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Growth Performance of *Heterobranchus longifilis*, (Valenciennes, 1840) Fingerlings Fed with *Nymphaea lotus* (Linné, 1753)

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Abstract

A 56-day feeding trial was performed to examine the effects of using *Nymphaea lotus* leaf meal (NLM) on growth performance, feed utilization, body composition, and survival rate of African catfish (*Heterobranchus longifilis*). Five treatment groups in triplicates with an average of the initial weight of 0.26±0.07g were fed diets incorporated with graded levels of NLM (0, 10, 20, 30 and 40%). The obtained results showed that higher final body weight (FBW), daily weight gain (DWG), and specific growth rate (SGR) were observed with fish fed the control diet (CD) and NLM at the level of 20% compared to those of fish fed other diets. Protein efficiency ratios (PER) were not altered with dietary treatment. In addition, feed conversion ratio (FCR) of fish groups fed up to 30% of NLM were not varied ($p>0.05$), meanwhile, daily feed intake of fish fed NLM diets exhibited lower values compared to those of fish fed CD. Final whole body proximate compositions of catfish showed no significant differences ($P>0.05$) except for the body total fat and ash contents. Fat contents of fish fed diets incorporated with NLM were significantly lower than those of fish fed the control diet (CD) ($P<0.05$). Meanwhile, ash contents of fish fed diets incorporated with high levels of NLM (30, 40%) were significantly lower than those of fish fed the control diet (CD) ($P<0.05$). Survival rates ranged between 72 - 80% and there were no differences among all groups ($P > 0.05$). Therefore, the results of the present study suggest that up to 20% of NLM protein can be incorporated in the diet of African catfish with no adverse effects on growth performance, feed utilization, and survival rate.

Keywords: *Nymphaea lotus*, protein source, catfish, *Heterobranchus longifilis*, growth performance

1. Introduction

Aquaculture promotion depends on many factors. Among these factors, feed is always at the top, and account for more than 50% of the total input costs (Craig & Helfrich, 2002). Among commonly used feed ingredients, fish meal is considered to be the best ingredient due to its compatibility with the protein requirement of fish and the major protein and lipid sources for aquafeeds (Abowei & Ekubo, 2006).

Since protein is the most expensive component used in artificial feeds, it is necessary to determine quantitative feed requirements in order to reduce the cost of fish feed. Therefore, there is an urgent need for low-cost, nutritionally balanced diets that can support increased production (Sivani et al., 2013). In addition, the reduced availability and rising cost of fishmeal have made it necessary to find cost-effective alternatives to fishmeal (Anvo et al., 2016; Otchoumou et al., 2011) .

Replacing fishmeal with a complex blend of plant proteins could reduce fish exposure to an individual anti-nutritional factor and improve growth performance (Borgeson, 2005). On the other hand, green plants have long been recognized as the potential source of the cheapest and most abundant protein (Fasuyi & Aletor, 2005). Much work has been done to include a large number of unconventional protein sources for use in feed or fish. In this respect, aquatic weeds or forage plants rich in protein can be considered as alternative sources of protein in aquatic feeds.

Nymphaea, aquatic weed, was a medicinal plant long used as natural therapeutic agents for the treatment of various illnesses since ancient times (Munglue, 2014). In aquaculture, herbal plants may provide active ingredients that would help to enhance the growth and the immune system of fish (Citarasu, 2014; Maqsood et al., 2011). Sivani et al., (2013) found that fish diets mixed with *Nymphaea* meal as an alternative protein source can produce better growth rates and survival in common carp (*Cyprinus carpio* L.) when compared to the basal diet. Munglue (2014) reported that *Nymphaea pubescens* stamens extract can be applied in fish diets as a natural feed additive to enhance fish growth, feed utilization efficiency and the immune system of fish.

In Côte d'Ivoire, the ability of African catfish *Heterobranchus longifilis* to use aquatic plants such as water lilies for growth and survival remains to be determined. Thus, this study was designed to evaluate the effects of the dietary inclusion of *Nymphaea lotus* leaf meal (NLM) on growth performance, feed utilization, body composition, and survival rate of African catfish (*Heterobranchus longifilis*) fingerlings.

