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Evaluation of raw and fermented water hyacinth (*Eichhornia crassipes*) incorporated diets on growth and feed efficiency of young grass carp (*Ctenopharyngodon idella*)

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Abstract: The evaluation of raw and fermented water hyacinth *Eichhornia crassipes* (Mart.) Solms as dietary ingredient for young grass carp *Ctenopharyngodon idella* (Val, 1844) was carried out by adding 20% of each of the alternative ingredients separately to the control diet to completely compensate barley, a portion of wheat bran and 20% of fish meal. Results indicated that all growth and feed efficiency parameters among treatments were similar and there were no significant difference ($p>0.05$) in specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) of fish fed any of the diets. The diet containing fermented water hyacinth (WF) was found to be more efficiently utilized by fish and of highest digestibility values for all nutrients, which indicated that the digestibility was stimulated by incorporation of fermented water hyacinth. The raw water hyacinth diet (WR) produced significantly ($p<0.05$) highest satiation level associated with lowest digestible energy. Evacuation rate was not affected significantly ($p>0.05$) by the inclusion of raw or fermented water hyacinth to the diets. The protein, lipid and ash levels in carcass were higher in fishes fed the control diet (C). In conclusion, incorporation of raw or fermented water hyacinth up to 20% level was found to be suitable and there were no adverse effects on the growth and feed efficiency of experimental fishes.

Keywords: Water hyacinth, Grass carp, fermentation, growth, feed efficiency.

Introduction

Aquaculture has undeniable benefits through supplying of high quality and accessible food and the generation of millions of jobs and billion dollars in budget for the developing countries (Martinez-Porchas and Martinez-Cordova, 2012). The cost of ingredients and

aquafeed is increasing day by day in developing as well as in advanced countries due to inflation and high inputs in production, therefore, there is a persistent need to search for inexpensive and high quality alternative ingredients to substitute partially or

completely the costly feed ingredients, such as fish meal, soy bean meal and oil seed meals to be used in fish feed as protein or energy source (A-Rahman Tibin *et al.*, 2012; Mahmood *et al.*, 2018).

Aquatic plants, both marine and freshwaters, have been used extensively worldwide as supplementary feeds in fish farming since early days of freshwater fish culture and still play an important role as livestock fodder (Wersal and Madsen, 2012; Saha and Ray, 2011).

The water hyacinth *Eichhornia crassipes*, one of the rich sources of organic resources, has received attention for its control as well as utilization (Wersal and Madsen, 2012). It has been considered as one of the most abundantly growing aquatic weeds in tropical and sub-tropical countries and potential ingredient for herbivorous or omnivorous freshwater fishes in simple farming systems where it is available at low cost (Mohapatra, 2015). It is assumed as cosmopolitan invasive aquatic plant, which often causes both economic and ecological harms to a natural or managed ecosystem (Madsen, 2014; Hossain *et al.*, 2015).

The availability of aquatic plants (including water hyacinth) as ingredients in fish feed is difficult to reach its full potential due to many reasons such as their high content of fiber and the presence of anti-nutritional factors (A-Rahman Tibin *et al.*, 2012; Velasquez, 2014). Fermentation is a simple and cheap process where an increase in the nutrient level through microbial synthesis may occur (Bairagi *et al.*, 2002). This technique could be used to transform aquatic plants into utilizable forms for feeding and enhancement of their nutrient value by reduction or removal of anti-nutritional factors and crude fiber (Hasan and Chakrabarti, 2009; Saha and Ray, 2011).

Fishes, both juveniles and adults of many species, rely on aquatic plants at some points during their lives (Dibble and Kovalenko, 2009). Grass carp (*Ctenopharyngodon idella*) is primarily a herbivorous fish which has strong preference for aquatic vegetation (Sangeetha and Rajendran, 2016).

The aim of the present study was to investigate the growth, feed efficiency, digestibility, satiation level, evacuation, and body composition of young *C. idella*, which fed on formulated diets containing raw or fermented *E. crassipes*.

