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Assessment of Hydroponically Grown Macrophytes for Their Suitability as Fish Feed

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Abstract: Water hyacinth, water lettuce and parrot's feather plants were examined for their ability to remove nutrients from aquaculture wastewater at two retention times. During the experiment, the aquatic plants grew rapidly in the hydroponics system and appeared healthy with green color. At hydraulic retention times (HRTs) of 6 and 12 days, the average water hyacinth, water lettuce and parrot's feather yields were 83, 51 and 51 g (dm) m^{-2} and 49, 29 and 22 g (dm) m^{-2}, respectively. The aquatic plants were able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P reductions ranged from 21.4 to 48.0%, from 71.1 to 89.5%, from 55.9 to 76.0%, from 49.6 to 90.6%, from 34.5 to 54.4% and from 64.5 to 76.8%, respectively. Generally, the reductions increased with longer retention times and were highest in compartments containing water hyacinth followed by compartments containing water lettuce and parrot's feather. The nutritive value of the three wastewater grown plants was assessed to determine the suitability of using the plants as a component in fish feed. The three wastewater grown plants did not contain sufficient amounts of protein and fat to meet the dietary requirements of fish and shellfish. They also contained high concentrations of K, Cu, Fe, Mn, Se and Zn, which can lead to reduced feed intake, weight gain and growth rates in fish and shellfish.

Keywords: aquaculture, wastewater, water hyacinth, water lettuce, parrot's feather, fish feed, nutrition

INTRODUCTION

Aquaculture is defined as the controlled cultivation and harvest of aquatic organisms. It is the fastest growing food production sector in the world[1]. In aquaculture, good nutrition is essential for the production of a healthy, high quality product. Generally, cultured fish do not consume natural prey and forages, but are provided with a manufactured feed formulated to contain a range of essential and non-essential nutrients from a variety of raw ingredients. To sustain a high rate of increase in aquaculture production, a new source of manufactured fish feed is required[2].

Aquaculture feeds are amongst the most expensive animal feeds and typically account for half of the total cost of aquaculture production, with protein being the most expensive component[2-4]. Due to their high nutritional content, marine protein meals such as fish meal, squid meal and shrimp meal have long been the main protein sources used in feeds for most aquaculture species. Marine meals are generally incorporated into feeds at levels between 30 and 60%[5]. However, with the increasing cost and periodic shortages of marine meals on the global markets, the aquaculture industry is interested in reducing its dependence on fish meal through the development of alternative protein sources. The sustainability of the aquaculture industry depends on the reduction of wild fish inputs into fish feed[6]. However, alternative protein sources should be: (a) economically competitive, (b) capable of being produced in large quantities, (c) contain balanced amino acid profiles and required crude protein levels and (d) not compromise the growth and health of the fish[2]. Plant meals, which are considerably cheaper than marine meals, are being studied as a partial replacement for marine meals in aquaculture feeds[4, 7].

The primary aim of this study was to evaluate the feasibility of using hydroponically grown water hyacinth, water lettuce and parrot's feather plants on wastewater from a recirculating aquaculture system as a component of fish feed as determined by their nutritive value. The specific objectives of the study were to evaluate: (a) the effect of retention time on plant growth

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and yield, (b) the effectiveness of these plants in reducing the pollution load of the aquaculture wastewater as measured by TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P and (c) the nutritional value of these plants (energy, carbohydrates, crude protein, crude fat, crude fiber, Ca, Cl, Mg, P, K, Na, S, B, Cu, Fe, Mn, Mo, Se and Zn) and (d) their suitability as a component in fish feed.

EXPERIMENTAL APPARATUS

The hydroponic system (Fig. 1) consisted of a frame, growth troughs and aeration, lighting, cooling, irrigation, supernatant collection and control units.

The frame (Fig. 2) was constructed of angle iron with a width of 244 cm, a depth of 41 cm and a height of 283 cm. The back and the top were covered with 0.6 cm thick plywood sheets. The frame consisted of three shelves (76 cm apart). Each shelf was divided vertically into two cells by dividers made of 1.2 cm thick plywood sheets. The frame supported the growth troughs and all other systems.

The plant growth unit consisted of six troughs. Each trough was made of galvanized steel and was divided into three compartments. Each compartment held a tray that acted as the plant support medium and consisted of a wire-mesh base (16 openings cm^{-2}) with 5 cm high metal sides. The dimensions of each trough and plant supporting tray are shown in Figure 2. The trays were positioned in the troughs so that the plant roots were in contact with the liquid waste. The placement of trays was maintained by means of supports welded into the corners of each compartment 5 cm below the top edge of the trough.

An aeration unit was installed in each compartment to provide oxygen to the immersed roots of the growing plants. The main air supply was connected to a manifold (PVC pipe of 2.54 cm outside diameter) on each shelf using PVC tubing of 0.635 cm outside diameter. The air flow from the main supply to the manifold on each shelf was controlled by a pressure regulator (Model 129121-510, Aro, Brayn, OH). Six aeration units were connected to the manifold on each shelf using PVC tubing of 0.635 cm outside diameter. Each aerator consisted of a main tube with three perforated stainless steel laterals coming off it at right angles to the main. Each lateral was approximately 30 cm long whereas the main was 26.5 cm long.

The lighting unit was designed to provide approximately 360 hectolux of illumination per trough. This was achieved by a mixture of fluorescent and incandescent lamps. Six 34 W cool white fluorescent lamps (122 cm in length) and two 60 W Plant Gro N Show bulbs were fastened above each trough.

A cooling unit was designed to continuously remove the heat produced by the lamps to avoid heating of the wastewater on the upper and middle shelves. For each of these two shelves, a 5 cm diameter PVC pipe, having 6 mm diameter holes spaced 6 cm apart and facing out, was placed under the backside of the troughs. Two metal blocks supported the front side of the trough. This provided a 5 cm space between the trough and the lighting unit of the shelf below it. A 5 cm diameter PVC pipe acting as a manifold was attached vertically to the left side of the frame, through which air was blown by means of a motor driven fan (Model AK4L143A type 821, Franklin Electric Company, Bluffton, IN).