2. Material and Methods

Fish

Catfish fingerlings with an average weight of 0.26 ± 0.07 g were obtained from the fish hatchery of the Oceanological Research Center in Abidjan (Côte d'Ivoire). The fish were acclimatized to experimental conditions for a period of 2 weeks before the start of the experiment, during which period they were fed a control diet (CD) with 35% protein manufactured at the Layo Aquaculture Research Station in Dabou. Prior to stocking the treatments, 50 fish were frozen (–20◦C) for subsequent whole-body proximate analysis. A total of 750 fish were then randomly divided into five different groups with three replicates containing 50 fish in each replicate in a completely randomized design.

Rearing conditions

Five isonitrogenous experimental diets (39.49±0.83) containing different inclusion levels of *Nymphaea lotus* meal (0, 10, 20, 30, and 40%) Table 1.

A 56-day feeding trial was conducted in 15 glass 50-L tanks containing 45 L of well-aerated water, 30% of which was replaced daily. Each treatment had three replications as per a completely randomized design. Fifty (50) *Heterobranchus longifilis* fingerling were randomly distributed in each tank after recording the individual wet weight (g). The feed was given *ad libitum* twice daily and the leftover feed was collected every day and weighted. Growth was assessed by sampling weekly, wherein the total weight of the sampled fish was recorded. At the end of the experiment, total weight and the number of surviving fish in each experimental unit were noted. Every day at 08:00 before feeding, water quality parameters temperature, dissolved oxygen were monitored (CRISON Oxi 330, WTW GmbH, Weilheim, Germany), and pH (pH 90, WTW GmbH, Weilheim, Germany).

Evaluation criteria for growth and feed utilization

The evaluation criteria were final weight gain (FWG), daily weight gain (DWG), feed conversion ratio (FCR), daily feed intake (DFI), protein efficiency ratio (PER), gross energy (GE) and nitrogen losses (NL) according to the following formulae:

-Daily weight gain (DWG) = (W2 –W 1)/t; W1 is the mean initial, W2 mean final fish weight and t the duration of the experiment.

-Feed conversion ratio (FCR) = feed fed (g dry weight)/weight gain (g).

-Protein efficiency ratio (PER) = weight gain (g)/protein consumed (g).

-Daily feed intake (DFI) = (daily feed consumed (g)/average total weight (g)) ×100.

-Gross energy = 22.2 × protein content + 38.9 × lipid content + 17.2 × carbohydrate content (kJ).

- NL= Nitrogen intake - Nitrogen gain

At the end of the experiment, fish from each experiment unit were killed and frozen (–20◦C) for further determination of whole-body composition. Proximate composition of diets and fish were analyzed using the following procedures: dry matter after drying at 105◦C for 24 h, fat by petroleum ether extraction (Soxtherm, Gerhardt, Germany), protein content (N × 6.25) by the Kjeldahl method after acid digestion, ash by combustion at 550◦C in a muffle furnace to a constant weight; crude fiber by acid/alkali digestion. The gross energy (GE) contents of the fish and diets were calculated from the fat and protein contents using the equivalents of 38.9 KJ.g−1 crude fats, 22.2 KJ.g−1 crude protein, 17.2 KJ.g−1 and carbohydrate (NFE).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Statistica, statistical software for Windows (release 7.1), and comparisons among treatment means were carried out by Duncan's test ($p < 0.05$) (Duncan, 1955).

Table 1. Formulation and proximate composition of the test diets

Ingredients (g/100g)	CD	Experimental diets			
		10%	20%	30%	40%
Fish meal	35.60	32.04	28.48	24.92	21.36
Soybean meal	34.00	38.00	39.00	45.00	47.00
Wheat bran	11.00	10.00	10.00	5.00	4.00
Maize meal	8.00	5.00	4.00	3.00	2.00
Nymphaea lotus meal (NLM)	0.00	3.56	7.12	10.68	14.24
Palm oil	8.40	8.40	8.40	8.40	8.40
Vitamin mixture[1]	1.50	1.50	1.50	1.50	1.50
Mineral mixture[2]	1.50	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00	100.00
Proximal analysis (%DM)					
Crude Protein	40.47	40.19	39.12	39.29	38.40
Total fat	13.82	13.53	13.23	12.96	12.68
Ash	10.04	9.48	8.92	8.32	7.69
Crude fiber	3.15	3.46	3.92	4.06	3.24
Nitrogen free extract (NFE)	30.07	31.18	32.91	33.80	35.50
Total carbohydrate	33.23	34.64	36.83	37.85	38.75
Digestible energy (KJ/100g)	16.42	16.41	16.29	16.36	16.29
P/E (mg protein / KJ ED)	24.65	24.57	24.01	24.02	23.58

[1]Composition for 1 kg of premix: Vitamin A 1,760,000 IU; Vitamin D3 880,000 IU; Vitamin E (dl-alphatocopheryl acetate) 22,000 mg; Vitamin B1 4,400 mg; Vitamin B2 5,280 mg; Vitamin B6 4,400 mg; Vitamin B1 236 mg; Vitamin C 151,000 mg; Vitamin K 4,400 mg; Vitamin B3 35,200 mg; folic acid 880 mg; choline chloride 220,000 mg; Pantothenic acid

D-14, 80 mg.

