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Evaluation of two freshwater macrophytes, *Ceratophyllum demersum* and *Potamogeton amplifolius*as feed ingredients for Nile tilapia (*Oreochromis niloticus*) fingerlings

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The present study was carried out to evaluate the potential of two common freshwater macrophytes (*Ceratophyllum demersum* and *Potamogeton amplifolius*) as feed ingredients for Nile tilapia fingerlings, in two consecutive experiments. The first experiment investigated the use of raw, dried *C. demersum* and *P. amplifolius* as sources of energy in the diets of fingerlings. The macrophytes were incorporated in 6 isonitrogenous and isocaloric diets at four levels as a replacement of dietary wheat bran. The test diets were fed to triplicate groups, stocked in 140 L culture aquaria in a recirculating system, three times a day to satiation, for 45 days. The results showed that control, macrophyte-free diet produced significantly improved growth rates and feed utilization efficiency than macrophytes-based diets.

In Experiment 2, fermented *C. demersum* and *P. amplifolius* were incorporated into six isonitrogenous and isocaloric diets. The diets were fed to triplicate groups of fingerlings (35 g) for 45 days. Growth rates and feed utilization efficiency of fish fed with fermented *P. amplifolius* at 33 % and 66 % inclusion level were not significantly different from fish fed the control diet. At 100 % inclusion level, fish performance was significantly reduced. On the other hand, fermented *C. demersum* produced extremely poor performance, compared to raw *ceratophyllum.* In conclusion, the present results indicated that fermentation improved the quality of *P. amplifolius*; but not *C. demersum*.

[**Keywords**: Fermentation, Feed utilization, Macrophytes, Nile tilapia]

Introduction

Global tilapia production has witnessed a sharp expansion during the past two decades, and is being cultured in more than 130 countries worldwide. Nowadays, tilapias are considered the second most important farmed finfish group in the world, after carps[1]. Global production of farmed tilapia has increased from less than a half million metric tons in the early 1990s to 58 million metric tons in 2016, representing 125 % of freshwater fish production and 107 % of total fish culture, with an average annual growth rate of 135 %[1]. Nile tilapia is the dominant farmed species; contributing 71 % to total tilapia production in 2016(ref. 1).

The global expansion and industrialization of tilapia production has led to stepwise improve in tilapia culture from traditional, low-input, semi-intensive systems to more intensive farming practices, with an increasing dependence on formulated diets. This has created a gap between feed supply and a farmer's demand. Therefore, the major challenge faced by tilapia culture industry is the production of sufficient quantity of high quality feeds. The sharp increase in feed ingredients in recent years has made the challenge more difficult, and the search for unconventional, locally available ingredients has become inevitable[2-5].

The potential of soft submerged aquatic macrophytes as feed ingredient for herbivorous/ omnivorous fishes; such as tilapia, have attracted the attention of many authors, with varying results, depending on cultured species and size and macrophyte species. The most commonly studied macrophytes were hornwort, oxygen weed, water velvet and pondweeds[6-10]. Hornwort (*Ceratophyllum demersum*) is a widely distributed fresh water macrophyte, belonging to family Ceratophyllaceae. The large-leaf pondweed (*Potamogeton amplifolius*) is also another well-known pondweed; which belongs to family Potamogetonaceae. Macrophytes have been used either fresh as a whole or dried as a partial component of a diet. Also, they have been tested as a partial or complete replacement of protein and/or energy sources in pelleted diets. The usage of

macrophytes in fish feeds depends -among other factors- on fish species and size, source, composition and processing of macrophytes, and culture systems[11].

A number of studies were carried out on the preference of different wild tilapia for different aquatic weeds. The feed preference of adult blue tilapia (*Oreochromis aureus*) for five aquatic plants was evaluated by Schwartz & Maughan[12]. The order of preference was *Najas guadalupensis* and *Chara* sp., filamentous algae (predominantly *Cladophora* sp.), *Potamogeton pectinatus* and *P. nodosus*, respectively. Similarly, when juvenile Nile tilapia were fed on different fresh macrophytes, *Elodea canadensis* was the most preferred, followed by *Potamogeton pectinatus* and *Spirodela polyrhiza* (with equal preference), whereas *Myriophyllum spicatum* showed the lowest preference[13]. The consumption of the water fern (*Azolla pinnata*) by Nile tilapia (*Oreochromis niloticus)*[14] and *Tilapia zillii*[15] deteriorated the growth rates over time. When *Azolla* was incorporated in test diets, they led to a reduction of fish performance beyond 25 % inclusion level[16] for Nile tilapia in South Lake (Guangdong province, China). They found that macrophyte consumption was size-specific, where larger ones consumed mainly macrophytes, while small tilapias were much more dependent on periphyton, seston, or detritus. Moreover, in another study, the authors also found that fresh and dried (pelleted) *Ceratophyllum* improved the growth rate of Nile tilapia reared in hapa-in-pond. Similarly Bag *et al*.[17], evaluated the use of three aquatic weeds namely, lemna (*Lemna minor*), water hyacinth (*Eichhornia crassipes*) and azolla (*Azolla pinnata*) as major feed ingredients in Mozambique tilapia (*O. mossambicus*) feeds. The limna-based diet produced significantly better performance than the other two macrophytes.