The wastewater application unit consisted of: (a) a wastewater storage tank, for storing the wastewater, (b) a pump, to transfer the wastewater from the storage tank to the growth troughs, (c) six valves, to control the amount of wastewater fed to each cell and (d) an irrigation system, for applying the wastewater onto the plant supporting trays in the growth troughs. The wastewater storage tank was constructed of plastic and had a capacity of approximately 100 L. A mixing shaft, with a 40 cm diameter impeller, was installed through the center of the cover of the tank to agitate the wastewater in the tank. Four 2.5 cm baffles were installed vertically along the inside wall of the tank to promote complete mixing. A 1 hp motor (Model NSI-10RS3, Bodine Electric Company, Chicago, IL) with speed reducer was mounted on the tank cover to drive the mixing shaft and impeller. The wastewater storage tank was connected to the pump using TYGON tubing of 3.175 cm outside diameter. A variable speed pump (Model 110-23E, TAT Pumps Inc., Logan, OH) with a capacity of 138 cm^3 rev^{-1} was used to transfer the wastewater from the storage tank to the irrigation system. The pump was connected to the irrigation system using PVC tubing of 1.905 cm outside diameter. Six valves were used to control the amount of wastewater fed to each growth trough. The timing and duration of opening/closing of the valves were controlled by an electronic circuit. Each wastewater applicator was fabricated from stainless steel pipe with holes punched along the lower edge to allow the wastewater to flow out. The wastewater entered the applicator at the center of the top edge. To overcome the problem of clogging, a water line with six solenoid valves was attached to the applicator and was used to