[2]Composition for 1 kg of premix: cobalt 20 mg; iron 17,600 mg; iodine 2,000 mg; copper 1,600 mg; zinc 60,000 mg; manganese 10,000 mg; selenium 40 mg. Nitrogen-free extract (NFE) = 100 – (% protein+% lipid + % moisture + % ash+ % fiber).

[3]Digestible energy (ED) = 18.8 × protein content + 37.7 × lipid content + 11.3 × carbohydrate content. DM: Dry matter; P/E: protein/energy ratio; DC: Diet control.

3. Results & Discussion

Growth performances, nutrient utilization, and survival rates of *Heterobranchus longifilis* fed test diets for 56 days are shown in Table 2. Higher final body weight (FBW), daily weight gain (DWG), and specific growth rate (SGR) were observed with fish fed the control diet (CD) and NLM at the level of 20% compared to those of fish fed other diets. Protein efficiency ratios (PER) were not altered with dietary treatment. In addition, feed conversion ratio (FCR) of fish groups fed up to 30% of NLM were not varied (p>0.05), meanwhile, daily feed intake of fish fed NLM diets exhibited lower values compared to those of fish fed CD.

The highest values of different studied parameters had obtained in fish fed control diet (CD) without *Nymphaea lotus* meal. The present result is in line with the results of several studies conducted with the inclusion of different aquatic weeds in different fish species (Mer et al., 2016). A possible explanation of this observed effect might be due to the presence of anti-nutrients in this aquatic weed based diets compared to control diet attributing to the less growth of fish post feeding diets test. This observation is in agreement with Kalita et al. (2008) findings. Also, the decreasing trend in growth performance may be linked to the low acceptance of feed, digestion related problems. Also, growth performance was favored by the optimum inclusion level of *Nymphaea lotus* meal in the experimental feed. The diet containing the plant meals extends the diet acceptability and growth feed and nutrient utilization efficiency up to 20%. Above the optimum level of inclusion of plant proteins, retarded growth of fishes was observed.

In contrast, Sivani et al. (2013) found that anti-nutritional factors were found to be present in *Nymphaea* weeds, and their levels were within tolerable limits and consumption of these plants would not result in any deleterious effect on the growth of *Cyprinus carpio*. The feeding of *Cyprinus carpio* with high levels of *Nymphaea* leaf meal (50% diet) has not yielded a positive result, optimum levels of incorporation 40% yielded better results in terms of growth. This difference between optimum levels could be explaining by the cooking of ingredients mixed in a pressure cooker for 30 min which had to reduce the anti-nutritional factors whereas in the present study, ingredients have not treated and the feed was powdered. Moreover, species difference could also contribute to the difference in the optimum level.

The high values of nitrogen losses recorded in the fish fed diet containing *Nymphaea* meal might be due at the presence of the anti-nutritional factors which could prevent the full use of the proteins contained in the water lily, thus a large part could be rejected or lost. Therefore, prior treatment of *Nymphaea* leaves would be necessary to reduce the amount of anti-nutritional factors for better utilization and total protein, and all other elements necessary for the suitable growth of fish as suggested by some authors.

Survival rates ranged between 72 - 80% and there were no differences among all groups (P > 0.05). *Nymphaea* was a medicinal plant long used as natural therapeutic agents for the treatment of various illnesses since ancient times. In aquaculture production, herbal plants may provide active ingredients that would help to enhance the growth and the immune system of fish (Citarasu, 2010; Maqsood et al., 2011). Phytochemical studies indicated that different solvent extracts of *Nymphaea* were found to contain alkaloids, saponins, tannins and flavonoids (Kumaradoss et al., 2010; Shajeela et al., 2012). There is evidence that fish diet containing *Nymphaea* leaf meal significantly increased the survival rate of *Heterobranchus longifilis* fingerlings. This variation trend of survival rate was also observed for all parameters studied (growth parameters, feed, nutrient utilization efficiency, body composition).