Fermentation may improve the nutritive value of feed ingredients, including macrophytes[8] .When molasse-fermented water hyacinth replaced wheat bran at two levels 10 and 20 % substitution levels, significant growth rate response of Nile tilapia fingerling[18] found that fermented water hyacinth was well accepted by catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), silver carp (*Hypophthalmicthys molitrix*) and by common carp (*Cyprinus carpio*). However, mrigal and silver carp showed the best growth rates, followed by rohu. Fermentation of water hyacinth may thus be a simple and efficient treatment for utilizing water hyacinths as a feed or manure in fish culture without the energy-consuming process of palletization[19]. Such findings were confirmed in a study realized by Sadique[20] who showed the significant effect of molasse-fermented water hyacinth on the growth rate and flesh quality of common carp fingerlings.

Several aquatic macrophytes are widely distributed in irrigation and drainage water bodies in Egypt, as well as in inland and coastal lakes. Water hyacinth (*Eichhornia crassipes*), *Ceratophyllum demersum* and *Potamogeton amplifolius* are the most dominant in these water bodies. However, despite the potentials these macrophytes may have as fish feed ingredients, they have not been evaluated yet. Therefore, the present study was carried out to evaluate the potential of fresh, dry and fermented *C. demersum* and *P. amplifolius* as feed ingredients for Nile tilapia (*Oreochromis niloticus*) fingerlings.

Materials and Methods

Fish and culture facilities

Nile tilapia (*Oreochromis niloticus*) fingerlings (n = 205) were brought from Barsik Fish Farm (Behaira Governorate), Egypt. The fish were transported to the laboratory in Nylon bags (100×30×30 cm) half-filled with water and completed with oxygen gas for fish respiration. After resting and acclimation to water temperature in the lab, for a few minutes, the fish were distributed randomly into the culture aquaria. Fish were fed on a commercial (30 % CP) diet for one week to adapt the laboratory conditions and artificial feeding. The fish were stocked into 140 l aquaria in a closed, self-cleaning recirculating indoor system. The culture system was provided with a biological filter, aeration through an air compressor, and heaters to maintain water temperature at 27 °C. Approximately 10 % of the water volume was replaced by new freshwater daily. Lighting in the culture unit was set at 12:12 L:D cycle. Water quality parameters, including dissolved oxygen (DO), ammonia (NH_4–N), nitrates (NO_3–N) and nitrites (NO_2–N) and pH, were monitored weekly. The average values of these parameters throughout the study were: DO = 64±13 mg l^{-1}, NH_4 –N = 006±0002 mg l^{-1}, NO_3 –N = 84±172 mg l^{-1}, NO_2 = 000 mg l^{-1} and pH = 80±009.

Macrophyte fermentation

The macrophytes were partially dried in an electric oven at 60 °C until about 50 % of their water content

was removed. They were then fermented as described by El-Sayed[8]. Each macrophyte was put in a clean, dry glass aquarium at room temperature (25-30 °C). Five percent sugar cane molasses and 2 ml orthophosphoric acid/kg were added to each aquarium, with continuous mixing. The aquaria were then covered with glass covers. The mixture of macrophytes was remixed every ten days to facilitate decomposition, for two months. After fermentation process, the macrophytes were sun-dried for two days, grinded into powder-like form, using an electric grinder. They were then stored into sealed and labeled plastic bags until used. The proximate composition of fermented macrophytes is given in Table 1.

Test diets

Twelve isonitrogenous (30 - 35 % CP), isocaloric (400 - 450 kcal GE/100 g) test diets were prepared. Fresh and fermented *C. demersum* and *P. amplifolius* were incorporated into the test diets, as an energy source, at 33 %, 66 %, and 100 % as a replacement for wheat bran (Table 2). The chemical analysis of the macrophytes, experimental diets and whole fish was done per the CIFA[21] guideline. The test diets for each experiment were fed to groups of Nile tilapia fingerlings in triplicates, three times/day (08:00, 12:00 and 16:00 hrs), 7 days a week, for 45 days. Each group of fish was bulk weighed at the start and after every 15 days throughout the experimental period.