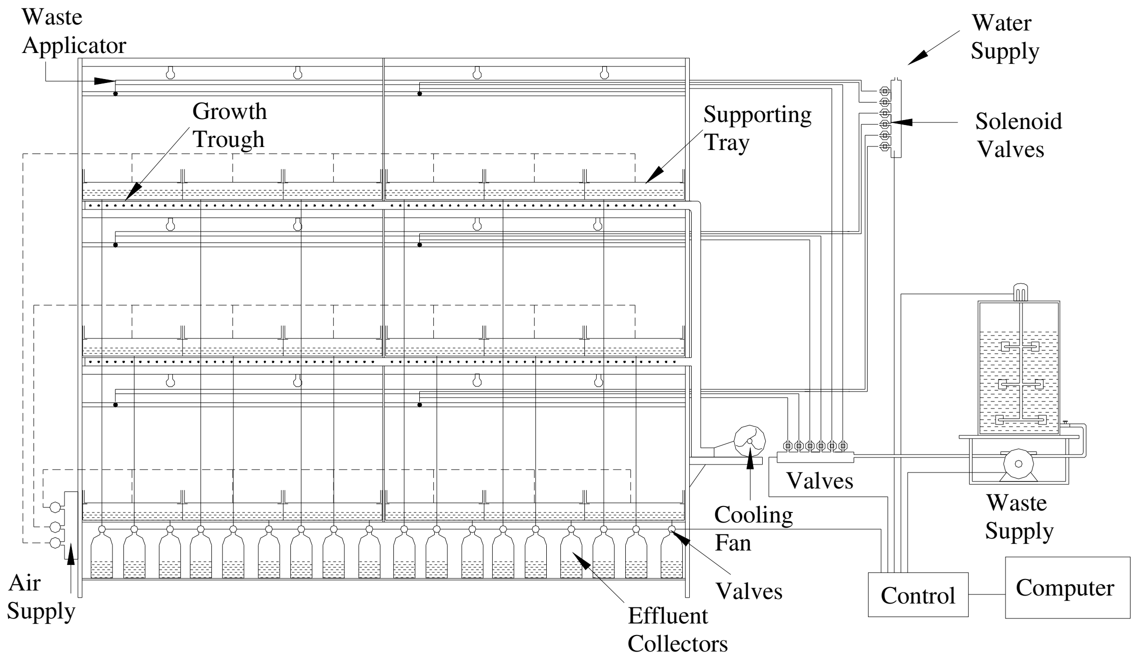

Fig. 1: The hydroponics system

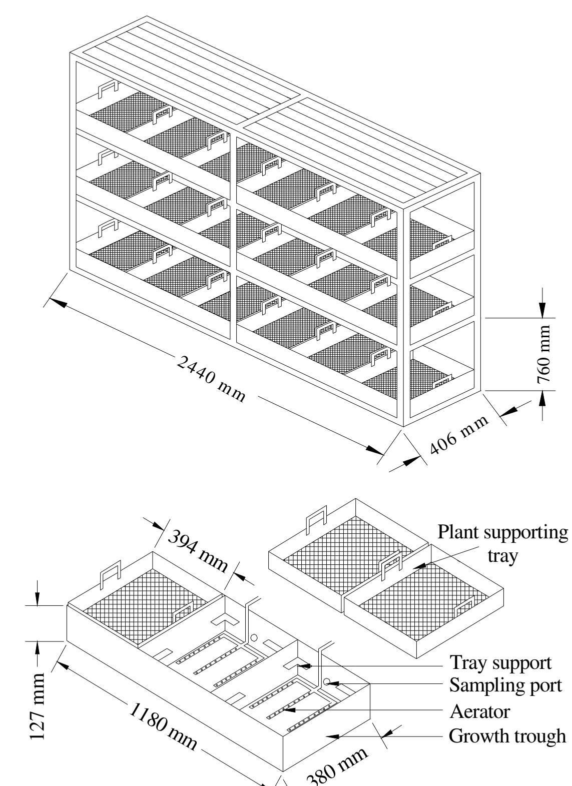

Fig. 2: The frame, growth trough and plant support tray

flush out the applicator after feeding periods. The wastewater application system was fully automated and consisted of a motor driven pulley arrangement on each shelf to which the applicator tubes were attached. The motors (Sigma Model 20-3424SG-24007, Faber Industrial Technologies, Clifton, NJ) ran at 6 rpm and were controlled by an electronic circuit. The system was set up so that each applicator traveled 122 cm (3 tray lengths). When a guide on an applicator hit a micro – switch located at each end of the shelf, the motor stopped. After a 3 second delay, the applicator traveled in the opposite direction. This process continued for the designated feeding time which was controlled by computer. Each compartment contained a sampling port located 2.0 cm from the bottom of the trough. Each sampling port was connected to a 2.7 L glass bottle using PVC tubing of 1.27 cm outside diameter and a valve.

A microcontroller (BASIC Stamp 2P24, Parallax, Inc., Rocklin, CA) was used to run the various components of the hydroponics system including the lighting, cooling, irrigation and supernatant collection units. Addressable latches were used to effectively increase the microcontroller's 24 input/output pins to the required number. The microcontroller was programmed using BASIC computer software (BASIC Stamp Windows Editor version 2.2.6, Parallax, Inc., Rocklin, CA). A real time clock (Dallas Semiconductor

X1226, Maxim Integrated Products, Inc., Sunnyvale, CA) and a 1-Farad supercapacitor provided nonvolitile timing. A separate program (BASIC Stamp Windows Editor version 2.2.6, Parallax, Inc., Rocklin, CA) was used to set the real time clock.

MATERIALS AND METHODS

Experimental Materials: The water hyacinth, water lettuce and parrot's feather plants were purchased from Dubé Botanical Gardens, River John, Nova Scotia. The wastewater used in the study was obtained from an intensive, recirculating aquaculture facility stocked with Arctic charr (*Salvelinus alpinus*) located in Truro, Nova Scotia. The chemical analyses for the aquaculture wastewater are presented in Table 1.

Table 1: Chemical analysis of aquaculture wastewater

Parameter	Value		
Total solids (mg L^{-1})	826.67	±	28.87
Suspended solids (mg L^{-1})	103.33	±	13.63
Total chemical oxygen demand (mg L^{-1})	157.97	±	9.32
Soluble chemical oxygen demand (mg L^{-1})	102.34	±	8.56
Ammonium-Nitrogen (mg L^{-1})	2.08	±	0.50
Nitrite-Nitrogen (mg L^{-1})	1.27	±	0.09
Nitrate-Nitrogen (mg L^{-1})	21.64	±	0.60
Total phosphorus (mg L^{-1})	6.30		
Orthophosphate (mg L^{-1})	4.49	±	0.18
Potassium (mg L^{-1})	74.67	±	0.32
Calcium (mg L^{-1})	59.90	±	0.95
Sodium (mg L^{-1})	114.67	±	0.58
Sulfur (mg L^{-1})	6.97	±	0.12
Chloride (mg L^{-1})	86.67	±	0.58
Magnesium (mg L^{-1})	5.06	±	0.07
Manganese (mg L^{-1})	0.20		
Iron (mg L^{-1})	0.03	±	0.01
Copper (mg L^{-1})	0.06		
Zinc (mg L^{-1})	0.20		
pH	7.00	±	0.13

Experimental Procedure: The effects of retention time (6 and 12 days) on the growth and yield of three aquatic macrophytes (water hyacinth, water lettuce and parrot's feather) and the pollution reduction of the wastewater were investigated. The nutritive values of the three hydroponically grown plants were assessed to determine their suitability as a component in fish feed. The day length at a latitude of 45°N during the crop growing season (May 1st to Sept 31st) is approximately 14 hours. Therefore, the lighting system was programmed to provide a daily photoperiod of 14 hours. The study was designed as a completely randomized 3x2 experiment with 2 replicates. This resulted in 12 treatments. Four compartments were utilized as controls and contained wastewater only.

On day 1, with the valves controlling the sampling ports in the closed position, each compartment was filled with 12 L of aquaculture wastewater. Water hyacinth, water lettuce and parrot's feather were washed with tap water and weighed using an analytical balance (Model PM4600, Mettler Instrument Corporation, Hightstown, NJ). Each compartment was then stocked with the appropriate plant to provide approximately 50% plant coverage. This resulted in initial average masses of 204, 144 and 41 g tray $^{-1}$ for water hyacinth, water lettuce and parrot's feather, respectively. The lighting system was activated and programmed to provide a daily photoperiod of 14 hours. The cooling system was programmed to operate with the lighting system. The aeration system was turned on and pressure regulators were adjusted to 0.340 atm.

During the growth period (days 2 – 24), plant appearance was observed and recorded daily. The valves controlling the effluent tubes were opened and samples of effluent were collected from each compartment and refrigerated at 4°C in labeled bottles until needed for chemical analyses. The required amounts of wastewater were applied to each compartment. Plants were removed from the compartments on day 24 and allowed to dry at room temperature (22°C) for 24 hours. The biomass was measured using an analytical balance (Model PM4600, Mettler Instrument Corporation, Hightstown, NJ) and recorded. Plant samples were collected from each compartment for nutritional analyses.

