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Nutritional value of water hyacinth (*Eichhornia crassipes*) leaf protein concentrate for aquafeeds

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Abstract. The present study evaluated the nutritive value of water hyacinth leaf protein concentrate (WHLPC) as a potential feed ingredient for aquafeeds in general and measured the Apparent Digestibility of the Ingredient (ADI) for dry matter in *Oreochromis niloticus* adults. Concentrating the water hyacinth meal resulted in a 248% increase in crude protein. The apparent dry matter digestibility (ADMD) of WHLPC was relatively high at 76.4%. Cadmium, copper, and lead increased after protein concentration but were still considered much lower than the allowable limits set by the European Union for animal feedstuffs. The most limiting amino acid was methionine followed by lysine. The chemical score of the WHLPC was estimated to be 38.9% while the Essential Amino Acid Index (EAAI) was 0.88; the latter index indicated that the WHLPC was a useful protein source and with amino acid supplementation or protein complementation could convert it to a good quality protein source for aquafeeds.
Key Words: *Eichhornia crassipes*, leaf concentrate, apparent digestibility of ingredient, chemical score, EAAI.

Introduction. Given the current level of per capita consumption of aquatic foods, it is projected that the world will require an additional 23 million tons by 2020 (FAO 2012). Considering that most of the world's major fish stocks are overfished and catches are either static or declining, aquaculture could be the only option to increase total fish production. However, aquaculture largely depends on quality of feeds, the sustainability of which is affected by the supply of animal and plant proteins, oils and carbohydrates. The key aquafeed ingredients such as fish meal, soybean meal, and various oilseed cakes are are in direct competition with terrestrial animal husbandry (Sumagaysay-Chavoso 2007). In Asia, these ingredients are produced in much lesser quantities than what is being consumed for feed manufacture (De Silva & Hasan 2007). The perennial shortage of this key raw material is highly problematic for feed manufacturers and can seriously affect aquaculture sustainability and profitability.

Increased awareness of the likelihood of fish meal scarcity has led to efforts in reducing dependence on this ingredient. One of the solutions is incorporating plant-based raw materials to satisfy the protein requirement of cultured species. Attention has been focused on replacing fish meal with soybean meal. A large volume of soybean meal used in aquafeed production is imported especially in the Philippines. One of the untapped resources are aquatic macrophytes which include water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), duckweed (*Lemna* spp.) among others. These aquatic plants are considered pests and pose many negative impacts on bodies of water. Water hyacinth is the most noxious among these and can be one of the bigger problems in managing water systems including the Philippines (Valencia 2012). Many water quality problems such as low dissolved oxygen, high carbon dioxide, and toxic nitrogenous compounds are caused by aquatic vegetation. Water-level manipulation, through clogging drains and intakes, in ponds and raceways can also be complicated by aquatic vegetation (Masser 2000). The use of water hyacinth in fish feed could reduce the present

dependence on other competitive agricultural crops included in compounding feeds and could put a nuisance aquatic plant into beneficial use.

The objective of the present study is to evaluate the nutritive value of water hyacinth leaf protein concentrate (WHLPC) as a potential feed ingredient for aquafeeds.

Material and Method. The present study was conducted in May to July, 2013 at the Multispecies Hatchery of the University of the Philippines Visayas in Miagao, Iloilo, Philippines.

Production of water hyacinth leaf protein concentrate. Water hyacinth was collected from a freshwater pond in Culasi, Antique, Philippines. Leaves were dried until about 10% moisture and were ground using a hammer mill.

WHLPC was produced using methods described by Virabalin et al (1993). Briefly, hyacinth leaf powder was soaked in water at 1:3 (w/v) ratio for 30 min and homogenized in a blender for 5 min. Sodium hydroxide (NaOH) solution was used to adjust the pH to 9.0 and allowed to stand. The resulting slurry was filtered through a cheese cloth, the filtrate collected, acidified with hydrochloric acid (HCl) to pH 2.0 and flocculate allowed to settle. Further flocculation was enhanced by heating in a water bath between 60 and 80^{o}C for 10 min the precipitate of which was collected, oven-dried at 60^{o}C, and ground to fine powder.

Proximate composition of the raw water hyacinth leaf powder and WHLPC were analyzed. 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^{o}C for 12 h. Crude protein was measured after block digestion and steam distillation using Foss Tecator^{TM} digestion system and Foss Kjeltec^{TM} 8200 auto-distillation unit. Crude fat was extracted using Foss Soxtec^{TM} 2050 automatic system and fiber was determined using Foss Fibertec^{TM} 2010 system. Amino acid composition of WHLPC was analyzed by a service laboratory (SGS Taiwan) using liquid chromatography with tandem mass spectrometry (LC-MSMS) following standard methods (AOAC 2003).

