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Eichhornia crassipes biomass as a dietary supplement for *Pterophyllum scalare* (Schultze, 1823)

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ABSTRACT. Current study evaluates the macrophyte biomass of water hyacinth, *Eichhornia crassipes,* in diet food to improve the growth and survival of angelfish, *Pterophyllum scalare*. The liquid (extract) and ground modes are employed. Specific growth rate, weight gain, feed intake and final body weight in basal diet and macrophyte extract biomass diets were similar (p > 0.05). Fish survival was high in all dietary treatments (> 90%), while survival percentage of 100% was reported in ground macrophyte biomass treatment. Ground macrophyte biomass may be included in the diet of *P. scalare* up to 5%. The inclusion of *E. crassipes* in the diet of *P. scalare* proved similar or better results than basal diet. The macrophyte proved to be a food strategy in angelfish diets that may be implemented in the species's diet at 32% levels of crude protein. The latter, easily found in tropical regions, affects directly the species's growth and survival rates.

Keywords: biochemical composition; growth performance; macrophyte biomass.

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Introduction

In-depth information on alternative ingredients, especially plant-based sources, may significantly reduce costs in the production of farm-made feeds for local fish production. Aquatic macrophytes have a potential for local aqua-feeds production and are widely used as feed for omnivorous and herbivorous fish. Several processing techniques to increase nutritive rates and decrease high moisture contents have been employed in dried, mashed, boiled, composted and fermented forms, coupled to the incorporation of plant meal in pelleted feeds (Hasan & Chakrabarti, 2009). Several authors recorded high growth responses of different fish species fed on trial diets with different inclusion rates in the meals of *Eichhornia crassipes* (Pillans, Franklin, & Tibbetts, 2004; Velásquez, Kijora, Wuertz, & Schulz, 2015).

Water hyacinth, *E. crassipes*, has high protein contents featuring a reasonable essential amino acid profile that may be used as a replacement in the diets of aquatic animals (Chavez, Ragaza, Corre Jr, Serrano Jr, & Traifalgar, 2016). In general, the aquatic plants contribute towards nutrients transformation by removing these compounds from water for their own growth (Sirakov, Velichkova, Stoyanova, Dinev, & Staykov, 2015). Increase of plant protein feedstuffs in fish diets will reduce feed cost and decrease dependence on fish meal as a fish protein feedstuff source (Mohapatra, 2015).

Owing to macrophytes's protein levels and their great abundance in tropical and subtropical countries, several significant research studies have been carried out to discover a potential use of aquatic plants, mainly *E. crassipes*. In fact, the aquatic plant its rich in protein ranging from 6 to 12% (Lee, Farid, Wendy, & Zulhisyam, 2016). Macrophytes may be used in different ways, as organic fertilizers (Sipaúba-Tavares & Braga, 2007), microalgae culture medium (Sipaúba-Tavares, Ibarra, & Fioresi, 2009; Sipaúba-Tavares, Segali, & Scardoelli-Truzi, 2015) and with alternative feed material (Chavez et al., 2016; Mohapatra, 2015; Tham, 2016). Worldwide efforts are now being directed to discover alternate good quality protein sources without any side effects for fish growth performance (Mohapatra, 2015).

Only limited information is available on the nutritional rates of these plants included in the feed given to ornamental fish (Francis, Makkar, & Becker, 2001). Freshwater ornamental fish trade is a million-dollar industry with angelfish *Pterophyllum scalare* (Schultze, 1823) known as the "king of aquaculture". Although *P. scalare* is an omnivorous fish, one of the main constraints to optimal commercial production is the lack of information on adequate diets during its different life stages (Patra & Ghost, 2015).

Growth performance and feed conversion ratio should be evaluated for the successful inclusion of plant-based ingredients in diets. Readily available and cheap, the plant meal proteins have been suggested as food additives to alternative fishmeal as an essential requirement in the economic sustainability, viability and future of aquaculture (Chavez et al., 2016).

The use of ground macrophyte biomass and macrophyte extract (liquid) diets are not yet common in the diet of ornamental fish, aimed at growth improvement. Current study investigates the effectiveness of different modes of *E. crassipes* (ground and extract) as food supplement to *P. scalare*. The assay tries to answer the issue whether *E. crassipes* biomass in different modes and the water quality would improve the growth and survival of *P. scalare*.

