepinus with average weight of 25.75± 0.25g were obtained from the hatchery of the Fisheries and Aquaculture Teaching and Research Farm, FUTA, Akure, Ondo State. The fish were distributed randomly into plastic tanks (40cm × 30cm × 35cm) at ten fish per tank. Each treatment was in replicate groups of fish. They were acclimated for seven days while being fed commercial diet. The juveniles were not fed for 24 hours before started on the experimental diet maintain a uniform stomach condition of the fish and to induce their appetite for the commencement of the feeding trial. During the feeding trial, fish were fed to satiation with their respective diets twice daily between 8:00amand 4:00pm. Feed were administered bit by bit to check the rate at which the fish picked the feeds. The weight of the fish in each tank was taken and recorded every week. Their agility (activeness) was checked at each feeding period and water changed regularly to ensure the water was conducive for growth and survival of the fish.

Plant material

Ocimum gratissimum (scent leave) was harvested from grace flower gardens opposite Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) in Ile-Ife, Osun State South-Western part of Nigeria. The leaves were collected and dried for 7 days under sun to reduce its moisture content.

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Experimental Diets

The feed ingredients for the experimental diet were purchased from K2 feed mill Lafe, Akure, Ondo State, Nigeria. The ingredients include yellow maize, soybean mea, fishmeal, vegetable oil, vitamin and mineral premixes and starch. Six diets (40%CP) were formulated to contain the treatment (*Ocimum gratissimum*) at (0, 10, 20, 30, 40 and 50 mg/g) and labelled as T1 to T6. The prepared scent leave powder were mixed thoroughly with other ingredients. The ingredient were weighed with an electronic weighing balance (Model PB3002). The dough was pelleted using a packed in polythene bags, sealed and marked according to treatments and stored in a container kept in a cool and dried place. A simple diet was taken for proximate analysis according to the method of AOAC (1990).

Table 1: Gross Composition (g/Kg) of the Experiment Diets for C. gariepinus

Ingredient	T1	T2	T3	T4	T5	T6
Fishmeal (72%)	265	265	265	265	265	265
Soya bean meal	425	425	425	425	425	425
Maize (10%)	210	210	210	210	210	210
Vegetable oil	60	60	60	60	60	60
Vitamin and mineral premix	20	20	20	20	20	20
Starch	20	20	20	20	20	20
O. gratissimum (g/mg)	0	10	20	30	40	50

Weighing of experimental fish

Fish were batch-weighed after acclimatization using an electronic balance (Model PB 3002) and recorded as mean weight. This continued bi-weekly during the feeding trials until termination of experiment.

Feeding of Experimental Fish

The diets were fed to the fish twice daily between 08:00 – 9:00 and 16:00 -17:00 hours to apparent satiation for 70 days. 75% of water in each tank was changed twice in a week but leftover feed and faeces were siphoned daily to avoid accumulation of excess ammonia and water quality deterioration.

Monitoring of Water Quality

Culture water was changed twice in a week at the early hour of the day (morning) to maintain good water quality. Temperature was measured with thermometer (YSI-DO 550 U.S.A), pH was measured with a pH meter (Hanna H198106 model), and dissolved oxygen was measured using dissolved oxygen test kit (JPP-607 model).

Growth and Nutrient Utilisation Indices

Calculation of the performance data was according to Takeuchi, (1988) and Tacon, (1990). At the end of the experiment, fish were counted and weighed. The growth parameters and feed utilisation were calculated as follows:

Weight Gained (g) = Final weight gained - Initial weight

Specific Growth Rate (SGR)

This was calculated from data on changes of body weight over a given time interval; SGW (% per day) = $\frac{(logW2-logW1)\times 100}{T2-T1}$

Feed Intake

This was obtained by adding daily mean feed intake (DFI) of fish under each treatment for the experiment period.

Feed Conversion Ratio (FCR) = Feed intake (g)/ weight gained (g)

Feed Efficiency Ratio (FER) = Weight gained (g) / feed intake (g)

Survival Ratio (%) = Number of fish harvested / number of fish stocked × 100

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Proximate Composition of Experimental Feed

Proximate analysis was carried out on the formulated feed and experimental fish according to AOAC, 2010 to determine the moisture ash, crude protein, crude lipid, crude fibre and nitrogen free extract (NFE). Gross energy (GE) was calculated as 5.65,9.45, 4.1kcal/g for protein, fat and carbohydrate respectively (NCR,1993).

Statistical Analyses

Data collected was subjected to one-way analyses of variance (ANOVA) as described by Steel Torrie, 1990 followed by Duncan New Multiple Range Test (Duncan, 1955) to separate differences among the means. The statistical analyses was performed with the aid of the computer software SPSS (Statistical for Social Science Version 22).

