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GROWTH AND NUTRIENT UTILIZATION OF *HETEROBRANCHUS BIDORSALIS* FED *EUCALYPTUS GLOBULUS* LEAF MEAL SUPPLEMENTED DIETS

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Abstract
The present study evaluates the growth performance and nutrient utilization of Heterobranchus bidorsalis fingerlings fed varying inclusion levels of Eucalyptus globulus leaf meal. H. bidorsalis fingerlings of initial mean weight of 12.24g ± 0.01 were evaluated for a period of 70 days. Five iso-nitrogenous experimental diets were formulated at varying inclusion levels; (0 (control), 0.5, 1.0, 1.5, and 2.0) g/100g of E. globulus leaf meal. Fish were randomly distributed into tanks at 10 fish/tank with each treatment in triplicate. At the expiration of the feeding trials, results showed significant differences (p<0.05) in growth and nutritional performance of H. bidorsalis fingerlings with increasing inclusion of E. globulus leaf meal. H. bidorsalis fingerlings fed 1.0g/100g diet of Eucalyptus globulus recorded the best growth performance with respect to body weight gain, feed conversion ratio (FCR) and specific growth rate (SGR). (P<0.05). A differential equation ($y = 13.433x^4 - 46.633x^3 + 38.922x^2 + 0.8983x + 14.56$; $R^2 = 1$) showed that optimum growth occurred at E. globulus supplementation level of approximately 0.85 g/100g. The study therefore showed that E. globulus leaf meal supplementation significantly improved growth and nutrient utilization parameters in the treated groups.

***Key words**: Heterobranchus bidorsalis, Eucalyptus globulus, growth performance, nutrient utilization*

Introduction
The quantity and quality of feed supply is a major determinant of a successful aquaculture venture since feed accounts for about 70 % of the total cost of aquaculture production with protein being the most required and most expensive component in fish diets (Garza de Yta, 2012). The use of phyto-additives as natural growth promoters is currently practiced in the aquaculture industry as they are considered to be less harmful to the environment than synthetic agrochemicals. This has renewed the interest in the research on phytoadditives as an ecologically safe alternative (Isman, 2006).

Recently, there have been series of research focused on developing new dietary supplementation strategies for aquaculture using phyto additives as growth promoters such as *Ocimum gratissimum* leaf powder (Gbadamosi and Salako 2015), Garlic and Onion powder (Saleh *et al.,* 2015), *Telfaria occidentalis* Leaf Powder, (Dada, 2017), *Zingiber officinale* root powder (Ibidunni *et al*., 2017), *Aloe barbadensis* leaf (Adegbesan *et al.,* 2018), and *Acacia auriculiformis* Leaf powder (Afe and Omosowone, 2019). Some of these additives used in fish feed are medicinal plants that can enhance efficiency of feed utilization and animal productive performance (Mohamed *et al.,* 2003).

H. bidorsalis is a highly economic species that performs better than other species in the family *Clariidae*. *H. bidorsalis* has a whitish appearance ventrally and dark olive dorsally (Adesulu and Syndenham, 2002). It is distinguished from other species in its genus by the presence of a dorsal fin which is longer than its adipose fin and the presence of black spot at its tail end (Reed *et al.,* 1967). *H. bidorsalis* can grow up to 1.2 m in length and 30 kgrelative to members of the genus *Clarias* and this is a proof that it has significant potentials for aquaculture (Reed *et al.,* 1967). It performs better in captivity as it attains maturity between 10–12 months of intensive culture (Fagbenro *et al.*, 1993; Adebayo and Fagbenro, 2004).

Eucalyptus globulus belongs to the family Myrtaceae; consisting of about 900 species and sub-species (Gilles *et al.,* 2010). It originated from Australia and has spread all over the world due to its adaptability, ease of cultivation and rapid growth. The major products obtained from the *Eucalyptus* include oil, cellulose, gum and wood. The essential oil extracted from the leaves is popularly used in pharmaceutical, cosmetics and food industries. Eucalyptus oil possess antimicrobial, antifungal, antiseptic, anti-inflammatory, wound healing, disinfectant, and expectorant properties (Estanislau *et al.,* 2001: Batish *et al.,* 2008). Sadlon and Lamson (2010) reported the use of *E. globulus* leaf in reducing nasal congestion during winter. *E. globulus* leaf has been reported to be rich in phytochemicals such as propanoids, tannins, alkaloids and flavonoids (Dixit *et al*., 2012). Despite the fact that the essential oils derived from *E. globulus* leaves are widely used, there is limited information on the use of the leaves in aquafeeds. Therefore this study evaluating the effect of *E. globulus* leaf powder on growth performance and nutrient utilization of *H. bidorsalis* fingerlings.