Analyses: All effluent samples were analyzed for: total solids (TS), total chemical oxygen demand (COD), ammonium – nitrogen (NH_4^+-N), nitrite – nitrogen (NO_2^--N), nitrate – nitrogen (NO_3^--N), phosphate – phosphorus (PO_4^{3-}-P) and pH. The TS, COD, NO_2^--N and PO_4^{3-}-P analyses were performed according to procedures described in Standard Methods for the Examination of Water and Wastewater[8]. The NH_4^+-N measurements were performed using the Kjeltec Auto Analyzer (Model 1030, Tecator, Höganäs, Sweden) according to the Kjeldahl method. The NO_3^--N analysis was performed according to the phenoldisulfonic acid technique described in Methods of Soil Analysis[9]. The pH of the wastewater was measured using a pH meter (Model 805MP, Fisher Scientific, Montreal, QC). Plant tissue analyses (energy, carbohydrates, crude protein, crude fat, and crude fiber) were performed at Maxxam Analytics Inc., Mississauga, Ontario according to procedures described in Official Methods of Analysis of AOAC International[10]. The elemental composition (Ca, Cl, Mg, P, K, Na, S, B, Cu, Fe, Mn, Mo, Se and Zn) of the wastewater and plant tissue was determined in the Minerals Engineering Center, Dalhousie University using flame atomic adsorption spectroscopy.

RESULTS AND DISCUSSION

Plant Appearance: Initially, the plants in all compartments appeared healthy with green color. During the first seven days of the experiment, the plants in all compartments grew rapidly. The older water hyacinth and water lettuce plants produced numerous daughter plants by vegetative propagation. The long creeping stems of parrot's feather grew rapidly across the water surface forming numerous branches at the nodes. By day 8 of the experiment, the surface area of compartments containing water hyacinth and water lettuce were completely covered, while compartments the surface area of containing parrot's feather were approximately 60% covered. Figure 3 shows water hyacinth, water lettuce and parrot's feather plants on day 14 of the experiment.

Plant Yield: At hydraulic retention times (HRTs) of 6 and 12 days, the average water hyacinth, water lettuce and parrot's feather yields were 83, 51 and 51 g (dm) m^{-2} and 49, 29 and 22 g (dm) m^{-2}, respectively (Table 2). The effects of plant type and hydraulic retention time on plant yield were tested using a two-way analysis of variance (ANOVA) and a Duncan's multiple range test using SPSS (SPSS 14.0.1, SPSS Inc., Chicago, IL). The yield was significantly influenced by plant type and HRT (Tables 3 and 4). Water hyacinth produced the highest yields followed by water lettuce and parrot's feather. The plant yields increased as retention time was decreased due to the additional nutrients provided to the plants[11-13]. Jo et al.[14] evaluated the growth of water hyacinth and water lettuce plants for 30 days on effluent from an intensive, recirculating aquaculture system and reported biomass yields of 6402.5 and 10188 g m^{-2} for water hyacinth and water lettuce, respectively. Sooknah and Wilkie[13] investigated the use of water hyacinth and water lettuce plants for reducing the nutrient content of an anaerobically digested dairy manure. After 31 days of batch growth, the researchers reported biomass yields of 1608 and 30 g (dm) m^{-2} for water hyacinth and water lettuce, respectively.

(a) water hyacinth

(b) water lettuce

(c) parrot's feather

Fig. 3: The aquatic plants at day 14 of the experiment

Table 2: Average plant yields and growth rates after 24 days of growth in aquaculture wastewater

Plant	HRT (days)	Yield ($g\ m^{-2}$)	Growth rate ($g\ m^{-2}\ day^{-1}$)
Water hyacinth	6	83 ± 8.4	3.47 ± 0.35
	12	49 ± 2.6	2.05 ± 0.11
Water lettuce	6	51 ± 1.3	2.13 ± 0.05
	12	29 ± 4.1	1.20 ± 0.46
Parrot's feather	6	51 ± 1.5	2.11 ± 0.06
	12	22 ± 3.4	0.91 ± 0.14

Table 3: Results of a two-way ANOVA for plant yields as affected by plant type and hydraulic retention time

Source	DF	SS	MS	F	P
Total	11	4848.48			
Model	5	4635.76			
Plant type	2	2133.99	1067.00	30.10	0.001
HRT	1	2431.86	2431.86	68.59	0.000
Plant type × HRT	2	69.91	34.95	0.99	0.426
Error	6	212.72	35.45		

Note: Differences are considered significant at the $p \leq 0.05$ level (95% confidence interval).

Table 4: Results of a Duncan's multiple range test for plant yields as affected by plant type and hydraulic retention time

Parameter	Average Yields ($g\ dm\ m^{-2}$)	Duncan Subsets ($\alpha = 0.05$)
Plant type		
Water hyacinth	66.25	A
Water lettuce	40.00	B
Parrot's feather	36.29	B
HRT (days)		
6	61.75	A
12	33.28	B

Note: Treatments with different numbers are significantly different at the $p \leq 0.05$ level.

Plant Productivity: The average water hyacinth, water lettuce and parrot's feather growth rates were 3.47, 2.13 and 2.11 g (dm) m^{-2} day^{-1} and 2.05, 1.20 and 0.91 g (dm) m^{-2} day^{-1} for HRTs of 6 and 12 days, respectively (Table 2). DeBusk et al.[15] evaluated the use of a water hyacinth based treatment system for nutrient removal from a secondarily treated municipal wastewater and reported an average plant productivity of 16 g (dm) m^{-2} day^{-1}. Wen and Recknagel[16] examined the use of parrot's feather for treatment of agricultural drainage waters and reported an average growth rate for parrot's feather of 7.12 g (dm) m^{-2} day^{-1}. In this study, the growth rates are lower than those reported by other investigators because aquaculture effluents are characteristically high in volume, but low in nutrient content. In comparison, municipal and agricultural wastewaters are relatively low in volume and high in nutrient content[17].

