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Protein concentrate of water hyacinth partially replaces soybean meal in the diet of the Nile tilapia *Oreochromis niloticus* juveniles

[1]Gaily J. S. Hontiveros, [2]Barry L. M. Tumbokon, [2,3]Augusto E. Serrano Jr.

[1] College of Fisheries, Mindanao State University, General Santos City, Philippines; [2] Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines; [3] National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao, Iloilo, Philippines. Corresponding author: A. E. Serrano Jr., serrano.gus@gmail.com

Abstract. A 90-day feeding trial was conducted to determine the effects of water hyacinth leaf protein concentrate (WHLPC) on survival, growth, feed conversion efficiency and carcass composition of the Nile tilapia *Oreochromis niloticus* juveniles. Four experimental diets containing WHLPC replacing soybean meal at 0, 15, 30 and 45% were fed to triplicate groups of tilapia fingerlings. Data shows that survival rates (85-88%), feed intake, weight gain (WG), specific growth rate (SGR) and feed conversion ratios were all statistically similar among dietary treatments. However, carcass protein was higher in fish fed diets at 15 and 30% replacement levels than in those fed the control; those fed diets with 45% replacement was similar with those fed the control diet. In contrast, carcass lipid in fish fed the control diet was statistically similar with that in fish fed diets with 30 and 45% substitution levels. Increasing levels of WHLPC replacement did not significantly affect the levels of ash and NFE in fish carcass at the end of the growth trial. Conclusion: WHLPC could partially replace soybean meal in the diet of the Nile tilapia up to 45% which was equivalent to 11.7% inclusion in the diet.
Key Words: Nutrition, leaf protein concentrate, plant protein source, carcass composition.

Introduction. Previously, we have shown that concentrating the water hyacinth meal resulted in a 248% increase in crude protein (Hontiveros & Serrano 2015). In the same study, the apparent dry matter digestibility (ADMD) of water hyacinth leaf protein concentrate (WHLPC) was high at 76.4%, with chemical score of 38.9 due to the limiting amino acid methionine. Its Essential Amino Acid Index (EAAI) was 0.88 that indicated that it was a useful protein source.

Soybean meal used in aquafeed production is imported in Asia including the Philippines and replacing it with an equivalent locally available plant material may reduce the price of aquafeeds in general. Water hyacinth is a great candidate to partially replace soybean meal in aquafeeds due to its abundance in some areas in the Philippines and its being considered as a nuisance that clogs waterways and results in flooding. However, its high fiber, low protein content is a factor that works against it as a candidate ingredient. Concentrating the protein and reducing fiber can be achieved by chemical and mechanical means to produce a leaf protein concentrate whose nutritive value could be dramatically improved (Hontiveros & Serrano 2015). Other problems with plant proteins in general include amino acid deficiency or imbalance (Lund et al 2011), presence of anti-nutritional factors, and the presence of crude fiber, cellulose, hemicellulose, and lignin. Fish do not generally possess cellulase or other gut flora which hydrolyze cellulose in macrophytes (Saha & Ray 2011). These are problems that could be encountered at very high levels of incorporation but when used as a partial substitute, plant protein could be incorporated without negative effect on the performance of cultured species. This has been shown in shrimp *Penaeus monodon* (Chavez et al 2014).

The aim of the present study was to determine the effects of increasing the proportion of replacement of soybean meal with WHLPC on survival, growth performance, feed utilization, carcass composition, and nutrient retention in *Oreochromis niloticus* juveniles.

Material and Method

Growth trials were done from August to October 2013 at the UPV Multispecies Hatchery in Miagao, Iloilo, Philippines.