,**Materials and Methods**

Plant Materials

Fresh *E. globulus* leaves were collected from the field within the main campus of the Federal College of Agriculture, Jalingo, Taraba State, Nigeria. *E. globulus* leaves were identified and authenticated at the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure. 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 for inclusion in experimental diets.

Experimental Fish

Apparently healthy *H. bidorsalis* fingerlings (12.24 ± 0.01) g used in this study were obtained from the hatchery of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure. The fish were acclimated to laboratory conditions for 7 days during which they were fed a commercial diet - Durante of 40 % crude protein. Healthy fish were randomly distributed into 15 plastic tanks of dimension 40 ×30×35 cm^3 at a stocking density of 10 fish / tank.

Preparation of experimental diets

Five iso-nitrogenous diets of 40 % crude protein were prepared to fulfill the requirements of *H. bidorsalis* fingerlings according to Fagbenro *et al.,* 1993. The basal diet served as control (EG1) along with four others containing graded levels of *E. globulus*; 0.5 g / 100 g (EG2), 1.0 g / 100 g (EG3), 1.5 g / 100 g (EG4) and 2.0 g / 100 g (EG5) (Table 1). All dietary ingredients were milled into a small particle size and thoroughly mixed in a Hobart A-2007 mixer and pelletizer (Hobart Ltd, London, UK) to obtain a homogeneous mass with cassava starch added as a binder. The resultant mash was pressed without steam through a 2 mm diameter die attached to the Hobart pelleting machine. Pellets produced were air dried, broken up, sieved and kept refrigerated until the start of the feeding experiment.

Experimental set-up

This study was carried out at the Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure, Central Research Laboratory. The experiment is a completely randomized design consisting of five treatments, with different supplementation levels of powdered leaves of *Eucalyptus globulus.* The graded levels of *E. globulus* are 0.0 g (control), 0.5 g, 1.0 g, 1.5 g and 2.0 g per 100 g for each diet denoted a, EG1, EG2, EG3, EG4 and EG5 in diets 1, 2,3,4 and 5 for *H. bidorsalis* respectively. Each experimental diet was fed to five groups of fish in three replicates for seventy days. Fish were fed between 08:00-09:00h and 18:00-19:00h GMT. All groups were fed respective experimental diets at 5 % body weight / day. Each group of fish were batch weighed fortnightly to monitor growth and adjust feeding rates accordingly

Table 1: Ingredients and Proximate composition of experimental diets (on dry weight basis)

Ingredients	EG1(control)	EG2	EG3	EG4	EG5
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
cassava starch (binder)	2.0	2.0	2.0	2.0	2.0
E. globulus leaf powder	0	0.5	1.0	1.5	2.0
Proximate composition (%)					
Moisture	8.93	7.92	8.01	8.16	7.93
Ash	8.81	8.89	9.13	8.89	9.33
Crude lipid	10.96	11.01	11.03	11.05	11.06
Crude fibre	2.08	2.44	2.46	2.48	2.71
Crude protein	40.26	40.27	40.25	40.23	40.24
Carbohydrate	28.96	29.47	29.12	29.19	28.73
Gross energy(Kcal/g)	4.50	4.52	4.51	4.51	4.50

*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).

Experimental Analyses

Chemical compositions of dry *E. globulus* leaf, experimental diets and pooled fish samples were analyzed using methods described by Association of Official Analytical Chemists, AOAC (2005). Some water quality parameters such as temperature (°C), hydrogen-ion concentration (pH) and dissolved oxygen concentration (DOC) were monitored weekly using the Mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607model) respectively Fish in each experimental unit were batch-weighed (10 fish/tank or batch) fortnightly using top load balance (METLER TOLEDO, PB 8001 LONDON). The following growth and feed utilization parameters were evaluated following appropriate procedure:

Weight Gain

Weight gain = $W_2 - W_1$; where W_2 is the final weight of fish and W_1 is the initial weight of fish in each tank

Percentage Weight Gain

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

Specific Growth Rate

$$= \frac{100\left(\ln W_2 - \ln W_1\right)}{t}$$

Specific Growth Rate (SGR) ($\%d^{-1}$)

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

Survival Rate (%) = $N_1 / N_0 \times 100$

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)

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

Protein Efficiency Ratio (PER)

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

Protein Productive Value (PPV)

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

Feed efficiency ratio (FER)

FER = weight gain/ feed intake.