Table 1 — Proximate composition of *C. demersum* and *P. amplifolius* before (BF) and after (AF) fermentation and wheat bran (WB) used in the experiment

Component (% DM)	*C. demersum*			*P. amplifolius*			(WB)
	BF	AF	Change %	BF	AF	Change %	
Crude protein	92	14	± 52	111	25	± 125	13
Ether extract	19	22	± 16	23	26	± 13	43
Fiber	158	142	± 11	154	108	± 43	15
Nitrogen free extract	636	585	± 9	663	549	± 21	625
Ash	95	111	± 17	49	67	± 37	52
[1]G E (kcal/100 g)	338	351	± 4	346	397	± 15	345

[1]GE, gross energy, calculated based on 565, 95 and 41 (Kcal/100 g) for protein, lipid, and carbohydrate, respectively

Table 2 — Composition and proximate analyses (percent dry weight) of the test diets

Ingredients (%)	Control	Fermented						Raw (Non-fermented)					
		C. demersum			*P. amplifolius*			*C. demersum*			*P. amplifolius*		
	0%	33 %	66 %	100 %	33 %	66 %	100 %	33 %	66 %	100 %	33 %	66 %	100 %
Fish meal[1]	10	10	10	10	10	8	9	10	10	10	10	10	10
Soy bean meal	54	54	54	54	54	55	55	40	40	40	40	40	40
Wheat bran meal	27	18	9	--	18	10	--	26	12	--	26	12	--
C. demersum meal	---	9	18	27	--	--	--	13	26	38	--	--	--
P. amplifolius meal	--	--	--	--	9	18	27	--	--	--	13	26	38
Soy bean oil	3	3	3	3	3	3	3	4	5	5	4	5	5
Fish oil	2	2	2	2	2	2	2	3	3	3	3	3	3
Vit & min mix[2]	2	2	2	2	2	2	2	2	2	2	2	2	2
Binder[3] (CMC)	2	2	2	2	2	2	2	2	2	2	2	2	2
Total	100	100	100	100	100	100	100	100	100	100	100	100	100
Crude protein	3531	3618	3522	3547	3578	3651	3625	3050	3012	3144	2886	3010	3130
Ether extract	581	725	587	513	514	668	527	664	722	710	608	623	553
Crude fiber	348	260	371	424	346	278	298	795	919	989	446	538	698
Ash	790	1287	1913	2422	1050	1256	1548	1297	1665	2013	1187	1312	1479
NFE[4]	4750	4110	3607	3094	4512	4147	4002	4043	3599	3175	4465	4287	4328
GE[5] (kcal/100 g)	449	442	403	376	436	440	420	400 9	387	376	403	401	404

[1]60 % crude protein (Nile tilapia meal). [2]Contains (per g): retinal palmitate, 20000 IU; cholecalciferol, 5000 IU; DL-a-tocopherol, 10 mg; ascorbic acid, 25 mg; Vit K3, (Menadione), 35 mg; thiamin hydrochloride, 2 mg; riboflavin, 48 mg; pyridoxine hydrochloride, 25 mg; cyanocobalamine, 25 mcg; biotin, 10 mcg; nicotinic acid, 25 mg; folic acid, 05 mg; Dcalciumpantothenate, 75 mg; sodium sulphate, 50 mg; potassium chloride, 30 mg; manganese sulphate, 15 mg; zinc sulphate, 15 mg; copper sulphate, 2 mg; ferrous sulphate, 15 mg. [3]Carboxymethyle cellulose, used as binder. [4]NFE, nitrogen-free extract, determined by difference. [5]GE, gross energy, calculated based on 565, 95 and 41 (Kcal/100 g) for protein, lipid, carbohydrate, respectively.

Body composition analysis

At the termination of the study, all fish in each tank were netted, weighed, and frozen for final body composition analyses. Initial body analyses were performed on a sample of fish, which were weighed and frozen prior to the study. Proximate analyses of body water, protein, lipid, and ash were performed according to standard methods[21].

Statistical analysis

Results of fish growth rates, feed utilization efficiency and body composition of an experiment were subjected to one-way analyses of variance (ANOVA). Orthogonal polynomial procedures were used to compare means at P = 005. Least significant difference (LSD) was used to test differences among treatment means when F-values from ANOVA were significant.