The carcass composition of experimental *Heterobranchus longifilis* fingerlings is shown in Table 3. It was observed that except crude lipid and protein content, the other tested components and gross energy content of African catfish before and post feeding trial did not differ significantly (P > 0.05) irrespective of the supplied feed. The proportion of crude lipid was higher in fish fed diet CD (Control Diet) as compared to other feeds (P < 0.05).

Table 2. Growth parameters, Feed and nutrient utilization efficiency of *Heterobranchus longifilis* fed different levels of *Nymphaea lotus* meal for 56 days

Parameters	CD	Experimental diets			
		10%	20%	30%	40%
FBW (g)	3.94 ± 0.46^{b}	2.39 ± 0.22^{a}	3.15 ± 0.69^{ab}	2.60 ± 0.54^{a}	2.30 ± 0.34^{a}
DWG (g d^{-1})	0.07 ± 0.01^{b}	0.04 ± 0.00^{a}	0.06 ± 0.01^{ab}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}
SGR (% d^{-1})	4.86 ± 0.21^{c}	3.98 ± 0.17^{ab}	4.45 ± 0.37^{bc}	4.11 ± 0.35^{ab}	3.84 ± 0.26^{a}
DFI (g $fish^{-1}day^{-1}$)	0.18 ± 0.07^{b}	0.12 ± 0.01^{a}	0.14 ± 0.02^{a}	0.13 ± 0.02^{a}	0.11 ± 0.01^{a}
FCR	2.48 ± 0.05^{a}	2.85 ± 0.23^{ab}	2.53 ± 0.30^{ab}	2.78 ± 0.25^{ab}	2.93 ± 0.14^{b}
PER	1.00 ± 0.02	0.87 ± 0.07	1.02± 0.12	0.92± 0.09	0.89± 0.05
NL (g $kg^{-1}day^{-1}$)	1.02 ± 0.07^{a}	1.49 ± 0.11^{bc}	1.11 ± 0.26^{ab}	1.44 ± 0.27^{bc}	1.60 ± 0.22^{c}
Survival	72.00 ± 4.00	79.33 ± 0.01	80.00 ± 2.00	77.33 ± 6.11	77.33 ± 6.43

Values are means ± SD, standard deviation (n = 3). Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with the same superscript are not significantly different ($P > 0.05$). Absence of letters indicates no significant difference between treatments. CD: Control diet, FBW: Final body weight, WG: Daily weight gain, SGR: Specific growth rate, DFI: Daily feed intake FCR: Feed conversion ratio, PER: Protein efficiency ratio, NL: nitrogen losses

Table 3. Body composition of *Heterobranchus longifilis* fed different levels of *Nymphaea lotus* meal

Parameters	CD	Experimental diets			
		10%	20%	30%	40%
Moisture (%)	79.16 ± 0.66	79.70 ± 0.73	82.04 ± 3.29	81.62 ± 2.44	82.21 ± 1.66
Crude protein (%FM)	12.16 ± 0.43	11.26 ± 0.45	11.30 ± 2.14	11.45 ± 1.54	10.38 ± 1.01
Total fat (%FM)	2.58 ± 0.10[b]	1.99 ± 0.12[a]	2.13 ± 0.04[a]	1.95 ± 0.26[a]	1.74 ± 0.13[a]
Ash (%FM)	2.24 ± 0.15[b]	2.08 ± 0.09[ab]	1.93 ± 0.38[ab]	1.79 ± 0.17[a]	1.72 ± 0.13[a]
Cross Energy (kJg^{-1})	3.70 ± 0.13	3.27 ± 0.12	3.34 ± 0.63	3.30 ± 0.44	2.98 ± 0.28

Composition of fish slaughtered at the beginning of the experiment (moisture 82.75%; crude protein 11.75%; fat 2.99%; ash 1.61%; gross energy 3.77 KJ.g-1). Values are means ± SD, standard deviation (n = 3). Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with the same superscript are not significantly different ($P > 0.05$). Absence of letters indicates no significant difference between treatments. CD: Control diet, FM: Fresh matter

4. Conclusion

To sum up, the present study is preliminary work for future studies evaluating the dietary inclusion of *Nymphaea lotus* leaf meal in aquatic feeds as a promising cheap source of protein. The results of the present study suggest that, up to 20% of NLM protein can be incorporated in the diet of African catfish with no adverse effects on growth performance, feed utilization, and survival rate but this level could be increased if the leaves *Nymphaea lotus* were previously treated to reduce the anti-nutritional factors.

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