Material and methods

Fish and rearing conditions

Current research comprises 60-day-old *P. scalare* juveniles, weighing 0.14 ± 0.1 g, acquired from the Ornamental Fish Laboratory (22°C 15'S; 47°C 19'W). Prior to the start of the experiment, specimens of *P. scalare* were fed on *Artemia* during one week to acclimatize the animals to the laboratory culture system. The fish, randomly divided in to 16 groups of 15 animals each (four treatments in four replicates) received basal diet (BD) and mixed diet (ground mode and liquid mode) during 60 days. Experimental units consisted of 16 rectangular glass aquariums (80 L^{-1}).

Water parameters

Continuous aeration was provided by two alternating air blowers to maintain dissolved oxygen. The water in the experimental aquariums was assessed weekly at 08:00-h, while conductivity, pH and dissolved oxygen were measured with YSIN 682 multi-sensor. Nitrite, nitrate, ammonia and total phosphorus compounds were quantified by spectrophotometry following Koroleff (1976) and Golterman, Clymo, and Ohnstad (1978).

Composition of plant nutrients

Biomass of macrophytes was retrieved and dried at 60°C till constant weight. The nutrients of *Eichhornia crassipes* biomass (ground mode and liquid mode) followed methods by Bataglia, Furlani, Teixeira, Furlani, and Gallo (1983).

Experimental diets

Diets in each treatment were prepared to meet the required minimum amounts of nutrients recommended for freshwater cichlids (National Research Council [NRC], 2011). All experimental diets were iso-energetic and iso-nitrogenous compounds with the same level of crude fiber. An analyses of basal diet treatment and macrophyte dietary treatment determined dry matter, ash, crude protein, crude fiber, ether extract and gross energy, as described by Association Official Analytical Chemist (AOAC, 2005) (Table 1).

The inclusion level for ground macrophyte biomass and macrophyte extract (liquid mode) was 5% and 3% dry weight, respectively. Fishmeal was included in each diet to improve palatability of the experimental diets. A control basal diet (BD) was formulated to contain 32.7% crude protein and 4,801 joule g^{-1} gross energy based on feedstuff values reported by Tamaru and Ako (1999). Three different diets were formulated by adding ground macrophyte biomass and macrophyte extract (liquid mode) to the BD containing 50g kg^{-1} of each treatment used (ground mode and liquid mode) designated as: (1) BD + MG = basal diet with ground macrophyte biomass; approximately 10 kg of macrophytes were washed and then dried in the sun for two days. After drying, the plants were placed in an oven at 60°C for 48 hours and 50 g kg^{-1} were added to the BD (control diet); (2) BD + ME = basal diet with macrophyte extract (liquid mode) and NPK fertilizer (20-5-20); the mixture had approximately 5 kg wet weight of *E. crassipes*, ground and boiled in distilled water for one hour; the hot extract (liquid mode) was filtered and autoclaved at 120°C, for 20 minutes; after autoclaving, the extract was retrieved (8-L), cooled and added to 14.2 mL NPK (Sipaúba-Tavares et al., 2009); approximately 1-L of macrophyte extract (liquid mode) was added to the basal diet, replacing the water of the basal diet; (3) BD + MG + ME = basal diet with ground macrophyte biomass and macrophyte extract (liquid mode); 50 g of ground macrophyte biomass plus 1-L of macrophyte extract (liquid mode) were also added. Table 1 shows ingredients, formulation and proximate composition of dietary treatments.

The ingredients tested were ground in a hammer mill, mixed, moistened and pelleted in a meat grinder. The pellets were dried for 24 hours fractionated and sieved to obtain a diameter of 0.71-1.0 mm.

Growth performance and survival

Mean fish weight at the start and end of the experiment was calculated. Weight gain of fish was measured individually and feed intake (amount of feed intake during the experimental period) was also assessed. Growth performance parameters were calculated by the formula:

$$\text{SGR}(\%\ \text{day}^{-1}) = \frac{\ln \ln\ (\text{Final weight})\ - \ln \ln\ (\text{Initial weight})}{\text{number of days}} * 100$$

$$\text{Survival rate}\ (\%) = \frac{\text{number of surviving fish}}{\text{total number of fish stocked}} * 100$$

$$\text{Weight gain (g)} = \text{fish mean fish weight} - \text{initial mean fish weight}$$

$$\text{Feed conversion ratio (FCA)} = \frac{\text{amount of feed supplied}}{\text{final biomass}}$$

Data analysis

All data underwent one-way analysis of variance (ANOVA) with Statistica 8.0 to test the effects of the experimental diets and water. Fisher's exact test was used for difference among means. Difference was significant at $p < 0.05$.