Results

With respect to fish growth rates, feed utilization efficiency and body composition, the results of the experiment indicated that the inclusion of macrophytes (*Ceratophyllum demersum and Potamogeton amplifolius*) in Nile tilapia diets significantly affected their growth rates and feed utilization efficiency (Table 3). The control diet [containing wheat bran (WB) as an energy source] produced significantly better (P < 005) weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). The results of inclusion of raw (non-fermented) *C. demersum* and *P. amplifolius* in Nile tilapia diets at all WB-substitution levels significantly reduced growth rates and feed utilization efficiency. This finding suggests that raw *C. demersum* (RC) and raw *P. amplifolius* (RP) macrophytes are not good sources of energy for Nile tilapia fingerlings. However, *C. demersum* was slightly better utilized than *P. amplifolius*. Fermentation improved the quality of *P. amplifolius* (FP), and led to improved performance up to 66 % inclusion level. On the contrary, fermentation of *C. demersum* (FC) had resulted in extremely poor performance of Nile tilapia fingerlings.

Body composition of Nile tilapia fed the test diets in the present study was not significantly affected by dietary treatments (Table 4).

Discussion

The relatively high fiber contents of *C. demersum* and *P. amplifolius* may have also been responsible for poor digestibility which in turn may have led to poor growth. In support, Edwards[22] reported that high fiber levels in the diet are known to retard the growth of fish. Boyd[23] also noted that the coarseness of macrophytes, due to the encrustation by calcium carbonate on their external surfaces, makes them unpalatable.

The present results agreed with those reported on tilapia fed on *C. demersum*[24], where the poor growth has been attributed to the poor digestibility of this macrophyte. Poor performance was also recorded when *C. demersum* was fed to Nile tilapia[6,25,26].

Similarly, Appler[27] demonstrated that most of the aquatic plants including algae contain 40 % or more

Table 3 — Effects of fresh and fermented dietary macrophytes on weight gain and feed utilization efficiency of fingerling Nile tilapia

Treatment	IW[1]	FW[2]	Percent gain[3]	SGR[4]	FCR[5]	PER[6]
Control	57	151^{a}	1670^{a}	22^{a}	13^{a}	26^{a}
RC33	61	135^{ab}	1220^{ab}	18^{ab}	22^{bc}	15^{bc}
RC66	69	106^{b}	540^{e}	10^{c}	40^{d}	08^{d}
RC100	63	89^{c}	410^{e}	08	39^{d}	08^{d}
FC33	39	77^{cd}	990^{d}	15^{b}	16^{ab}	22^{ab}
FC66	38	80^{cd}	1120^{c}	17^{ab}	19^{b}	18^{b}
FC100	39	65^{d}	650de	11^{c}	39^{d}	08^{d}
RP33	58	96^{c}	647de	11^{c}	24^{c}	15^{bc}
RP66	61	78^{cd}	272^{f}	05^{d}	30^{cd}	11^{c}
RP100	61	69^{d}	116^{f}	03^{d}	56^{e}	06^{d}
FP33	64	159^{a}	1471^{b}	20^{a}	13^{a}	27^{a}
FP66	64	165^{a}	1566^{b}	20^{a}	12^{a}	28^{a}
FP100	64	114^{b}	790^{d}	13^{b}	20^{b}	18^{b}

Values in the same column with different superscripts are significantly different (P = 005). [1]Initial weight (IW); [2]Final weight (FW); [3]Percent gain = 100(final weight- initial weight)/initial weight; [4]Specific growth rate (SGR) = 100 (ln final weight-ln initial weight)/time [days]; [5]Feed conversion ratio (FCR) = dry feed offered/fish weight gain; and [6]Protein efficiency ratio (PER) = Fish weight gain (g) / protein intake (g).

Table 4 — Effect of fresh and fermented dietary water hyacinth on body composition (% day weight) of Nile tilapia fingerlings

Treatment	Water content	Ether extract	Protein	Ash
Initial	709	227	659	137
Control	696	163	678	181
RC33	750	221	616	176
RC66	745	232	591	189
RC100	773	239	574	203
FC33	732	197	619	187
FC66	742	172	648	175
FC100	712	184	621	195
RP33	758	211	623	186
RP66	800	227	582	180
RP100	802	216	601	183
FP33	698	161	623	215
FP66	682	171	628	185
FP100	680	175	623	200

carbohydrates, of which only a small fraction consists of mono-saccharides and di-saccharides. Low digestibility of plant materials has been attributed to a preponderance of complex carbohydrates[17]. The same findings could be true in the present study, where *C. demersum* and *P. amplifolius* contained more than 50 % of nitrogen free extract, part of which is mono-saccharides and di-saccharides, which may interpret the limited utilization of these macrophytes leading to poor growth. However, more work is needed to verify the mono-saccharides and disaccharides contents of these plants and their digestibility and assimilation by Nile tilapia.