Statistical Analysis

All data generated were subjected to one-way ANOVA using the general linear mode function of Statistical Package for Social Science (SPSS), version 22.0 sooftware for windows and recorded as mean standard error. Statistical differences of treatment were determined using New Duncan's Multiple Range Ad-hoc Test at 95% confidence level (Dytham, 1999). Fourth degree polynomial regression analysis was used to determine *H. bidorsalis* fingerlings responses to varying levels of *E. globulus* in the formulated diet.

Results

The water quality parameters measured during the experiment showed that temperature ranged between 24.20 -25.80 °C, dissolved oxygen concentration ranged between 5.10 – 6.10 mg / l while pH was between 7.10 - 7.33.

The proximate compositions of *H. bidorsalis* fingerlings prior to feeding and after feeding with experimental diets are shown in Table 2. *H. bidorsalis* fingerlings used in this study had initial moisture content of 7.31 %, ash content of 5.56 %, crude lipid content of 16.76%±0.00, crude protein content of 46.44±0.00 and Nitrogen free extract content of 23.9%±0.01.

There was an increase in crude protein of fish when the final was compared with the initial. Fish fed *E. globulus* supplemented diets had a significantly ($p \leq 0.05$) higher crude protein content than fish fed the control diet (EG1). The highest ash, crude lipid and crude protein contents (4.26, 20.57, and 50.86 % respectively) were recorded in the fish fed with 2.0 g / 100g of *E. globulus* leaves (EG5. The lowest ash content and crude protein contents (3.33 and 48.10% respectively) were recorded in fish fed control diet (EG1) and the lowest crude lipid content (19.07 %) was recorded in the fish fed control diet (Eg1).

Table 2: Proximate composition of *Heterobranchus bidorsalis* fed *E. globulus* supplemented diets (%)

PARAMETERS (%)	INITIAL	EG1(control)	EG2	EG3	EG4	EG5
Moisture	7.31±0.01	7.91±0.00^{a}	8.08±0.02^{a}	8.45±0.05^{b}	7.82±0.11^{a}	8.42±0.16^{c}
Ash	5.56±0.01	3.33±0.00^{a}	4.16±0.05^{b}	4.37±0.02^{c}	4.01±0.02^{b}	4.26±0.09^{c}
Crude Lipid	16.76±0.00	19.07±0.00^{a}	19.30±0.06^{a}	19.80±0.05^{b}	20.41±0.05^{c}	20.57±0.04^{c}
Crude Protein	46.44±0.01	48.10±0.00^{a}	50.17±0.07^{b}	50.24±0.03^{b}	50.72±0.14^{c}	50.86±0.27^{c}
NFE*	23.92±0.01	21.59±0.00^{d}	18.29±0.11^{c}	17.14±0.12^{b}	17.04±0.27^{b}	15.89±0.01^{a}

NFE*= Nitrogen free extract

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

Results obtained from the growth and nutrient utilization indices of *H. bidorsalis* fingerlings fed *E. globulus* leaves powder at varying inclusion levels are shown in Table 3. The result showed significant differences ($P<0.05$) in the final weight, weight gain, percentage weight gain (PWG), protein productive value (PPV), and energy utilization of *H. bidorsalis* fingerlings fed with the experimental diets. Fish fed 1.0 % *E. globulus* powder had the highest percentage weight gain and specific growth rate of 173.45% and 1.41%/day respectively.

The feed conversion ratios among the treatments were significantly different ($p<0.05$) to those observed in the control (EG1). However, the best feed conversion ratio (FCR) was recorded in fish fed EG3 followed by fish fed EG4 powder inclusion level, which was not significantly different from other dietary treatments. Mortalities were recorded in fish fed Control diets, EG4 and EG5 with percentage survival of 70.00, 83.33 and 93.33 % respectively while fish fed EG2 and EG3 had 100 % survival. The optimum dietary *E. globulus* 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 weight gain and *E. globulus* concentration (g/100g) in the fish diets. A differential equation ($Y = -0.54x^4+1.9x^3-1.945x^2+0.665x$; $r = 1$) shows that optimum growth occurred at *E. globulus* level of approximately 0.85 g/100g (Figure 1).