Results

Relative concentration of dry matter in the basal diet and in the other dietary treatments reached a minimum of 89%. Dry matter, gross energy and crude protein contents were similar in all diets. Ash content and ether extract ranged from 9.3% to 11.7% and from 5.3% to 6.2%, respectively (Table 1).

Table 1. Ingredients, formulation and proximate composition of dietary treatments for *Pterophyllum scalare*.

Ingredients (g kg^{-1})	Dietary treatments			
	Basal diet	Macrophyte diets		
	BD	BD+ME*	BD+MG*	BD+MG+ME*
Soybean meal	287.05	287	174.48	174.70
Corn	282.78	282.80	273.69	273.60
Fish meal	65.54	65.60	75.92	75.90
Wheat bran	6.72	6.70	42.03	42.00
Poultry by-product meal	80	80	80	80
Rice bran	33.95	33.90	-	-
Corn starch	80	80	80	80
Corn gluten (60%)	120	120	180	180
Dicalcium phosphate[1]	6.80	6.80	4.30	4.30
Soybean oil	22.16	22.20	19.58	19.50
Mineral-vitamin mix[2]	5	5	5	5
BHT[3]	5	5	5	5
Eichhornia crassipes[4]	-	-	50	50
Kaolin[5]	5	5	10	10
Proximate composition				
Dry matter (%)	89.8	90.1	89.6	89.9
Ash (%)	9.3	10.9	9.8	11.7
Crude protein(%)	32.7	32.3	32.5	32.6
Crude fiber (%)[6]	40	40	40	40
Ether extract (%)	5.3	5.4	6.0	6.2
Gross energy (J g^{-1})[7]	4801	4756	4786	4820

[1]$CaHPO_4$: calcium supplement. [2]Mineral –vitamin mix (units/kg base diet): 5000000 IU vitamin A: 200 000 IU vitamin D3: 5000 IU vitamin E: 1000 mg vitamin K3:15000 mg vitamin C; 4000 mg vitamin B12; 1500 mg vitamin B1; 1500 mg vitamin B2; 1500 mg vitamin B6; 50 mg biotin; 500 mg folic acid; 4000 mg pantothenic acid; 12.25 g B.H.T.; 40 g choline; 5000 mg Fe; 500 mg Cu; 1500 mg Mn; 10 mg Co; 50 mg I; 10 mg Se and 5000 mg Zn. [3]BHT: butyl-hydroxytoluene or butylated hydroxytoluene, is a lipophilic organic compound with antioxidant properties. [4]*Eichhornia crassipes* obtained locally. [5]Kaolin or China clay is almost colorless, with fine particles and pure color. [6]Crude fiber: calculated data. [7]Gross energy: calculated by oxygen pump calorimeter.

Table 1 shows the results of animal performance of the angelfish. Specific growth rate, weight gain, feed intake and final body weight were higher ($p < 0.01$) in BD and BD + ME treatments. Contrastingly feed conversion ratio and survival rates were higher in BD + MG and BD + MG + ME treatments (Table 2).

Fish showed lower weight gain in BD + MG and BD + MG + ME treatments. Fish survival was higher in all treatments (> 90%) whilst the highest survival percentage (100%) was recorded in BD + MG dietary treatment, which was different from the survival percentage of the other treatments (Table 2).

High survival percentage in BD + MG treatment may be related to high nutrient contents in the ground macrophyte biomass which ranging between 0.01 g kg^{-1} (B) and 22.7 g kg^{-1} (K). Nutrient contents over 20 g kg^{-1} were due to N and K; other nutrients were below 23 g kg^{-1} (Table 3). Most nutrient contents in the macrophyte extract were below 0.0001 g kg^{-1}, except N, P and K above 35 g kg^{-1}. In fact, K and P were three to twenty times higher in the macrophyte extract (Table 3).