Materials and Methods

Plant collection: Water hyacinth plant was collected from Al-Gharraf River (Qalat Sikar city, 100 km north of Dhi-Qar governorate, Southern Iraq). The plant was thoroughly washed with tap water to remove adhering dirt. Leaves and leaf stalks were separated from roots and then chopped into small pieces (1-2 cm), dried by sun until their water content was dropped to about 50%, and then they were divided into two parts, one incorporated in the diets as raw (dried by oven at 60 °C for 48 h and finely grinded) and the other as fermented.

Fermentation: The fermentation was carried out according to El-Sayed (2003) by adding 5% of molasse and 2 ml of acetic acid (Al-Kanaani, 2014) per each kilogram of water hyacinth with continuous mixing and stored in plastic bags inside plastic containers for 50 days in the room (35±2.2 °C). Then, the containers were regularly overturned each couple days to facilitate decomposition. Finally, the fermented plant was dried by oven at 60 °C for 48 h, finely grinded, bagged, and stored in refrigerator until used.

Diets formulation: Three test diets were formulated by using locally available feed ingredients and adding either raw (WR) or fermented (WF) water hyacinth (Table 1) at 20% level to compare with control (C) diet. The experimental diets were formulated to provide 40% crude protein and approximately 410 Kcal/ 100 g gross energy (Table 2) by adding 20% of each of the alternative ingredients separately to the control diet to completely compensate barley, a portion of wheat bran and 20% of fish meal.

Growth and feed efficiency: Forty-five young (4.03±0.16) *C. idella* were collected from Marine Science Center fish ponds, transported to the laboratory, randomly distributed into nine plastic

aquariums (22 liter capacity) at a stocking density of five fishes per tank, acclimatized and fed the experimental diets for a period of two weeks.

Each of the trial diet was fed to triplicate groups of the fishes, at 3% of their body weight, twice a day, at 9.00 a.m. and 4.00 p.m., for 12 weeks. Water quality parameters were checked periodically (Table 3). Fish

weight were recorded every four weeks intervals, at the end of the experiment. Fish weight and the amount of the feed consumed in each tank were recorded to calculate the following growth indices:

Specific growth rate (SGR) %/ day = $((\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}) \times 100) / \text{days of rearing}$

Table (1): Chemical composition of raw and fermented water hyacinth incorporated in diets.

	Moisture	Proteins	Lipids	Carbohydrates	Ash
Raw water hyacinth diet (WR)	2.73±0.50	20.04±0.86	4.76±0.32	61.92±0.66	10.56±0.95
Fermented water hyacinth diet (WF)	2.16±0.37	18.35±0.80	4.89±0.32	62.36±2.37	12.26±1.51

Table (2): Content, proximate chemical composition (%), gross energy (Kcal/ 100 g) and P:E ratio (mg protein/ Kcal) of experimental diets.

Ingredients	Control diet (C)	Raw water hyacinth diet (WR)	Fermented water hyacinth diet (WF)
Fish meal	25.0	20.0	20.0
Soy cake	35.0	35.0	35.0
Wheat bran	26.0	21.0	21.0
Barley	10.0	0.0	0.0
WH raw	0.0	20.0	0.0
WH fermented	0.0	0.0	20.0
Vitamins and minerals premix	2.0	2.0	2.0
Carboxymethyl cellulose	2.0	2.0	2.0
Proximate chemical composition			
Moisture	7.86	6.52	6.36
Crude protein	40.44	39.19	38.7
Ether extract	3.15	3.41	3.41
Carbohydrates	38.91	40.09	41.27
Ash	7.65	8.79	8.26
Gross energy	410.6	411.0	413.1
P:E ratio	98.5	95.4	93.7

Feed conversion ratio (FCE) = food consumed (g)/ weight gained (g)

Protein efficiency ratio (PER) = weight gained (g)/ protein consumed (g)

Protein productive value (PPV) % = (protein gained (g)/ protein consumed (g)) x 100

Table (3): Water quality parameters of aquariums (Mean ± SD, n = 24).