Production of WHPC. Water hyacinth was collected from a freshwater pond in a domestic area in Culasi, Antique, Philippines. Leaves were shade dried until moisture was less than 10% and were pulverized in a hammer mill. Water hyacinth leaf protein concentrate (WHLPC) was prepared as described earlier (Hontiveros & Serrano 2015). Briefly, dried water hyacinth leaves were soaked in fresh water at 1:3 (w/v) for about 30 min and homogenized in a blender for about 5 min. Sodium hydroxide (NaOH) solution was added to adjust the pH to 9.0. The slurry was filtered through cheesecloth and the filtrate was acidified with hydrochloric acid (HCl) to pH 2.0 and was allowed to stand to flocculate. The juice was heated at 60-80^{o}C for 10 min and the precipitate formed collected, oven-dried at 60^{o}C, and ground to its final powder form.

Diet preparation. Experimental diets (Table 1) were formulated to meet the requirements of Nile tilapia (NRC 1993). WHLPC replaced 15%, 30%, and 45% of soybean meal by weight in Diets 1, 2, and 3, respectively. At these levels, protein contribution of defatted soybean meal in the diets was replaced with WHLPC at 3.9, 7.8, and 11.7% dietary inclusion. A diet containing no WHLPC served as the control. The ingredients were mixed with distilled water, pelleted, and subsequently oven-dried at 60^{o}C for 12 h. Feeds were then sieved to produce pellets (0.5 to 2 mm) and stored in polyethylene bags at -20^{o}C until use.

Experimental animals. Nile tilapia (*O. niloticus*) fry were obtained from the hatchery of Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC/AQD), Tigbauan, Iloilo and transported to the university hatchery. Prior to the growth trial, fry were acclimatized to the laboratory conditions for two weeks and were fed the control diet at 30% body weight, four times daily (8 AM, 11 AM, 2 PM, 5 PM).

The feeding trial was conducted in a freshwater recirculating system with a biological filter, sedimentation tank, and sediment filter (5 µm). Using a completely random design, each dietary treatment was assigned to three replicate tanks with three replicates per diet. Fry (0.045 ± 0.001 g) were weighed and randomly distributed to 12 tanks (95 L) at a density of 20 fry tank^{-1}. Formulated diets were given by hand 4 times daily at 8 AM, 11 AM, 2 PM, 5 PM for 90 days. Daily feeding rate ranged from 20 to 6% over the trial period based on the bulk weight of fish recorded every 10 days.

Tanks were cleaned daily before the first feeding and about 70% of the system volume was replaced with dechlorinated freshwater (0 ppt) every 3 days. Continuous aeration was provided and water quality parameters were monitored regularly. Water temperature and pH were measured twice daily (8 AM and 2 PM) using a laboratory mercury thermometer and hand-held digital pH meter, respectively. Levels of dissolved oxygen by titration and total ammonia nitrogen, and nitrite were measured by colorimetry using test kits (Advance Pharma Co., Ltd., Bangkok, Thailand).

Table 1

Composition (g kg^{-1} dry matter) and proximate analysis of experimental diets containing varying replacement levels of water hyacinth protein concentrate

Ingredient	*Diet 1 (0%)*	*Diet 2 (15%)*	*Diet 3 (30%)*	*Diet 4 (45%)*
Danish meal	255.8	255.8	255.8	255.8
Squid meal	81.0	81.0	81.0	81.0
Defatted soybean meal	260.0	221.0	182.0	143.0
WHPC	-	39.0	78.0	117.0
Copra meal	152.5	152.5	152.5	152.5
Cod liver oil	10.0	10.0	10.0	10.0
Soybean oil	10.0	10.0	10.0	10.0
Corn starch	187.5	187.5	187.5	187.5
Vitamin mix[a]	13.0	13.0	13.0	13.0
Mineral mix[b]	30.0	30.0	30.0	30.0
Antioxidant[c]	0.2	0.2	0.2	0.2
Proximate composition (analyzed)				
Dry matter	939.9	930.9	934.4	932.3
Crude protein	420.0	406.8	407.2	401.5
Crude lipid	74.7	72.6	75.6	75.2
Crude fiber	38.9	45.2	38.2	49.1
Ash	94.7	98.6	97.6	99.5
Nitrogen free extract (NFE)	371.6	376.7	381.4	374.7