Effluent Quality: Table 5 shows the influent and effluent total solids (TS), chemical oxygen demand (COD), ammonium – nitrogen (NH_4^+-N), nitrite – nitrogen (NO_2^--N), nitrate - nitrogen (NO_3^--N) and phosphate – phosphorus (PO_4-P) concentrations and the removal efficiencies for each water quality parameter. The aquatic plants were able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P reductions ranged from 21.4 to 48.0%, from 71.1 to 89.5%, from 55.9 to 76.0%, from 49.6 to 90.6%, from 34.5 to 54.4% and from 64.5 to 76.8%, respectively. Generally, the reductions increased with longer retention times and were highest in compartments containing water hyacinth followed by compartments containing water lettuce and parrot's feather.

Sooknah and Wilkie[13] compared the potential of water hyacinth and water lettuce plants for reducing the nutrient content of an anaerobically digested dairy manure and reported suspended solids, COD and NH_4-N reductions of 56.7, 65.8 and 99.8%, 92.0, 80.5 and 99.6% and 80.6, 79.6 and 99.2% after 31 days of growth in the control and in the compartments

Table 5: Water quality parameters

Parameter	HRT (days)	Treatment	Influent[a] (mg L^{-1})	Effluent[b] (mg L^{-1})	Reduction (mg L^{-1})	Reduction (%)
TS	6	Control	827 ± 29	650 ± 28	177	21.4
		Water hyacinth	827 ± 29	500 ± 26	327	39.5
		Water lettuce	827 ± 29	585 ± 13	242	29.3
		Parrot's feather	827 ± 29	650 ± 18	177	21.4
	12	Control	827 ± 29	600 ± 12	227	27.4
		Water hyacinth	827 ± 29	430 ± 21	397	48.0
		Water lettuce	827 ± 29	450 ± 16	377	45.6
		Parrot's feather	827 ± 29	525 ± 21	302	36.5
COD	6	Control	158 ± 9.3	34.7 ± 0.6	123.3	78.1
		Water hyacinth	158 ± 9.3	16.6 ± 1.0	141.4	89.5
		Water lettuce	158 ± 9.3	27.7 ± 1.6	130.3	82.5
		Parrot's feather	158 ± 9.3	24.7 ± 1.0	133.3	84.4
	12	Control	158 ± 9.3	45.7 ± 1.2	112.3	71.1
		Water hyacinth	158 ± 9.3	24.7 ± 3.0	133.3	84.4
		Water lettuce	158 ± 9.3	27.7 ± 2.4	130.3	82.5
		Parrot's feather	158 ± 9.3	33.7 ± 1.9	124.3	78.7
NH_4^+-N	6	Control	2.08 ± 0.50	1.38 ± 0.11	0.70	33.8
		Water hyacinth	2.08 ± 0.50	0.54 ± 0.06	1.58	76.0
		Water lettuce	2.08 ± 0.50	0.67 ± 0.21	1.41	68.0
		Parrot's feather	2.08 ± 0.50	0.75 ± 0.25	1.33	64.0
	12	Control	2.08 ± 0.50	1.43 ± 0.10	0.66	31.9
		Water hyacinth	2.08 ± 0.50	0.50	1.58	76.0
		Water lettuce	2.08 ± 0.50	0.58 ± 0.11	1.47	72.0
		Parrot's feather	2.08 ± 0.50	0.92 ± 0.14	1.16	55.9
NO_2^--N	6	Control	1.27 ± 0.09	0.84 ± 0.05	0.43	33.9
		Water hyacinth	1.27 ± 0.09	0.30 ± 0.07	0.97	76.4
		Water lettuce	1.27 ± 0.09	0.44 ± 0.04	0.83	65.0
		Parrot's feather	1.27 ± 0.09	0.64 ± 0.10	0.63	49.6
	12	Control	1.27 ± 0.09	0.60 ± 0.10	0.67	52.7
		Water hyacinth	1.27 ± 0.09	0.12 ± 0.08	1.15	90.6
		Water lettuce	1.27 ± 0.09	0.32 ± 0.09	0.95	74.5
		Parrot's feather	1.27 ± 0.09	0.49 ± 0.07	0.78	61.4
NO_3^--N	6	Control	21.64 ± 0.6	16.11 ± 0.4	5.53	25.6
		Water hyacinth	21.64 ± 0.6	12.18 ± 0.2	9.46	43.7
		Water lettuce	21.64 ± 0.6	12.60 ± 0.2	9.04	41.8
		Parrot's feather	21.64 ± 0.6	14.17 ± 0.8	7.47	34.5
	12	Control	21.64 ± 0.6	16.22 ± 0.2	5.42	25.0
		Water hyacinth	21.64 ± 0.6	9.87 ± 0.2	11.77	54.4
		Water lettuce	21.64 ± 0.6	10.19 ± 0.3	11.45	52.9
		Parrot's feather	21.64 ± 0.6	10.62 ± 0.3	11.02	50.9
PO_4-P	6	Control	4.49 ± 0.18	2.77 ± 0.25	1.72	38.4
		Water hyacinth	4.49 ± 0.18	1.52 ± 0.14	2.97	66.2
		Water lettuce	4.49 ± 0.18	1.57 ± 0.14	2.92	65.0
		Parrot's feather	4.49 ± 0.18	1.59 ± 0.01	2.90	64.5
	12	Control	4.49 ± 0.18	2.55 ± 0.06	1.94	43.3
		Water hyacinth	4.49 ± 0.18	1.04 ± 0.18	3.45	76.8
		Water lettuce	4.49 ± 0.18	1.11 ± 0.28	3.38	75.3
		Parrot's feather	4.49 ± 0.18	1.49 ± 0.12	3.00	66.8

[a] day 1
[b] day 24

Table 6: A comparison between the nutritional composition of wastewater grown aquatic plants and the nutrient requirements of aquatic organisms[20-28].