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

PARAMETERS	EG1(control)	EG2	EG 3	EG4	EG5
Initial Mean Weight (g)	12.24±0.02^a	12.25±0.02^a	12.21±0.01^a	12.23±0.02^a	12.26±0.01^a
Final Mean Weight (g)	26.80±2.66^a	32.00±0.63^b	33.39±0.63^b	26.33±4.19^a	26.18±3.08^a
Mean Weight Gain (g)	14.56±2.64^a	19.75±0.61^b	21.18±3.88^b	14.10±4.22^a	13.91±3.09^a
% Weight Gain (%)	118.82±21.33^a	161.25±4.71^b	173.45±31.79^b	115.38±34.71^a	113.48±25.20^a
Survival (%)	70.00±0.00^a	100.00±0.00^b	100.00±10.00^b	83.33±12.02^a	93.33±6.67^{ab}
SGR (%)	1.12±0.14^a	1.38±0.03^a	1.41±0.17^a	1.06±0.22^a	1.06±0.18^a
Feed Intake (g)	35.53±0.10^a	40.49±0.08^c	36.85±0.07^b	33.28±0.09^a	34.50±0.04^a
FCR	2.44±0.48^a	2.05±0.06^a	1.74±0.40^a	2.36±0.64^a	2.48±0.77^a
PPV (%)	11.61±0.01^a	22.87±0.00^b	25.62±0.01^c	31.96±0.00^d	31.84±0.01^d
PER	1.02±0.08^a	1.21±0.04^a	1.43±0.06^a	1.05±0.09^a	1.00±0.02^a
FER	0.41±0.07^a	0.49±0.02^a	0.64±0.10^a	0.42±0.13^a	0.40±0.09^a
Energy Utilization (Kj/g)	97.91±0.01^b	95.45±0.01^a	98.93±0.01^b	98.29±0.01^b	107.08±0.01^c

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, FER: feed efficiency ratio.

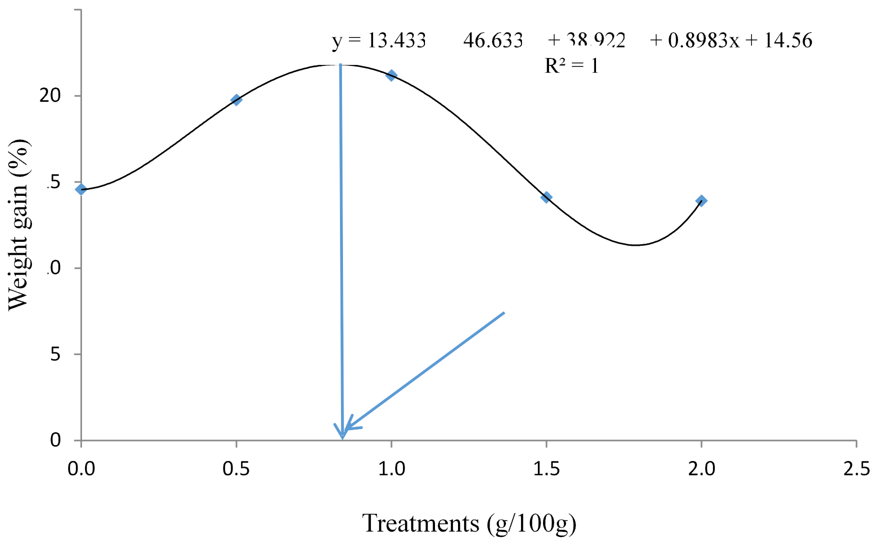

Figure 2: Fourth order degree polynomial regression analysis of weight gain to dietary *E. globulus*

Discussions

The study showed an improvement in growth and feed utilization parameters in all fish groups fed *E. globulus* supplemented diets at all inclusion levels. This indicates that the protein contents of the experimental diets improved the fish growth positively.

The pH, temperature and dissolved oxygen concentration values recorded in the present study were within acceptable limits as opined by Viveen *et al*., (1985) indicating that the physico-chemical parameters of the culture media had no influence on results obtained in the present study.

There was a significant increase in whole body crude protein content in treated groups over the control. This increase could be due to the inclusion of *E. globulus* leaf powder in the diets, leading to an increase in muscle free amino-acids to improve protein synthesis. Crude protein, lipid and ash contents in fish fed experimental diets showed better improvements when compared with what was reported by Solomon and Oluchi (2018) when *C. gariepinus* juveniles were fed with *Telfaria occidentalis* and *Moringa Oleifera* leaves supplemented diets. The significant increase observed in crude lipid and protein contents of fish fed diets containing *E. globulus* leave powder in this study were similar to the observations of Turan and Akyurt (2005) in *C. gariepinus* fed with diets containing Red clover. This suggests that *E. globulus* leaf powder contains growth improvement properties.