Parameters	Moisture
Temperature (°C)	24.40±0.66
Oxygen (mg/l)	7.95±0.32
Salinity (ppt)	1.82±0.15
pH	8.15±0.29

Satiation level: Aquariums were cleaned prior to feeding sessions to remove feces. The fishes were fed an excess amount of diets at 9:00 a.m. each day for three weeks. After sixty minutes, each tank was siphoned to remove residual feed which trapped by outlet mesh screen in order to calculate real feed intake by using the following equation:

Satiation level (%) = (food consumed (g)/ body weight (g)) x 100

Evacuation time and rate: Evacuation time was estimated by observing the time between feeding and the ending of feces production depending on using stained diets with two different colors: red (carmine 1%) and green (chromic oxide 1%). The evacuation rate was assessed by the following equation:

Evacuation rate (mg food/ h) = (food consumed (g)/ evacuation time (min))/ 60

Digestibility: The apparent digestibility coefficients (ADCs) of the diets were conducted according to Felix and Alan Brindo (2014), by incorporation of 1% chromic oxide as indicator in each diet. Fishes were acclimated to the experimental diets during the first couple days and no feces were collected. The experiment was last for three weeks. Diets were given daily at 9.00 a.m. and one hour after food consumption, uneaten

feed and feces were removed by siphoning. Feces collected from triplicate treatments on 20 min. intervals were pooled, dried by air and stored for further analysis. The amount of chromic oxide present in the feeds and fecal samples was estimated by digestion with concentrated nitric and perchloric acids and the absorption was measured in the atomic absorption at 357.9 nm. The apparent digestibility coefficients (ADCs) were calculated according following equations:

Total apparent digestibility % = 100 – (100 x (% indicator in food/ % indicator in feces))

Nutrient apparent digestibility % = 100 – (100 x (% indicator in food/ % indicator in feces) x (% nutrient in feces/ nutrient in food))

Chemical composition: Determinations of chemical composition were obtained for feed ingredients, diets, feces and fishes by using physio-chemical methods according to Porto *et al.* (2016). Moisture was determined by weight loss in oven set at 105 °C. Ash was obtained by igniting a known amount of the sample in furnace at 550° C to constant weight. Protein determined by the Kjeldahl method, was used in the determination of total nitrogen, to convert the result into crude protein. The factor 6.25 was used. Total lipids were extracted by using petroleum ether in Soxhlet method. The total caloric value was calculated from the calorie corresponding coefficients for proteins, lipids and carbohydrates, 5.5, 9.1 and 4.1 kcal/ g, respectively. Carbohydrates were estimated by difference according to the following equation:

Carbohydrates = 100 – (moisture % + protein % + lipid % + ash %)

Results

Growth and feed efficiency: The experiment showed that the test diets were well accepted by fishes, and no fishes were died during the growth trial. Growth performance and feed efficiency parameters of young *C. idella* are shown in Table (4). All parameters among treatments were similar and there were no significant

Table (4): Growth and feed efficiency related parameters of young grass carp fed with experimental diets (Mean \pm SD, n = 3), means in the same column with different letters are significantly (P< 0.05) different).

Treatments	Initial weight (g)	Final weight (g)	Weight increment (g)	SGR (% / day)	FCR	PER	PPV (%)
C	20.18 \pm 0.21	54.11 \pm 2.38	33.93 \pm 2.29	1.15 \pm 0.05a	2.18 \pm 0.06a	1.18 \pm 0.03a	15.94 \pm 2.04a
WR	19.58 \pm 0.43	53.43 \pm 3.25	33.85 \pm 3.16	1.17 \pm 0.07a	2.34 \pm 0.14a	1.17 \pm 0.07a	13.18 \pm 1.02a
WF	19.80 \pm 0.41	49.45 \pm 4.17	29.65 \pm 4.18	1.06 \pm 0.10a	2.33 \pm 0.16a	1.16 \pm 0.08a	14.08 \pm 1.37a

Table (5): Nutrient digestibility (%) of experimental diets fed to young grass carp (Mean \pm SD, n = 3), means in the same column with different letters are significantly (P< 0.05) different).