Preparation of the test diets. A reference and test diets were formulated with chromic oxide (Cr_2O_3) used as an inert marker at a concentration of 10 g kg^{-1} in the reference diet (Table 1). Ingredients were purchased from the Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC/AQD). The test diet was prepared as a 70:30 mixture of the reference diet to the test ingredient. Dry ingredients were thoroughly mixed first before the liquid component (i.e. oil and distilled water). The resulting dough was pelleted and subsequently oven-dried at 60^{o}C for 12 h. Feeds were then cut and sieved to appropriate size (2 mm) pellets and stored at -20^{o}C until use.

Table 1

Composition of the reference and test diets in g kg^{-1}

Ingredient	*Ref. diet*	*Test diet*
Danish meal	255.8	
Squid meal	81.0	
Defatted soybean meal	260.0	
Copra meal	152.5	
Cod liver oil	10.0	
Soybean oil	10.0	
Corn starch	177.7	
Vitamin mix[a]	13.0	
Mineral mix[b]	30.0	
Chromic oxide (Cr_2O_3)[c]	10.0	
Reference diet		700.0
Water hyacinth leaf protein concentrate (test ingredient)		300.0

[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] Sigma-Aldrich chromic (III) oxide.

Digestibility set up and fecal collection. Male Nile tilapia (*Oreochromis niloticus*) were obtained from the University of the Philippines Visayas Freshwater Aquaculture Station, Miagao, Iloilo and transported to the University hatchery. Prior to the digestibility trial, fish were acclimatized to the laboratory conditions and fed a commercial diet (27% crude protein, 6% crude lipid) at 4% body weight for 7 days. Four tanks (67 L) in a static system were randomly assigned to each diet. Sixteen adult fish (46.17 ± 6.34 g) were divided into 8 experimental tanks (Figure 1) and allowed to acclimate for 3 days. Fish were fed with the reference or test diet to apparent satiation twice daily (08:00 and 14:00 h). Tanks were cleaned before the first feeding; about 70% water was replaced daily with previously chlorine-treated freshwater (0 ppt). Continuous aeration was provided and water quality parameters were monitored regularly. Water temperature and pH were measured twice daily (08:00 and 14:00 h). Levels of dissolved oxygen were determined by titration, and total ammonia nitrogen and nitrite were analyzed by colorimetry using test kits (Advance Pharma Co., Ltd., Bangkok, Thailand).

Figure 1. Digestibility set up used in the study.

The digestibility trial lasted for 8 weeks from May to July 2013. Fecal collection started 3 days after feeding adult Nile tilapia with the reference or test diet to allow evacuation of all previously ingested material (Koprucu & Ozdemir 2005) and repeatedly done daily until the required fecal amount in the analysis had been collected. Fish were netted and feces were collected by manual stripping following the procedure of Stone et al (2008). Gentle pressure was applied to the abdomen using a moderate squeezing motion with the thumb and forefinger. Three times weekly, manual stripping was done and fish were promptly returned to the experimental tank. Fecal matter was pooled by treatment, weighed, and stored at -20^{o}C until analysis. Triplicate samples of the diets and fecal matter were homogenized and analyzed for moisture and ash contents of the samples according to standard methods (AOAC 1995). Moisture was measured by drying to a constant weight in an oven at 105^{o}C. Ash content was determined after incineration in a muffle furnace at 550^{o}C for 12 h. Samples were then acid-digested and chromium (III) was estimated using Varian SpectrAA 55 double beam flame atomic absorption spectrometer.

Analytical methods and calculations. The apparent digestibility coefficients (ADC) of the diets and test ingredient were calculated according to the equations described by Cho et al (1985):

$$\text{ADC of reference or test diet} = 1 - (F/D \times Dcr/Fcr)$$

where F = percent of nutrient in feces; D = percent of nutrient in diet; Dcr = percent of Cr_2O_3 in diet, and Fcr = percent of Cr_2O_3 in feces.

$$\text{ADC of test ingredient, ADCI} = ADCT + [((1 - s)\ DR)/s\ DI]\ (ADCT - ADCR)$$

where ADCI = ADC of test ingredient; ADCT = ADC of test diet; ADCR = ADC of reference diet; DR = percent dry matter in the reference diet; DI = percent dry matter in the test ingredient; s = proportion of test ingredient in test diet (i.e. 0.3 in this study), and 1 – s = proportion of reference diet in test diet (i.e. 0.7 in this study).