[a] Vitamin premix (kg^{-1} of diet): Vitamin A, 15600 IU; Vitamin D3, 2600 IU; Vitamin E, 260 IU; Vitamin B1, 104 mg; Vitamin B2, 104 mg; Vitamin B6, 65 mg; Vitamin B12, 26 µg; Niacin, 520 mg; Calcium pantothenate, 260 mg; Biotin, 0.52 mg; Folic acid, 23.4 mg; Ethoxyquin, 6.5 mg.
[b] Mineral premix (kg^{-1} of diet): Iron, 1200 mg; Manganese, 300 mg; Zinc, 1200 mg; Copper, 120 mg; Iodine, 54 mg; Cobalt, 600 µg; Selenium, 6 mg.
[c] Antioxidant: butylated hydroxytoluene (BHT).

Proximate analysis. Proximate composition of the WHLPC and tilapia carcass at the termination of the experiment was analyzed at the Quality Control Laboratory of Oversea Feeds Corporation, San Fernando, Cebu, Philippines. Moisture was measured using a thermo-balance (Mettler Toledo HB43 halogen moisture analyzer). Ash content was determined after incineration in a muffle furnace at 550°C for 12 h (AOAC 1995). Crude protein was measured after block digestion and steam distillation using Foss Tecator™ digestion system and Foss Kjeltec™ 8200 auto-distillation unit. Crude fat was extracted using Foss Soxtec™ 2050 automatic system and fiber was determined using Foss Fibertec™ 2010 system.

Calculations. Growth performance and nutrient utilization were measured by calculation of the following response parameters:

Survival (SR, %) = (final number of fish/ initial number of fish) x 100 Weight gain (WG, g) = final weight - initial weight

Specific growth rate (SGR, % day^{-1}) = [(In final weight - In initial weight) / number of culture days] x 100

Feed conversion ratio (FCR) = total dry feed intake (g)/wet weight gain (g)

Nutrient retention (NR, % intake) = 100 x [(% final carcass nutrient x final ABW) – (% initial carcass nutrient x initial ABW)] / total nutrient intake

Energy retention (% intake) = 100 x [(% final carcass energy x final ABW) – (% initial carcass energy x initial ABW)] / total energy intake

where physiological fuel values used were 5.64, 9.44, and 4.11 kcal g^{-1} for protein, lipid, and carbohydrate, respectively (NRC 1993).

Statistical analysis. A Shapiro-Wilks W test was used to asses normality of data and Levene's test was performed to check homogeneity of variance before emplying analysis of variance (ANOVA). Apparent dry matter digestibility of diets and data from the growth trial (i.e. survival, weight gain, SGR, FCR, carcass composition, nutrient and energy retention) were analyzed by one-way analysis of variance (ANOVA). Where significant difference existed, treatment means were compared using Tukey's HSD test at $p<0.05$. Values were expressed as means ± S.E.M. All statistical calculations were performed using SPSS for Windows (version 16).

Results and Discussion. After a 90-day feeding trial, survival rates were high with a range of 85 to 88% and were statistically similar among treatments (Table 2). Similarly, weight gain (WG) and specific growth rate (SGR) did not significantly change with increasing replacement of soybean meal with water hyacinth leaf protein concentrate (WHLPC). Feed intake was statistically similar in all tilapia fed the dietary treatments, indicating that all diets were palatable. Also, feed conversion ratio (FCR) did not differ significantly among fish fed the various dietary treatments. However, carcass protein was higher in fish fed diets with 15 and 30% soybean replacement than in those fed the control and 45% soybean this was similar with that in fish fed the control diets (Table 3). In contrast, carcass lipid in fish fed the control diet was statistically similar with that in fish fed diets with 30 and 45% substitution levels. Increasing levels of WHLPC replacement did not significantly affect the levels of ash and NFE in fish carcass at the end of the growth trial.