3. RESULTS

The result of the proximate carried out on the experimental feed at the beginning and end of the experimental feed was shown in Table 2. There was significant difference (P<0.05)

Histology of the Gastro Intestinal Tract and brain of African Catfish *C. Gariepinus* Fed with *O.gratissimum* at different inclusion level.

The histology of the intestine of African catfish *C.gariepinus* fed with *O.gratissimum* inclusion in T1 (control) shows moderate stunting vili compared to the other treatments which have no visible lesions. However, there were significance differences (p>0.05) when T1 (control) was compared with T2, T3, T4, and T6, but none between T1 and T5 which also exhibit slightly reduced stunted villi.

The histology of the brain of *C.gariepinus* fed *O.gratissimum* shows that there was significance difference (p>0.05) when T1 was compared with other five treatments. However, the histology revealed that T4 (30mg/g) shows higher population of gilal, ghrelin and orexigenic cells.

Proximate Composition of Experimental Diets

The result of the proximate carried out on the experimental feed at the beginning and end of the experimental feed was shown in Table 2. There was significant difference (p<0.05) in the moisture content when T1 (control) was compared with T2, T3, T4, T5 and T6 respectively. However, there was no significance difference (p>0.05) between T3 and T4. For crude protein, the highest was recorded for T1 (Control diet) with no supplement. However, there was no significant different (p>0.05) in the crude protein of experimental feed with 10mg/g in T2 and 30mg/g in T4 inclusion of *Ocimum.gratissimum*. there was a significant difference (p<0.05) in the crude protein of experimental feed with 0mg/g (T1), 10mg/g (T2), 20mg/g (T3), 30mg/g (T4), 40mg/g (T5) and 50mg/g (T6) inclusion of *Ocimum.gratissimum*. The highest crude lipid was recorded in feed with 40mg/g (T5). There was no significant difference (p>0.05) in the crude lipid of feed T2, T3 and T6. However, there was significance difference (P<0.05) when T1 was compared with other five treatment. The highest crude fibre was recorded in the diet with 20mg/g (T3) inclusion of *Ocimum. Gratissimum*. There was no significant difference (P>0.05) in the Crude fibre of (T1, and T2). There was no significant difference (P>0.05) in the crude fibre of T5 and T6. There was significant difference in Crude fibre of feed with 0mg/g, 10mg/g and 30mg/g(T1, T3 and T4).

Table 2: Proximate analysis of experimental feed.

Treatment	T1	T2	T3	T4	T5	T6
MC	10.84 ± 0.00^{b}	9.41 ± 0.00^{a}	9.85 ± 0.00^{ab}	9.70 ± 0.01^{ab}	10.88 ± 0.00^{bc}	11.03 ± 0.00^{c}
AC	8.71 ± 0.00^{b}	7.95 ± 0.05^{ab}	8.70 ± 0.00^{b}	7.06 ± 0.05^{a}	7.95 ± 0.00^{ab}	8.50 ± 0.00^{b}
LC	11.00 ± 0.00^{b}	10.62 ± 0.02^{a}	10.71 ± 0.00^{a}	11.0 ± 0.04^{bc}	11.85 ± 0.00^{c}	10.82 ± 0.00^{a}
CP	40.68 ± 0.00^{c}	39.73 ± 0.04^{b}	40.46 ± 0.00^{b}	39.97 ± 0.04^{b}	39.98 ± 0.00^{b}	38.03 ± 0.00^{a}
CF	4.72 ± 0.00^{ab}	4.62 ± 0.00^{ab}	4.87 ± 0.00^{b}	4.86 ± 0.09^{b}	4.30 ± 0.00^{a}	4.42 ± 0.00^{a}
NFE	25.03 ± 0.00^{a}	27.67 ± 0.07^{c}	25.38 ± 0.00^{b}	27.34 ± 0.23^{c}	25.04±0.00a	27.20 ± 0.00^{c}

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

MC-Moisture content LC – Lipid content CP- Crude protein NFE- Nitrogen free extract