No difference ($p > 0.05$) was reported among dietary treatments with regard to water parameters, except nitrogen compounds. Nitrate was higher ($p < 0.05$) in BD and BD+ME dietary treatments; nitrite in BD treatment; ammonia in BD + ME dietary treatment. Nitrate had the highest nutrient contents among the treatments, whereas total phosphorus was lowest (0.04 mg L^{-1}). Conductivity was higher and ranged between 41 and 167 µS cm^{-1}; pH was acid and ranged between 5.4 and 7.7; DO was above 5 mg L^{-1} (Table 4).

Table 2. *Pterophyllum scalare* growth performance in different dietary treatments.

Growth performance	Dietary treatments			
	Basal diet	Macrophyte diets		
	BD	BD+ME*	BD+MG*	BD+MG+ME*
Specific growth rate (% day^{-1})	3.1 ± 0.1^a	3.0 ± 0.2^a	2.7 ± 0.1^b	2.8 ± 0.2^b
Weight gain (g)	2.3 ± 0.2^a	2.2 ± 0.2^a	1.4 ± 0.1^b	1.7 ± 0.2^b
Feed intake (g)	3.0 ± 0.04^a	2.9 ± 0.1^{ab}	2.8 ± 0.04^b	2.8 ± 0.2^b
Feed conversion ratio (%)	1.3 ± 0.14^b	1.3 ± 0.1^b	2.0 ± 0.2^a	1.7 ± 0.3^{ab}
Survival (%)	91.8 ± 3.3^b	90.1 ± 4.0^b	100 ± 0.01^a	96.7 ± 3.9^{ab}
Initial body weight (g)	0.14 ± 0.01^a	0.15 ± 0.02^a	0.14 ± 0.01^a	0.14 ± 0.01^a
Final body weight (g)	2.3 ± 0.2^a	2.3 ± 0.2^a	1.5 ± 0.1^b	1.8 ± 0.3^b

**Eichhornia crassipes* obtained locally. Data represent mean ± SD of three replicates per dietary treatments (BD, basal diet inert food (control); BD+ME=basal diet with macrophyte extract; BD+MG=basal diet with ground macrophyte (dried); BD+MG+ME=basal diet with ground macrophyte (dried) and macrophyte extract). Values in the same row with different superscripts are different ($p < 0.05$).

Table 3. Composition of *Eichhornia crassipes* in different forms (ground and extract) used in the dietary treatments.

Macrophyte	Nutrients										
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Ground (g kg^{-1})	21.2	2.8	22.7	7.5	3.9	2.7	0.01	0.02	3	1.5	0.2
Extract** (g L^{-1})	35.1	56.4	73	*	*	*	*	*	*	*	*

*less than 0.0001g L^{-1}. ** macrophyte extract with NPK (20-5-20).

Table 4. Average, minimum and maximum values for pH, dissolved oxygen, conductivity, ammonia, nitrate, nitrite and total phosphorus for different dietary treatments of *Pterophyllum scalare*.

Parameters	Dietary treatments			
	Basal diet	Macrophyte diets		
	BD	BD + ME*	BD + MG*	BD + MG + ME*
pH	6.5^a	6.4^a	6.6^a	6.7^a
	6 - 7	5.4 - 7.1	5.4 - 7.3	5.6 - 7.7
Dissolved oxygen (mg L^{-1})	6.2^a	6.2^a	6.1^a	5.9^a
	5.6 - 6.9	5.7 - 6.5	5.7 - 6.4	5.3 - 6.5
Conductivity (µS cm^{-1})	102^a	101^a	101^a	97^a
	41 - 167	489 - 156	43 - 167	43 – 163
Ammonia (mg L^{-1})	0.14^b	0.16^a	0.14^b	0.13^b
	0.01 - 0.3	0.01 - 0.7	0.01 - 0.3	0.01 - 0.3
Nitrate (mg L^{-1})	1.1^a	1.0^a	0.9^b	0.9^b
	0.5 - 1.6	0.5 - 1.5	0.4 - 1.3	0.4 - 1.3
Nitrite (mg L^{-1})	0.16^a	0.13^b	0.12^b	0.13^b
	0.01 - 0.5	0.001 - 0.4	0.001 - 0.4	0.001 - 0.4
Total phosphorus (mg L^{-1})	0.04^a	0.04^a	0.04^a	0.03^a
	0.01 - 0.07	0.01 - 0.08	0.01 - 0.07	0.01 - 0.06

**Eichhornia crassipes* obtained locally. Values in the same row with different superscripts are different ($p < 0.05$).

Discussion

Fish fed on macrophytes in the extract mode (BD+ME) had the best growth rate when compared to those fed on BD + MG and BD + MG + ME. Velásquez et al. (2015) reported better survival rate in the Nile tilapia fed on fermented macrophyte when compared to sun-dried macrophytes.