The highest weight gain, percentage weight gain and specific growth rate observed in fish group fed diet containing 1.0 % *E. globulus* (EG2) agrees 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. This could be as a result of antioxidants such as flavonoids and phenols present in the leaves of *E. globulus.*

The 1.0 g / 100g treatment resulted in a significantly higher growth rate than the 1.5 and 2.0 g / 100g supplementation levels and the growth rate considerably decreased beyond 1.0 g / 100 g supplementation level, indicating that the higher concentrations (1.5 and 2.0 g / 100 g *E. globulus* / kg feed) had lesser effects on the growth of *H. bidorsalis* and this may be due to the presence of saponins and phenolic compounds which have bitter taste that could have reduced the feed intake.

Similar observation was reported by Gbadamosi and Osungbemiro (2016) in *Clarias gariepinus* fed varying inclusion level of *Moringa Oleifera le*af meal. Turan (2006) also observed similar results when *Oreochromis aureus* was fed with diets containing *Trifolium pretense*. The fish fed experimental diets in this 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. Contrasting results were reported by Farhadi *et al.,* (2017) who observed that body weight gain in broilers fed dietary supplementation of *E. globulus* leaf powder decreased within 7 - 28 days of age and that broilers performance were not affected by dietary inclusion of *E. globulus* at 7 - 42 days old.

Results of this study however contradict the reports of Jimoh *et al.,* (2014) who observed a decrease in growth and nutrient utilization parameters of *Clarias gariepinus* fed diets containing *Chrysophyllum albidum* seed meal. It also disagrees with the observations of Adewole and Faturoti (2017) who reported that *Clarias gariepinus* had highest mean weight gain when fed diets containing 0.25 % *O. gratissimum* Leaf meal. Furthermore, this is also in contrast with the reports of Ochang *et al.,* (2017) who reported decreased weight gain in *C. gariepinus* as the inclusion level of *Afzelia africana* increased.

The best FCR was obtained in the EG2 group having an inclusion level of 1.0% *E. globulus*. This is in contrast with the reports of Jimoh *et al*., (2014) who reported best FCR in the control group of *C. gariepinus* over those fed *Chrysophyllum albidum* seed meal supplemented diets. This suggests that supplementation of fish diets with *E. globulus* optimized protein use for the growth which can decrease the quantity of feed necessary for fish growth and hence, production costs.

The average Protein Productive Value (PPV%) of *H. bidorsalis* fed the experimental diets showed that fish treated with *E. globulus* had significantly higher PPV ($P \leq 0.05$) than those fed control diet. The values obtained for PPV and PER in this study were higher than those reported by Abdel-Hakim *et al*., (2010) when they fed *Oreochromis niloticus* with diets supplemented by either fresh or dried garlic. However, the values obtained for PER are lower than those reported by Oyegbile *et al*., (2017) when *Delonix regia* seed meal was included in the diets of *H. bidorsalis*. Better values recorded for both PPV and PER in the treatments over the control in this study indicates better nutrient utilization which could be attributed to the inclusion of *E. globulus* leaf meal in the diets. Energy Utilization (%) in fish fed *E. globulus* supplemented diets were higher than in those fed the control diet.

Percentage survival in fish fed *E. globulus* supplemented diets were significantly higher ($P \leq 0.05$)

than those fed the control diet, this could be because of the proper water management and suitability of *E. globulus* in the diet of *H. bidorsalis*. The result on survival agrees with the reports of Dada and Sonibare, (2015) when *Clarias gariepinus* was fed diets supplemented with *Chromolaena odorata.* However, Gbadamosi and Osungbemiro (2016) reported 100 % survival in African catfish *C. gariepinus* fed varying inclusion level of *Moringa oleifera*. This study showed that *E. globulus* supplementation generally enhances nutrient utilization, as reflected by the improvements in weight gain, food conversion ratio, specific growth rate, protein and feed efficiency ratios.

Conclusion

Dietary supplementation of *E. globulus* leaf powder in this study improved growth performance and nutrient utilization in *H. bidorsalis* fingerlings probably due to its growth promoting properties and there were no indications of negative effect of *E. globulus* on the water quality parameters. Therefore, *E. globulus* can be used a supplement in the diet of *H. bidorsalis* with an optimum dose of between 0.85-1.0g/100g. *E. globulus* is locally available and would provide a cheap source of dietary supplement to fish farmers.

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