Growth and Nutrient Utilization of *C.gariepinus* Fed with Experimental Diets

The growth parameter of the experimental fish are presented below in Table 4. The initial body weight gain varied approximately from 2.57g to 2.62g. However, there were no significant diffeences (P>0.05) in the initial body weight recorded among the treatment. The final body weight varied approximately from 10.17g to 14.17g with T1 (control) having the lowest while T4 had the highest. However, there were significant differences (P>0.05) in the final body weight recorded when T1 (control) was compared with other treatments. The body weight gain varied from 7.55g in T1 and 11.60g in T4. However, there were significant differences (P>0.05) in the body weight gain recorded when T1 was compared with other treatments. The feed intake varied from approximately 14.33g in T6 and 17.64.6g in T4. However, there were significant differences (P>0.05) recorded when T1 (control) was compared with T2, T3, T4, T5 and T6. The feed conversion ratio varied from 1.52 in T4 and 2.21 in T1. However, there was significant differences (P>0.05) in the feed conversion ratio recorded when T1 (control) was compared with other treatments. The feed efficiency ratio also varied from 0.45 in T1 and 0.65 in T4. However, there was significant differences (P>0.05) in the feed efficiency ratio recorded when T1 was compared with other treatments. The specific growth rate varied from 1.56 in T1 and 1.90 in T4. However, there were no significant differences (P<0.05) in the specific growth rate recorded when T1 (control) compared with other treatment. The percentage survival varied from 73.20% in T1 and 83.33% in T4. However, there were significant differences (P>0.05) in the percentage survival recorded between T1 and other five treatments. There was significant differences (P<0.05) in the percentage survival recorded when T1 (control) is compared with T2, T3, T4, T5, and T6.

Table 3: Growth performance and nutrient utilization of *Clarias gariepinus* fed with experimental diets.

Parameter	T1(control)	T2	T3	T4	T5	T6
IW(g)	2.62 ± 0.266^a	2.58 ± 0.83^a	2.59 ± 0.93^a	2.57 ± 0.37^a	2.57 ± 0.77^a	2.57 ± 0.70^a
FW(g)	10.17 ± 1.83^a	13.25 ± 0.00^b	13.36 ± 0.37^b	14.17 ± 0.67^c	14.01 ± 0.13^c	11.43 ± 77^{ab}
WG(g)	7.55 ± 0.57^a	10.67 ± 0.83^b	10.77 ± 0.86^b	11.60 ± 0.30^c	11.4 ± 0.64^c	8.86 ± 00.70^{ab}
FI	16.71 ± 0.97^{ab}	16.82 ± 1.30^{ab}	16.96 ± 1.28^b	17.64 ± 1.62^c	17.55 ± 0.14^c	14.33 ± 0.15^a
FCR	2.21 ± 0.15^c	1.57 ± 0.17^b	1.57 ± 0.70^b	1.52 ± 0.51^a	1.53 ± 0.15^a	1.60 ± 0.15^b
FER	0.45 ± 0.05^a	0.63 ± 0.05^b	0.63 ± 0.05^b	0.65 ± 0.03^c	0.65 ± 0.02^c	0.62 ± 0.05^b
SGR	1.56 ± 0.02^c	1.83 ± 0.00^a	1.84 ± 0.01^a	1.90 ± 0.02^a	1.89 ± 0.01^a	1.69 ± 0.01^b
Survival (%)	73.20 ± 0.33^a	80.00 ± 2.00^b	83.33 ± 2.48^c	83.33 ± 2.52^c	80.30 ± 0.00^b	80.30 ± 0.00^b

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

IW= Initial weight, FW= Final weight Gain, WG= Weight Gain, FI= Feed Intake, SGR= Specific growth rate, FCR= Feed conversion ratio.

Water Quality Parameters

The water quality parameters measured in the culture media is presented in Table 5: Water Temperature ranges between 26.4^0C and 26.6^0C. However, there were no significance differences (P>0.05) in the temperature recorded among the treatments. Dissolved oxygen ranges between 4.7mg/l (T3) and 5.3mg/l (T2). However, there were significant differences (P>0.05) in the dissolved oxygen level recorded when T1 (control) was compared with T2, T3, T4, T5 and T6, but none between T1 and T3. Hydrogen ion concentration ranges between 6.8 and 6.9. However, there was no significance difference (P>0.05) in the Hydrogen ion concentration recorded among the treatment. The water quality parameters recorded are within recommendation ranges for Clarias culture.

Table 4: Water quality parameters of the culture water.

Parameters	T1	T2	T3	T4	T5	T6
Temperature (^{0}C)	26.5 ± 0.05^a	26.6 ± 0.09^a	26.5 ± 0.09^a	26.4 ± 0.02^a	26.4 ± 0.0^a	26.4 ± 0.0^a
pH	6.9 ± 0.01^a	6.8 ± 0.03^a	6.9 ± 0.01^a	6.9 ± 0.01^a	6.9 ± 0.01^a	6.9 ± 0.01^a
DO (mg/l)	4.9 ± 0.02^a	5.3 ± 0.01^b	4.7 ± 0.06^a	5.1 ± 0.02^{ab}	5.2 ± 0.00^b	5.0 ± 0.21^{ab}

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4. DISCUSSION

In the present study *C.gariepinus* fed *O.gratissimum* supplemented diets showed improved growth performance indices over control with an optimum inclusion level of 30mg/g. this is similar to the past work of Afe et al.,(2019) where *Ocimum gratissimum* improved the growth of *H.bidorsalis*. 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.