El-Sayed (2003) recommended the use of macrophytes as fish feed due to their economic efficiency and to the fact that they were a source of dietary protein mainly for tilapia. Velásquez et al. (2015) recommended the use of fermented plant material. In fact, fermentation reduces the adverse effects of anti-nutrient substances and fiber. In current study, the macrophyte extract (liquid mode) results were satisfactory for the performance of *P. scalare*. Fiber was similar among the dietary treatments and did not affect the growth of the ornamental fish.

In current study, the specific growth rate 2.7 – 3.1% day^{-1} of *P. scalare* was achieved with the inclusion of 3% (macrophyte extract) and 5% (ground macrophyte) in the diets. Velásquez et al. (2015) reported similar results for specific growth rates 2.8 and 2.9% day^{-1}, achieved respectively with 25% and 15% fermented macrophytes inclusion in diets for Nile tilapia. However, Abdel- Tawwab (2008) reported highest specific growth rate of 8% day^{-1} for Nile tilapia fed on diets supplementation with sun-dried *Azolla pinnata* and 25% inclusion. According to Koca, Diler, Dulluc, Yigit, and Bayrak (2009) when *P. scalare* was fed on diet containing higher content protein, a growth rate was reported.

Sithara and Kamalaveni (2008) used *Azolla* as protein supplementation and reported 20-25% crude protein. Aquatic macrophytes are highly nutritious with 20-30% protein contents (Islam et al., 2015). Results of current experiment demonstrate that crude protein was higher (>32%) than that reported by these authors. Kumar et al. (2005) showed that 45% crude protein with a protein energy ratio of 112.62 mg protein/kcal is the best for the indoor rearing of *P. scalare* juveniles.

Another important factor for fish development comprises minerals which are important supplements for higher growth and survival rates. Phosphorus and Ca are essential and necessary for skeleton formation and for the regulation of acid-base balance (Yousefian et al., 2012). Nitrogen and K levels above 20 g L^{-1} were observed in the composition of *E. crassipes*, although N, P and K were high in liquid mode (extract). *Eichhornia crassipes* has high nutrient contents essential for microalgae growth. Although the ground mode showed different composition when compared to those in the liquid mode (extract), high N, P and K contents were enough to maintain good growth performance and survival (over 90%) for *P. scalare*. The nutrient composition of *E. crassipes* is generally related to nutrient availability in the habitat where the plants grow (Sotolu & Sule, 2011).

The inclusion of the plant with other ingredients may provide good results for fish production. In current assay, the final weight of *P. scalare* with BD+ MG and BD + MG + ME dietary treatment was higher than that in basal diet (control), however BD + ME dietary treatment was similar to control diet. When Koca et al. (2009) employed three different dietary treatments featuring live food (*Daphnia magna*), mixed diet (live food + commercial flakes) and commercial flakes, they reported that the final weight of *P. scalare* was lower (1.43-1.99 g) when compared with that in current experiment. Pillans et al. (2004) investigated *Siganus fuscescens* in laboratory conditions and reported that the ingested macrophytes depended on the combination of nutritional demands, with digestion playing an important role in the assimilation of the aquatic plant.

All macrophytes treatments (BD + ME, BD + MG and BD + MG + ME) did not affect the water quality which was similar to that in control diet (BD). Moreover, the high concentration of nitrite and nitrate in BD and BD + ME did not interfere in fish development.

Conclusion

Eichhornia crassipes in the food diet to *P. scalare* showed similar or higher results than that with basal diet. Angelfish fed on diets supplemented with *E. crassipes* by extract (liquid mode) had a growth performance similar to fish fed on diets without macrophyte supplementation. Different results occurred with treatment composed of ground macrophyte that was the only diet with 100% survival rate. Since there is very scanty information on ingestion and survival rates of *P. scalare* fed on basal diet supplemented with *E. crassipes*, further cost/benefit analysis and assessment on the reliability of the aquatic plant on the performance of this ornamental fish should be carried out.

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