Amino acid results were expressed as grams per 100 g determined amino acid for protein. The essential amino acid (A/E) ratio (Arai 1981) of each essential amino acid (EAA) was calculated as the percentage of the total EAA. The chemical score of the WHLPC was determined by the following formula:

Chemical score = % limiting Essential Amino Acid (EAA) in WHLPC/
% corresponding EAA in chicken egg

The essential amino acid index (EAAI) of the two diets was determined from the formula:

$$\text{Essential Amino Acid Index} = \sqrt[n]{[aa_1/AA_1 X aa_2/AA_2 \ldots aa_{10}/AA_{10}]}$$

where aa_1 is the A/E ratio in the feed [(EAA/total EAA)×100], AA_1 is the A/E ratio in the chicken egg [(EAA/total EAA)×100]. Chicken egg protein is considered by nutritionists to be the most complete protein and therefore use it as the standard dietary protein in evaluating other protein sources for a generalized animal diet. EAAI is the geometrical mean of the ratio of all essential amino acids in the evaluated protein relative to their content in a highly nutritive reference protein, viz., whole egg (Oser 1959).

Results. Proximate composition of the WHLPC is shown in Table 2. Concentrating protein by the combined acidification and heating resulted in an increased protein content from about 9% (unpubl. data) to about 22.4%. However, crude fiber content remained very high at about 55%.

Since aquatic macrophytes could play as adsorbent of heavy metals and thus could pose risk when accumulated in food organisms such as culture fish and shellfish, three heavy metals were analyzed and is presented in Table 3. Increases in cadmium, copper, and lead were noted after protein concentration but were still considered much lower than the allowable limits set by the European Union (EC 2002).

The amino acid profile of the WHLPC is shown in Table 4 and indicated a very high Essential Amino Acid Index but a considerable low chemical score when compared to amino acid profile of the whole chicken egg. The most and second limiting essential amino acids were methionine and lysine (about 39% and 72% of the corresponding EAA in chicken egg), respectively.

The *in vivo* ADC of the reference and test diets were not significantly different from each other (Table 5). The Apparent Digestibility Coefficient of Ingredient (ADCI) for the dry matter of WHLPC was determined to be about 76% in tilapia.

Table 2

Nutrient composition (dry basis) of water hyacinth leaf protein concentrate

Component	*(g kg^{-1})*
Dry matter	919.0
Crude protein	223.5
Crude lipid	70.7
Crude fiber	54.8
NFE	511.2
Ash	139.7

Table 3

Heavy metal content (dry basis) of water hyacinth leaf meal and leaf protein concentrate

Metal	*Detected level (mg kg^{-1})*	
	Water hyacinth leaf meal	*Water hyacinth leaf protein concentrate*
Cadmium	0.20 ± 0.02	0.31 ± 0.02
Copper	5.25 ± 0.14	22.09 ± 0.88
Lead	0.39 ± 0.03	0.44 ± 0.06

Values reported are means ± S.E.M. of three replicates.

Table 4

Amino acid composition (% of protein), A/E ratio*[1], Essential Amino Acid Index (EAAI) and chemical score of the water hyacinth leaf protein concentrate

	WHLPC (% CP)	A/E		Chemical score
		WHLPC	Chicken Egg*[2]	
Essential amino acids				
Arginine	6.58	13.07	10.6	107.68
Histidine	2.22	4.41	4.2	91.06
Isoleucine	5.47	10.86	11.0	86.78
Leucine	9.56	18.98	15.4	108.18
Lysine	5.06	10.05	12.2	72.36
Phenylalanine	6.01	11.93	10.0	104.67
Methionine	1.31	2.60	5.9	38.89
Threonine	5.27	10.46	8.9	102.68
Tryptophan	1.42	2.82	2.6	95.20
Valine	7.46	14.81	11.9	108.68
Non-essential amino acids				
Alanine	6.49			
Aspartic acid	10.21			
Cystine	0.38			
Glycine	6.51			
Glutamic acid	7.31			
Proline	5.62			
Tyrosine	2.92			
Serine	10.21			
EAAI		0.88		
Chemical score of the WHLPC				38.89

*1 - the essential amino acid (A/E) ratio of each essential amino acid (EAA) was calculated as the percentage of the total EAA (10 EAAs); *2 - from FAO (1981).

Table 5

Apparent dry matter digestibility (ADMD) coefficients of diets and test ingredient evaluated in the digestibility study

	Apparent digestibility coefficient (%)	
	Diet	*Ingredient (ADCI)*
Reference diet	98.8 ± 0.2	76.4 ± 11.2
Test diet	92.1 ± 3.3	76.4 ± 11.2

Values reported are means ± S.E.M. of three replicates.