Table 2

Growth performance and feed utilization of Nile tilapia fry fed diets containing increasing inclusion of water hyacinth leaf protein concentrate (WHLPC)
Values reported are means ± S.E.M. of three replicates

Parameters	*Diet 1 (0%)*	*Diet 2 (15%)*	*Diet 3 (30%)*	*Diet 4 (45%)*
Initial body weight (g)	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Final body weight (g)	5.74 ± 0.96	4.05 ± 0.44	4.09 ± 0.39	4.09 ± 0.67
Survival (%)	86.7 ± 1.7	86.7 ± 3.3	88.3 ± 4.4	85.0 ± 5.0
Weight gain (g)	5.69 ± 0.96	4.00 ± 0.44	4.04 ± 0.39	4.05 ± 0.67
Specific growth rate (% day^{-1})	6.00 ± 0.21	5.49 ± 0.08	5.52 ± 0.10	5.59 ± 0.19
Feed intake (g $fish^{-1}$)	6.29 ± 1.48	5.18 ± 0.78	4.50 ± 0.41	5.34 ± 0.79
Feed conversion ratio (FCR)	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.0

Table 3

Whole body composition (g kg^{-1}) of Nile tilapia fed diets containing increasing inclusion of water hyacinth leaf protein concentrate (WHLPC)

Parameter	*Diet 1 (0%)*	*Diet 2 (15%)*	*Diet 3 (30%)*	*Diet 4 (45%)*
Dry matter	95.1 ± 0.5	94.4 ± 1.1	94.5 ± 2.4	94.65 ± 1.7
Crude protein	58.1 ± 1.3^{ab}	61.6 ± 8.4^{a}	58.3 ± 9.8^{ab}	57.52 ± 4.8^{b}
Crude lipid	24.5 ± 0.9^{a}	22.2 ± 2.5^{b}	23.7 ± 4.7^{ab}	24.8 ± 1.0^{a}
Ash	15.5 ± 0.2	15.3 ± 0.9	16.2 ± 3.3	15.3 ± 0.3

Values reported are means ± S.E.M. of three replicates. Means with common superscripts in the same row are not significantly different (p<0.05). NS: not significant.

Gradual substitution of soybean meal with WHLPC did not significantly affect protein, lipid and energy retention in all treatments (Table 4).

Table 4

Nutrient and energy retention (% of intake) in carcass of Nile tilapia fed diets containing increasing inclusion of water hyacinth leaf protein concentrate (WHLPC)

Parameter	*Diet 1 (0%)*	*Diet 2 (15%)*	*Diet 3 (30%)*	*Diet 4 (45%)*
Protein	26.4 ± 1.6	26.2 ± 2.0	24.8 ± 2.6	23.6 ± 0.4
Lipid	64.7 ± 3.9	51.3 ± 3.9	54.5 ± 5.6	54.7 ± 0.9
Energy	23.5 ± 1.4	21.5 ± 1.6	21.2 ± 2.2	20.8 ± 0.3

Values reported are means ± S.E.M. of three replicates. NS: not significant.

The present study demonstrated that partially substituting WHLPC for soybean meal up to 45% in the diet of *Oreochromis niloticus* did not reduce survival, feed intake, growth rate and even carcass composition. These findings was supported previously by the ingredient's high dry matter digestibility at 79.4% and high essential amino acid index (EAAI) of 0.88 indicating that WHLPC was a good protein source (Hontiveros & Serrano 2015). The low chemical score of the WHLPC in the previous study did not manifest in all the biological parameters measured in the present study since it was not a major source of protein in the various experimental diets used. The limiting amino acids could have been supplemented by the other protein sources in the diet such as fishmeal, squid meal, soybean meal and copra meal.