Treatments	Total ADC	Protein ADC	Lipid ADC	Carbohydrate ADC	Ash ADC
C	64.44 \pm 2.27a	75.65 \pm 1.49a	72.84 \pm 2.97a	55.10 \pm 2.70a	24.26 \pm 1.95a
WR	63.49 \pm 2.43a	72.96 \pm 7.66a	75.67 \pm 6.83a	56.11 \pm 4.22a	23.04 \pm 1.41a
WF	66.72 \pm 2.39a	78.56 \pm 0.80a	79.60 \pm 1.10a	59.36 \pm 3.13a	25.01 \pm 4.51a

difference (p>0.05) in the growth and feed efficiency of fishes fed any of the diets. Best specific growth rate (SGR) and feed conversion ratio (FCR) were observed in fishes fed the raw water hyacinth diet (WR). However, highest protein efficiency ratio (PER) and productive protein value (PPV) were observed in fishes fed control diet (C).

Digestibility: All apparent digestibility coefficients (ADCs) values for dry matter and nutrients were not significantly different (P>0.05) among studied diets (Table 5). The diet containing fermented water hyacinth (WF) was found to be more efficiently utilized by the fishes and of highest digestibility values for all nutrients which indicate that the digestibility was stimulated by incorporation of fermented water hyacinth. However, digestibility results for protein and lipid were higher

than that for carbohydrates, while the ash digestibility was the lowest among nutrients.

Table (6): Satiation level (%) of young grass carp and digestible energy (Kcal/100g) in the experimental diets during experiment (Mean \pm SD, n = 15. Means in the same column with different letters are significantly (P< 0.05) different).

Treatments	Satiation level	Digestible energy
C	2.31 \pm 0.05a	290
WR	2.52 \pm 0.06b	278
WF	2.30 \pm 0.07a	301

Satiation level: Results revealed that satiation level was affected by digestible energy in the diets. Satiation level increased as dietary digestible energy decreased. The raw water hyacinth diet (WR) produced significantly (p<0.05) highest satiation level associated

with lowest digestible energy as shown in Table (6).

Table (7): Evacuation time (hh:mm) and evacuation rate (mg feed/ h) of experimental diets fed to young grass carp during experiment. Mean \pm SD (n= 6). Means in the same column with different letters are significantly (P< 0.05) different).

Treatments	Evacuation time	Evacuation rate
C	19:55	62.91 \pm 3.70a
WR	16:20	66.31 \pm 2.75a
WF	16:52	67.50 \pm 4.79a

Evacuation: Data on feed passage velocity through the digestive tract in terms of evacuation time and evacuation rate are presented in Table (7). The minimum

evacuation time was recorded in fishes fed the diet with fermented water hyacinth (WF). Whereas evacuation rate was not affected significantly ($p>0.05$) by the inclusion of raw or fermented water hyacinth to the diets.

Chemical composition: Young grass carp fed the raw water hyacinth diet (WR) showed higher value of moisture content. The protein, lipid and ash levels were higher (13.44 %, 4.98 % and 4.58 %, respectively) in fishes fed the control diet (C) (Table 8). However, there were no significant differences ($p>0.05$) in case of moisture and protein values among treatments. Control treatment (C) showed significant ($p<0.05$) difference with raw water hyacinth treatment (WR) in term of lipid content and with both other treatments in term of ash content.

Table (8): Proximate carcass composition (% wet weight) of young grass carp at the end of experiment (Mean \pm SD (n = 3). Means in the same column with different letters are significantly (P< 0.05) different.