Discussion. Results of the present study indicated that water hyacinth leaf meal concentrate exhibited a considerable potential as an aquafeed ingredient. As far as the authors are concerned, among the process of increasing the protein content of water hyacinth, the method used in the present study was similar with the method used by Chavez et al (2014) which exhibited the highest increase in protein. Saha & Ray (2011) have fermented the water hyacinth with *Bacillus megaterium* and *B. subtilis* and obtained a maximum increase of only 126% (from 13.37% to 16.88%). In the present study, the increase was about 248% (from about 9% to 22.35%). Furthermore, animal nutritionists

consider an ingredient as a protein supplement if it contains at least 20% crude protein, thus the WHLPC in the present study was prepared to be a protein supplement as traditionally defined.

Despite increases in the three heavy metal cadmium, copper and lead were observed (0.31, 22.1 and 0.44 mg $kg^{-1)}$, these values were considerably lower than the allowable limits set by the European Union for animal feed ingredients of 1, 25 and 5 mg kg^{-1}, respectively (EC 2002). However, this data was true at least from the samples taken from Culasi, Antique, Philippines; it is recommended that macrophytes should be sourced from known unpolluted sources.

The water hyacinth meal was moderately digestible in the Nile tilapia (76.4%) as an ingredient in the present study. This value was near the apparent protein digestibility values of the diets which included raw water hyacinth leaf meal of 80.1% 82.7% for meal and fermented leaf meal fed to *Labeo rohita* juveniles, respectively (Saha & Ray 2011). A-Rahman Tibin et al (2012) measured the ADCs of diets containing increasing proportion of water hyacinth meal (10-25%) to have an average value of 62.6%. But it should be noted that these values are ADCs of the diets and not of the ingredient; the present study measured the latter. At any rate, the water hyacinth leaf meal without any treatment process like fermentation (Saha & Ray 2011) or acid/heat treatment (in the present study) could lead to lower ADC values presumably because of the antinutritional factors it contained. Tannin and phytic acid contents in water hyacinth leaf meal were estimated to be 0.98% and 0.42%, respectively (Saha & Ray 2011). Tannins inhibit protease activity and possibly of activities of other digestive enzymes or by forming indigestible complexes with dietary protein (Krogdahl 1989). Phytic acid binds protein and minerals which could cause poor bioavailability of both nutrients (Cain & Garling 1995; Hossain & Jauncey 1989, 1993; Spinelli et al 1983).

Similar to other plant proteins, it was expected that there would be an imbalance in the amino acid content of water hyacinth concentrated meal. The most limiting amino acid observed was methionine with an A/E ratio to that of the chicken egg to be 38.9% which was also the chemical score; the second limiting amino acid was lysine (A/E ratio of 72.4%). The observed pattern was similar with the observations from various cereal grains and legume seeds showing either lysine or methionine to be the limiting amino acid (Lieder 1965). It could probably be the result of the acid- and heat-treatment of the water hyacinth leaf meal. Chemical score is based on the assumption that whole egg protein is of the highest biological value and thus the most suitable for growth which could be limited by the EAA in the diet whose ratio to its content in the whole egg protein is the lowest (Hepher 1988). Although chemical score is important in determining the relative value of dietary protein, the other essential amino acid could also have an effect on the nutritive value of the dietary protein as reflected in the essential amino acid index. The EAAI index of the WHLPC was estimated to be 0.88. Oser (1959) developed a criteria for protein quality of feedstuff which was later used by Peñaflorida (1989). The criteria classified good-quality protein sources to have an EAAI greater than or equal to 0.90, useful protein sources to have a value of 0.80, and inadequate when the value was below 0.70. Thus, WHLPC in the present study could be considered a useful protein source but perhaps when supplemented with the most limiting AA or combined with a complimentary protein source could be classified as good quality protein. In general, chemical scores and EAAI are very good indicators of biological value of dietary protein especially when use as sole source of protein. However, animal nutritionist almost always recommend varied sources of protein for reasons that each source could be complimentary with another source which ultimately could balance the essential amino acids. Knowing the EAA profile of a nonconventional protein source is critical in the formulation of a well balanced diet down to its EAA profile.

Conclusions. The present study showed that WHLPC could be a protein source for aquafeeds having a crude protein of more than 20%; it is relatively digestible in the Nile tilapia with ADC for dry matter of 76.4%. When sourced from an unpolluted source, concentrating the leaf meal by acid and heat-treatment resulted in slightly higher levels of heavy metals such as cadmium, copper and lead but were still much lower than the

allowable limit for animal feedstuff set by the European Union Council. Although the chemical score was low (38.9%) due to the most limiting amino acid methionine, its Essential Amino Acid Index of 0.88 resulted in the classification of the WHLPC as a useful protein and was very near the index of 0.90 for good quality protein. Amino acid supplementation or dietary protein complementation could easily make the WHLPC an excellent dietary protein source for aquafeeds.

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