Utilization of water hyacinth in aquafeeds was studied in rohu *Labeo rohita* (Saha & Ray 2011) and African catfish *Clarias gariepinus* (Sotolu & Sule 2011). Its use was also evaluated in diets for red tilapia fingerlings (A-Rhaman Tibin et al 2012) and Nile tilapia *O. niloticus* (Bag et al 2011; Sulieman & Lado 2011) but only as whole plant meal, leaf meal, or fermented leaf meal. In red tilapia, dried water hyacinth leaf could substitute for wheat bran meal up to 20% (on a dry weight basis) without negatively affecting growth, digestion and palatability (A-Rhaman Tibin et al 2012). However, the researchers have observed that digestibility and survival rate declined at higher replacement levels and were attributed to the fiber content in raw water hyacinth. In the Nile tilapia fingerlings, after feeding experimental diets containing 0, 10 and 15% sun-dried water hyacinth leaf meal for 105 days, Sulieman & Lado (2011) have observed that the diet with the lower amount of water hyacinth leaves (10%) had better growth performance, food conversion ratio and protein efficiency ratio. In *O. niloticus* fingerlings, inclusion of water hyacinth meal at 10% produced significantly poorer weight gain, specific growth rate and feed utilization efficiency than the water hyacinth-free diets after a 50-day feeding trial. However, fresh dry hyacinth subjected to fermentation with molasses, cow rumen content or yeast, fish were able to utilize the diets more efficiently (El-Sayed 2003).

Composted water hyacinth could provide the necessary nutritional requirements of *O. niloticus* (Edwards et al 1985). In their study, good growth and feed utilization efficiencies were obtained with diets containing up to 75% composted water hyacinth with no significant reduction in fish performance compared with the conventional pelleted tilapia feed. Furthermore, in the same study, they observed that fish performed better when given feeds with composted water hyacinth than those fed with dried water hyacinth perhaps because of the reduced fiber in the composted material. In channel catfish, hyacinth protein extract was incorporated in the diets at increasing amounts of up to 40%; the observed optimum range that promoted growth similar to that of the control diet was 5-10 % (Liang & Lovell 1971). This was in agreement with the present study in which the absolute percentage of the WHLPC ranged between 3.9-11.7% and the biological indices measured at the highest level was statistically similar with that of the control diet. In the shrimp *Penaeus monodon*, Chavez et al (2014) concluded that WHLPC could replace soybean meal until 75 % without affecting the overall performance of *P. monodon* post larvae. However, not only did the WHLPC component varied but also the starch in the diet which was reduced dramatically from 20.0% in the control diet to 2.5% in the highest WHLPC inclusion level. Thus, their conclusion on the replacement of soybean meal by the WHLPC could have been masked by the variable starch content of the experimental diets.

Concentrating leaf protein could optimize the protein/amino acid content in plant protein sources, reduce the level of anti-nutrients, and increase incorporation of plant protein in fish diet (Lund et al 2011). Leaf protein concentrates (LPC) contain more bioavailable source of dietary nutrients compared to other preparations of leaf material (Virabalin et al 1993). The same researchers have observed that plant cells are disrupted during the process of concentration thereby increasing the amount of protein, unsaturated fats, carotenes, xanthophylls, starch and minerals in the concentrate. The WHLPC in the present study was fairly digestible and its acceptability to the Nile tilapia was not reduced even at the highest incorporation of 11.7% of the diet or 45% replacement of soybean meal. This could be attributable to the process of concentrating nutrients used in the present study.

Conclusions. WHLPC could replace up to 45% of the soybean meal (or an equivalent inclusion of 11.7% in the diet) without negative effects on survival, growth, feed conversion efficiency and in general, carcass composition.

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Authors:
Gaily Jubie Sela Hontiveros, Mindanao State University, College of Fisheries, Philippines, General Santos City, e-mail: gailyjubie@gmail.com
Barry Leonard Malto Tumbokon, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Miagao, Iloilo, 5023, email: lbmtumbokon@gmail.com
Augusto Erum Serrano Jr., University of the Philippines Visayas, National Institute of Molecular Biology and Biotechnology, Philippines, Miagao, Iloilo 5023, e-mail: serrano.gus@gmail.com

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