The orexigenic effect of *Occimum gratissimum* on *clarias gariepinus* using the feed intake (FI) feed conversion ratio (FCR), feed efficiency ratio (FER) and specific growth rate (SGR) as marker showed that the optimum inclusion level of *Ocimum gratissimum* at 30mg/g was linked to improve acceptability and nutrient utilization in *C.Gariepinus*. This could be as a result of the ability of certain chemical reactions and enzymes capable of improving olfaction, palatability of the diet into a more absorbable nutrient for the host Taconet et al., (2009) described the feeding of phytogens as a way of improving the appetite and growth performance of the farmed fish. This study is also in related to Jianget et al., (2021) who reported that dietary stevioside supplementation increase feed intake by altering the hypothalamic transcriptome profile and gut microbiota in broiler chickens. 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 gradual increase in weight gain observed in the present study agreed with the reports of Saleh et al., (2015) who observed gradual improvement 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 were similar when compared with those reported by Gbadamosi and Salako (2014) in *C. gariepinus* fed *O.gratissimum* supplemented diets. It was concluded that *O.gratissimum* contained metabolites which improved gustation in *O.niloticus*. 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 cost. Soltan El-Laithy (2008).

The result obtained for FCR in this study also agreed with the reports of Gbadamosi et al., (2020) who fed C.gariepinus fingerlings diets containing *O. gratissimum* leaf-meal to check for the ameliorative effect when subjected to pathogenic and transportation induced stress. The inclusion of dietary *O.gratissimum* may significantly help to stimulate appetite and improve nutrition through production of vitamins, detoxifying toxic compounds in the diet and also breaking down of indigestible compounds (Hagi and Hoshino,2009).

The physicochemical parameters of water observed in this study were temperature, dissolved oxygen and PH and the values ranges between 26-26.6℃,4.7-5.3mg/I and 6.9 respectively and these values were within acceptable range between recommended for pisciculture (Viveen et al.,1985; Abdelhamid, 2010; Abdelhakim et al., 2002). However the optimum growth of African catfish *clarias gariepinus,* required 28-30℃, 5mg/I dissolved oxygen 6.5-9.0 PH (chapman2000) there was no significant difference in the water quality parameter observed this is similar to the findings of Wang and Zirong, (2006) who confirmed that there was no noticeable effect in the water parameters when Carp was fed with Basilicus species.

Histology was used in this work to access the orexigenic factors in African catfish *C.gariepinus* that was used for the experiment. This agrees with the work of Ayotunde et al., (2011) where histology was used to investigate changes in the gill, liver, intestine and skin of adult Nile tilapia *O.niloticus* exposed to Moringa oleifera seed at different inclusion level. The gastrointestinal tract of C.gariepinus showed apparently normal structure no visible lesions which is similar to the work of Najmeh et ai., (2019) who investigated the role of dietary spirulina platensis in improving mucosal immune responses and disease resistance of rainbow trout (Oncorhynchus mykiss)'', their result showed that spirulina platensis has no negative effect on the normal structure of the surface mucous in the intestine, skin, and gill. The brain of *C.gariepinus* fed with inclusion level of *O.garissimum* of 30mg/g and 40mg/g shows high density gilal and orexigenic cells which have been identified as sensitive neurons in fish brain responsible for olfaction and gestation (Gbadamosi and Salako, 2014). The Effect of dietary purslane (Portulaca oleracea L.) leaves powder on growth, immunostimulation, and health of Nile tilapia, *Oreochromis niloticus* against Aeromonas hydrophila infection was investigated and it was reported that P. oleracea improved growth and prevent anorexia in *O.niloticus* (Abdel-razek et al., 2019).

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5. CONCLUSION

In the present study, the results showing that there was improvement in the growth parameters, feed utilization and appetite stimulation ((orexigenic factors) of African catfish fed with *Ocimum gratissum* at different inclusion levels. The best supplementation level is in T4 which has inclusion level of *O. gratissimum* at 30mg/g and recorded growth performance and nutrient utilization. The highest inclusion ratio of *O. gratissimum* do not prove to be the best according to the result obtained from the experiment. Also the histological analysis of the brain and the gastrointestinal tract parameters showing that the inclusion level of 30mg/g has the protective and enhancing capability on the brain of the experimental fish without any negative effect on the gastrointestinal tract of the fish.

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