Parameter	Water hyacinth	Water lettuce	Parrot's feather	Fish Feed*
Energy (MJ/kg)	13.6	12.9	14.4	12 - 23
Nutrients (% dm)				
Carbohydrates	61.1	53.7	60.9	10 – 30
Fiber	17.25	19.78	11.39	1 – 12
Protein	15.02	17.07	16.51	32 – 52
Fat	2.41	2.76	3.77	4 – 28
Macroelements (mg kg^{-1})				
Calcium	2.60 ± 0.34	3.80 ± 0.16	2.33 ± 0.05	0.03 – 2.90
Magnesium	2.45	2.21	2.10	0.10 – 0.50
Nitrogen	0.42 ± 0.01	0.61 ± 0.05	0.28 ± 0.05	0.04 – 0.30
Phosphorus	0.47 ± 0.06	0.54 ± 0.05	0.30 ± 0.00	0.45 – 2.20
Potassium	2.11 ± 0.20	1.71 ± 0.07	1.57 ± 0.58	0.50 – 1.50
Sodium	1.33 ± 0.03	1.85 ± 0.04	0.73 ± 0.00	0.10 – 2.30
Sulfur	0.30 ± 0.01	0.46 ± 0.00	0.24 ± 0.05	0.30 – 1.70
Microelements (mg kg^{-1})				
Boron	35 ± 1.1	56 ± 2.4	27 ± 8	
Copper	165 ± 9.6	285 ± 64	108 ± 24	3 – 10
Iron	2008 ± 1512	2647 ± 1164	1203 ± 853	30 – 170
Manganese	1969 ± 1047	2610 ± 939	457 ± 115	2.4 – 120
Molybdenum	2 ± 0.4	3 ± 1.0	2 ± 0.1	
Selenium	4 ± 2.8	4 ± 1.7	6 ± 0.4	0.15 – 0.40
Zinc	5118 ± 884	3224 ± 723	1985 ± 34	15 – 240

containing water hyacinth and water lettuce, respectively. Jo et al.[14] evaluated the potential of water hyacinth and water lettuce plants for removal of NH_4^+-N, NO_2^--N and NO_3^--N from an intensive, recirculating aquaculture system effluent. Over a 48 hour period, the NH_4^+-N, NO_2^--N and NO_3^--N concentrations in the wastewater were reduced from 2.3 mg L^{-1} to 0.4 and 0.6 mg L^{-1}, from 0.197 mg L^{-1} to 0.024 and 0.029 mg L^{-1} and from 21.4 mg L^{-1} to 17.4 and 17.9 mg L^{-1} in aquaria containing water lettuce and water hyacinth, respectively. Jing et al.[18] investigated the use of water lettuce for nutrient removal from an artificially prepared wastewater. After a 30 day period, the researchers reported average PO_4^{3-}-P removal efficiencies in the controls and in the compartments containing water lettuce of 8.0, 33.3, 42.3 and 31.6% and 14.3, 53.9, 73.2 and 55.6% at hydraulic retention times of 1, 2, 3 and 4 days, respectively. Nuttall [19] examined the ability of parrot's feather for nutrient reduction from a secondarily treated municipal wastewater. Over a 13 month period, the researchers reported suspended solids removal efficiencies ranging from 12.8 to 65.0% and concluded that the continuous aeration of the lagoons agitated the wastewater and kept particles in suspension.

Nutrition: The plants grown at a hydraulic retention time of 6 days grew faster and produced higher yields and were therefore used for nutritional analysis. Six major components were considered when analyzing the wastewater grown water hyacinth, water lettuce and parrot's feather plants as potential fish feed: energy, carbohydrates, crude protein, crude fat, macroelements and microelements. Table 6 displays a comparison between the nutritional composition of the wastewater grown plants at a hydraulic retention time of 6 days and the nutritional requirements of aquatic animals.

Energy: Energy is defined as the ability or capacity to do work. Aquatic animals derive energy through the catabolism of dietary carbohydrates, lipids and proteins within the body. Energy is essential for the maintenance of life processes including: cellular metabolism, growth, reproduction and physical activity. The ability of a food to supply energy is, therefore, of great importance in determining its nutritional value to animals. The mean gross energy value for carbohydrates, lipids and proteins has been estimated to be 17.2 kJ g^{-1} (4.1 kcal g^{-1}), 39.8 kJ g^{-1} (9.5 kcal g^{-1}) and 23.4 kJ g^{-1} (5.6 kcal g^{-1}), respectively[29].

Two important differences exist in the energy metabolism in fish and shellfish compared to terrestrial farm animals. First, unlike warm-blooded animals, fish and shellfish are aquatic ectotherms, which means that they have no internal metabolic mechanism for regulating their body temperature and, therefore, do not have to expend energy to maintain a body temperature well above ambient conditions[30]. Second, the excretion of waste nitrogen requires less energy in fish and shellfish compared to terrestrial farm animals. Fish and shellfish do not have to convert ammonia, the end product of protein catabolism into less toxic substances (urea or uric acid) prior to excretion. Therefore, fish and shellfish can obtain 10 – 20% more energy from the catabolism of proteins compared to terrestrial farm animals[3].

Providing the optimum energy level in the diets of fish and shellfish is important for the development of a healthy product. Because fish feed to meet their energy requirements, excess dietary energy may result in high fat deposition in the fish, decreased feed intake and reduced weight gain. Similarly, a diet with low energy content may result in reduced weight gain because the animal will utilize nutrients for energy provision rather than for tissue synthesis and growth. A number of factors are known to influence the energy requirements of fish and shellfish including: water temperature, animal size, physiological status and water quality and stress[29].

The wastewater grown water hyacinth, water lettuce and parrot's feather plants had energy contents of 13.6, 12.9 and 14.4 MJ kg^{-1}, which meets the energy requirements of aquatic animals. Dominguez et al.[31] evaluated the chemical composition of water hyacinth plants grown on an anaerobically digested pig manure and reported an energy content of 15.4 MJ kg^{-1}. Lopes et al.[32] reported that the energy content of natural stands of water hyacinth and water lettuce plants ranged from 9.2 to 15.9 MJ kg^{-1} and from 9.8 to 14.5 MJ kg^{-1}, respectively. Steubing et al.[33] reported that the energy content ranged from 12.9 to 13.2 MJ kg^{-1} in natural stands of parrot's feather.