The reduced growth rates and feed efficiency of Nile tilapia fed macrophytes-based diets in the present study may have also been due to the effects of the anti-nutritional factors contained in these macrophytes. The existance of anti-nutritional factors within plant feedstuffs restricts their use in animal feeds[28].

On the contrary, some authors recommended the use of macrophytes as feed for fish[10,29,22.] They noted that *T. zillii* and *T. rendalli* are voracious feeders of submerged macrophytes. Buddington[30] reported that *T. zillii* preferred *Najas guadalupensis* as a food source to *Lemna*, *Myriophyllum spicatum* and *Potamogeton pectinatus.* Cassani[31] noted that grass carp prefer submerged, rather than floating macrophytes when they are supplied in fresh form[7,32]. Moreover, Hasanuddin *et al.*[33] confirmed the suitability of *Ceratophyllum* sp. for *Oreochromis niloticus.*

It is obvious from the current study that fermented *P. amplifolius* can be considered as a feed ingredient in the diets for Nile tilapia fingerlings up to 66 % level of incorporation. Growth performance indices including SGR, FCR, and PER, of Nile tilapia fingerlings were similar at 33 % and 66 % incorporation of fermented *P. amplifolius* to the reference diet. The utilization efficiency of fermented *P. amplifolius* was significantly better than raw *P. amplifolius.* This means that fermentation improved the quality of *P. amplifolius.*

The better performance of Nile tilapia fed with fermented *P. amplifolius* up to 66 % inclusion level may have been due to the increased level and improved nature of protein of this macrophyte. It has been reported that crude protein was significantly increased in the fermented aquatic macrophytes when compared to the unfermented macrophytes[19,34]. These authors noted increase of crude protein content in fermented *Lemna* and *Spirodelamay* through microbial synthesis. The present study found that crude protein content was also affected by fermentation. The effect of fermentation on the protein content was conditional and strongly depends on the plant species.

Fermenting *P. amplifolius* may have also contributed in the removal of the anti-nutrients that might have been present in this macrophyte. For example, Velásquez[35] reported that the anti-nutritional substances, including trypsin inhibitor, phytates, tannins (hydrolyzed and condensed), and oxalates in *Azolla* were significantly reduced by the lactic acid fermentation.

Fermentation has also significantly reduced the fiber content of *P. amplifolius* in the present study compared with the terrestrial plants; the fiber structure of aquatic macrophytes is relatively easier to be decomposed by microorganism[36]. Crude fiber was significantly lower in the fermented aquatic macrophytes when compared to the unfermented samples. The good performance of Nile tilapia fed with fermented *P. amplifolius* in the present study is also in agreement with the other results. El-Sayed[8] reported that the process of fermentation is necessary when water hyacinth (*Eichhornia crassipes*) is included at levels of 20 % or more in to Nile tilapia diets. Fermentation of duckweed had a significant positive effect on the growth performance, weight gain, specific growth ratio and protein efficiency ratio when applied to *Oreochromis niloticus* juveniles fed with low fishmeal diets[37].

Fermenting *C. demersum* led to a considerable reduction in fish performance compared to non-fermented format all inclusion levels and therefore, may not be considered as a feed ingredient in the diets for the Nile tilapia fingerlings. The cause of poor growth rates, PER, FCR, PPV and SGR of Nile tilapia fingerlings fed with fermented *C. demersum* is not known, particularly that the non-fermented *C. demersum* has resulted in better performance.

More research on the effects of processing on the quality of *C. demersum* as a feed ingredient for tilapia and other herbivorous fishes is needed. Other processing methods should also be tried in order to verify their effects on the quality of this macrophyte. Body composition of Nile tilapia fed with test diets in the present study was not significantly affected by

dietary treatments. This contrasts the findings of Edwards[38] who found that body protein, lipid, and ash were positively correlated with energy contents of dietary macrophytes.

The study concluded that, the inclusion of raw (non-fermented) *C. demersum* and *P. amplifolius* in Nile tilapia diets at all WB-substitution levels significantly reduced fish growth rates and feed utilization efficiency. This finding suggests that raw *C. demersum* and *P. amplifolius* macrophytes are not good sources of energy for Nile tilapia fingerlings. However, *C. demersum* was slightly better than *P. amplifolius*. This is due to the fermentation which improved the quality of *P. amplifolius* and led to improved performance up to 66 % inclusion level. On the contrary, fermentation of *C. demersum* had resulted in extremely poor performance of Nile tilapia fingerlings.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

The authors have equal participation in preparing the manuscript.

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