Treatments	Moisture	Proteins	Lipids	Ash
C	.00 \pm 2.01a77	13.44 \pm 1.33a	4.98 \pm 0.67b	4.58 \pm 0.34b
WR	.26 \pm 1.76a80	12.32 \pm 1.12a	3.92 \pm 0.35a	3.50 \pm 0.31a
WF	.85 \pm 1.98a79	12.15 \pm 1.29a	4.55 \pm 0.45ab	3.45 \pm 0.37a

Discussion

Growth and feed efficiency: The present study demonstrated that raw and fermented water hyacinth could be exploited as an ingredient for incorporation into the formulated diets for young grass carp and could be utilized effectively up to 20% level of incorporation without compromising growth and feed utilization efficiency. SGR, FCR, PER and PPV values were similar in all test diets, which may be due to their protein content and essential amino acids profile (Tham, 2012). These findings were supported previously by Hontiveros *et al.* (2015) in juveniles of Nile tilapia *Oreochromis niloticus*, Mohapatra (2015) in fry of common carp *Cyprinus carpio* and Saha and Ray (2011) in fingerlings of rohu *Labeo rohita*.

Digestibility: Results indicated that raw and fermented water hyacinth could be effectively used as a partial substitute for some feedstuffs in the diets of young grass carp without negatively affecting digestion process. Furthermore, fermented water hyacinth diet (WF) reported a slightly increase in nutrient digestibility compared to other diets. Ray and Das (1992) suggested that fermented macrophytes had a higher protein digestibility in relation to sundried macrophytes. Bairagi *et al.* (2002) showed that protein digestibility for raw duckweed meal was much lower in comparison to that obtained for the fermented meal due to the presence of antinutritional factors in the raw duckweed meal. Likewise, El-Sayed (2003) confirmed that fermentation of *Eichornia crassipes* might be necessary

when it is incorporated into Nile tilapia diets at levels up to 20%.

Satiation level: The raw water hyacinth diet (WR) produced highest satiation level. This could be due to the difference in the digestible energy content of diets. The high-energy diet caused a reduction in feed intake, probably because fishes eat to satisfy their energy requirements (Kim *et al.*, 2012). Tran-Duy *et al.* (2008) mentioned that despite the unclear mechanisms involved in feed intake regulation in fishes, dietary energy content has been recognized as a major factor. Correspondingly, Al-Tameemi (2007) explained that the increase of the satiation level could be due to the decrease of digestible energy content in the diets which affect the quantity of feed consumed.

Evacuation: The intestinal evacuation time of grass carp (12h) is much shorter than of other fishes. However, grass carp is herbivorous and stomachless and its natural food is of low energy and protein content, so it should be very necessary to improve the efficiency of digestion and absorption to shorten the passing time of food in intestine in order to obtain enough energy and protein (Nekoubin and Sudagar, 2013). In the current study, using of formulated diet may be the reason for increasing evacuation time. Fishes fed raw water hyacinth diet (WR) recorded highest evacuation time which reached more than twenty hours, and this could be as a result of the higher meal size comparing with the other diets (Gholami, 2015). Additionally, the increase in evacuation time in the fishes fed on WR diet could be explained by the lower digestible energy value (Tekünay and Davies, 2002).

Chemical composition: The whole body composition of young grass carp fed the experimental diets did not show any variations in moisture and protein. This is in an agreement with El-Sayed (2003), Nekoubin and Sudagar (2013) and Felix and Alan Brindo (2014). On the other hand, fishes fed control diet (C) resulted in a significant increase in lipid and ash content comparing with fishes fed raw and fermented water hyacinth incorporated diets. Similar results

were obtained by Ray and Das (1992) and Nekoubin and Sudagar (2013).

Conclusions

Incorporation of raw or fermented water hyacinth up to 20% level in the diets of grass carp *C. idella* was found to be suitable and there were no adverse effects on the growth and feed efficiency of experimental fishes.

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