Carbohydrates: Carbohydrates constitute the third most abundant group of organic molecules in the animal body[20]. They are produced by photosynthetic plants and contain carbon, hydrogen and oxygen in the ratio 1: 2: 1. Carbohydrates include sugars, starches, cellulose and other related compounds and serve as the principle source of metabolic energy in terrestrial farm animals. In fish and shellfish, no essential dietary requirement for carbohydrates has been established. Carbohydrates are included in fish and shellfish diets because they are an inexpensive source of dietary energy, serve as a binding agent during feed manufacturing and can increase feed palatability[3, 34].

Carnivorous fish species (salmonids) have a limited ability to digest complex carbohydrates due to the weak amylotic activity in their digestive tract. By contrast, warm water omnivorous and herbivorous fish species such as carp, channel catfish, tilapia and eel have been found to be more tolerant of high dietary carbohydrate levels. Unlike terrestrial farm animals, most fish species have a relatively short gastro – intestinal tract with little microbial colonization. As a result, the intestinal cellulase activity of fish is weak or absent. Consequently, dietary cellulose or crude fiber has no utilizable energy value and in dietary excess has a negative impact on growth and feed efficiency[29].

The wastewater grown water hyacinth, water lettuce and parrot's feather plants had carbohydrate and crude fiber contents of 61.1, 53.7 and 60.9% and 17.25, 19.78 and 11.39%, respectively. These exceed the dietary requirements of fish and shellfish. Abdelhamid and Gabr[35] reported that the chemical composition of natural stands of water hyacinth plants in terms of carbohydrates and crude fiber was 31.9 and 18.9%, respectively. El-Sayed[36] reported carbohydrate and crude fiber concentrations of 40.7 and 19.0% in natural stands of water hyacinth plants. Poddar et al.[37] reported that the chemical composition of water hyacinth plants in terms of carbohydrates and crude fiber was 49.41 and 16.34%, respectively. Carbohydrates are not normally included as a large part of the diet due to their low nutritional content and poor digestibility. Cooking, extrusion and expansion are methods used to improve digestibility of carbohydrates[25].

Crude protein: Proteins are high molecular weight organic compounds essential to the structure and function of all living cells. They consist of amino acids joined by peptide bonds and are composed of 50 – 55% carbon, 15 – 18% nitrogen, 20 – 23% oxygen, 6 – 8% hydrogen and 0 – 4% sulfur[3]. Protein is required in the diet to provide essential amino acids and nitrogen for the synthesis of non-essential amino acids and other nitrogen containing compounds [29, 34].

Fish and shellfish have a high dietary protein requirement which is generally attributed to their carnivorous/omnivorous feeding habit and their preferential use of protein over carbohydrates as a dietary energy source[29]. Protein requirements vary depending on species cultured, rearing environment and size and age of the cultured organisms. Generally, herbivorous fish have lower protein requirements than omnivorous and carnivorous species, fish reared in low density systems (pond aquaculture) have lower protein requirements than fish reared in high density systems (recirculating aquaculture) and larger, older fish have lower protein requirements than younger, smaller fish[38]. Protein is typically the largest and most expensive component of an aquaculture diet [4].

The wastewater grown water hyacinth, water lettuce and parrot's feather plants had crude protein contents of 15.02, 17.07 and 16.51%, respectively. These do not meet the dietary requirements of aquatic animals. These findings were quite comparable with those reported by other investigators. Wolverton and McDonald [39] conducted nutrient analyses on water hyacinth plants grown in four experimental sewage lagoons and reported that the crude protein content of the wastewater grown plants was in the range of 9.7 – 23.4%. Kawai et al.[40] evaluated the nutritional content of water hyacinths grown on primarily treated domestic sewage. After a 14 month period, the researchers reported a crude protein content of 17.5% in the water hyacinth plants. Jo et al.[14] reported that the crude protein contents of water hyacinth and water lettuce plants cultured in effluent from a recirculating aquaculture facility were 15.48 and 22.44%, respectively.

Since the macrophytes used in the study do not meet the protein requirements of aquatic animals, a protein supplement must be added. Fishmeal is one of the major ingredients in fish feed and is the most common protein source. Other common protein sources include meat and bone meal[25]. Either of these could be used to supplement the crop with protein at the required amount.

Crude fat: Lipids (fats) are a heterogeneous group of organic compounds found in plant and animal tissues that are readily soluble in organic solvents such as benzene, chloroform and ether, but are only sparingly soluble in water. Lipids are required for the long-term storage of metabolic energy, to supply essential fatty acids, as carriers of fat soluble vitamins and for structure and control[29]. Fatty acids are long chain organic acids having the general formula $CH_3(C_XH_Y)COOH$. The hydrocarbon chain is either saturated (only single bonds between adjacent carbon atoms) or unsaturated (double bonds between some of the adjacent carbon atoms) and usually contains an even number of carbon atoms (C_{14} to C_{24}) in straight chains[41]. In nature, fatty acids usually occur as triesters of glycerol and are called triacylglycerols or triglycerides[5]. Fatty acids are a major source of metabolic energy in fish for growth, reproduction and egg production [42].

The crude fat content of the wastewater grown water hyacinth, water lettuce and parrot's feather plants was 2.41, 2.76 and 3.77%, respectively. These do not meet the dietary fat requirement of fish and shellfish. Fish or vegetable oils can be added to the feed during manufacturing to increase the fat content in the diet. The results obtained from this study are comparable to those reported by other investigators. El-Sayed[36] reported that the crude fat content of natural stands of water hyacinths was 1.0%. Poddar et al.[37] reported a crude fat content of 1.61% in natural stands of water hyacinth. Jo et al.[14] evaluated the nutritional value of water hyacinth and water lettuce plants cultured in effluent from a recirculating aquaculture facility and reported crude fat contents of 4.75 and 4.61% for water hyacinth and water lettuce, respectively.

Macroelements: Macroelements are required by the body in relatively large amounts (> 100 mg kg^{-1} dry diet) and include calcium (Ca), chlorine (Cl), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na) and sulphur (S). These elements function in cellular metabolism, have important roles in osmoregulation and acid-base balance and serve as structural components of tissues[41]. The dietary macroelement requirements of fish and shellfish depends to a large extent upon the concentration of the element in the water body. This is because aquatic animals are able to directly absorb minerals through their gills, fins and skin from the surrounding water[43].

The wastewater grown water hyacinth plants meet the Ca, P, Na and S dietary requirements of aquatic animals and exceed the Cl, Mg and K requirements. The wastewater grown water lettuce plants meet the P, Na and S dietary requirements of aquatic animals and exceed the Ca, Cl, Mg and K requirements. The wastewater grown parrot's feather plants meet the Ca, Mg and Na dietary requirements of aquatic animals, exceed the Cl, Mg and K requirements and shellfish and do not contain sufficient quantities of P and S. These findings are comparable to those reported by other researchers. Abulude[44] reported that the nutrient composition of natural stands of water lettuce in terms of Ca, Mg, P, K and Na was 0.32, 0.16, 0.20, 0.80 and

0.20%, respectively. Tan[44] reported that the nutritive value of natural stands of water lettuce in terms of Ca, P, K, Na, Mg, S, and Cl was 0.88, 1.14, 2.38, 0.403, 0.760, 0.132 and 1.625%, respectively. Abdelhamid and Gabr[35] reported that the chemical composition of natural stands of water hyacinth plants in terms of P, Mg and Ca was 0.53, 0.17, and 0.58%, respectively. Poddar et al.[37] reported that the chemical composition of water hyacinths in terms of P, Ca and K was 0.53, 2.29 and 2.44%, respectively.

There was no evidence in the literature to suggest that the calcium, chlorine and magnesium concentrations observed in the wastewater grown plants would be detrimental to the healthy development of fish and shellfish[46-48]. However, studies have shown that excess dietary potassium can cause depressed growth, weight gain and nutrient utilization efficiency and reduced body fat and protein deposition in certain species of finfish[49-50].

Microelements: Microelements are required by the body in trace amounts (< 100 mg kg^{-1} dry diet) and include boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn)[43]. Microelements are involved in the regulation of cellular metabolism and are required for proper growth and development. The uptake and toxicity of dietborne metals in fish and shellfish are not well understood[51].

The three wastewater grown plants exceed the Cu, Fe, Mn, Se and Zn dietary requirements of aquatic animals. These findings are similar to those reported by others investigators. Qian et al. [52] evaluated the use of water lettuce and parrot's feather plants for removal of B and Se from an artificially prepared wastewater and reported B and Se concentrations of 400 and 250 mg kg^{-1} and 39 and 11 mg kg^{-1} in the shoot tissues of water lettuce and parrot's feather, respectively. Cordes et al.[53] investigated the uptake of Cu and Zn by water hyacinth plants grown in pulverized fuel ash leachate and reported Cu and Zn concentrations of 63.7, 239 and 667 mg kg^{-1} and 294, 418 and 336 mg kg^{-1} after 1, 7 and 14 days of batch growth, respectively. Soltan and Rashed[54] examined ability of water hyacinth to remove Cu, Mn and Zn from an artificially prepared wastewater and reported that the Cu, Mn and Zn concentrations in the wastewater grown plants ranged from 1750 to 2950 mg kg^{-1}, from 1950 to 2110 mg kg^{-1} and from 1850 to 5000 mg kg^{-1}, respectively. Cardwell et al.[55] reported Cu and Zn concentrations of 431.0 and 4296.1 mg kg^{-1} in roots of parrot's feather plants growing in contaminated urban streams. Sridhar[56] evaluated the ability of water lettuce plants to accumulate trace elements from a lake receiving organic and laboratory chemical wastes and reported an elemental composition in the plant tissue of 17.53, 11229.90, 210.99 and 7214.36 mg kg^{-1} for Cu, Fe, Zn and Mn, respectively.

Studies have shown that excess dietary Cu, Fe, Mn, Se and Zn can cause reduced feed intake, weight gain and growth rates in fish and shellfish[57-62]. (Berntssen et al., 1999; Lanno et al., 1985; Baker and Martin, 1997; Lorentzen et al., 1996; Lin and Shiau, 2005; and Eid and Ghonim, 1994).

CONCLUSIONS

The aquatic plants grew rapidly in the hydroponics system and appeared healthy with green color. At hydraulic retention times (HRTs) of 6 and 12 days, the average water hyacinth, water lettuce and parrot's feather yields were 83, 51 and 51 g (dm) m^{-2} and 49, 29 and 22 g (dm) m^{-2}, respectively. The aquatic plants were able to significantly reduce the pollution load of the aquaculture wastewater. The TS, COD, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P reductions ranged from 21.4 to 48.0%, from 71.1 to 89.5%, from 55.9 to 76.0%, from 49.6 to 90.6%, from 34.5 to 54.4% and from 64.5 to 76.8%, respectively. Generally, the reductions increased with longer retention times and were highest in compartments containing water hyacinth followed by compartments containing water lettuce and parrot's feather. The three wastewater grown plants do not contain sufficient amounts of protein and fat to meet the dietary requirements of fish and shellfish. They also contained high concentrations of K, Cu, Fe, Mn, Se and Zn, which can lead to reduced feed intake, weight gain and growth rates in fish and shellfish.

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