Evaluation of water hyacinth (Eichhornia crassipes) supplemented diets on the growth, digestibility and histology of grass carp (Ctenopharyngodon idella) fingerlings

Sajid Mahmood, Noor Khan, Khalid Javed Iqbal, Muhammad Ashraf and Anjum Khalique

Department of Fisheries & Aquaculture, University of Veterinary & Animal Sciences, Lahore, Pakistan; Department of Life Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan; Department of Animal Nutrition, University of Veterinary & Animal Sciences, Lahore, Pakistan

ABSTRACT

This study was conducted to evaluate the potential of water hyacinth as aquafeed for grass carp (Ctenopharyngodon idella). Fish were stocked in eight glass aquaria at 20 fish per aquaria. Three iso-proteinous diets (30% crude protein (CP)) were prepared with the addition of different parts of sun-dried water hyacinth, that is, whole plant meal diet (WPM), leaf meal diet (LM) and root meal diet (RM), along with control diet. Results revealed significant $P \leq 0.05$ differences in weight gain and length among treatments. Higher weight gain was observed in LM and lower in RM diets. CP contents of fish fed WPM were significantly higher $P \leq 0.05$, followed by LM than RM and control diets. Crude fat was significantly higher in fish fed RM followed by LM, while ash contents were significantly higher in control. Nutrient digestibility in the case of CP was significantly higher $P \leq 0.05$ for WPM, LM and control groups than RM, while fat digestibility was significantly higher $P \leq 0.05$ in RM diet followed by LM than WPM and control. Histological study showed no significant variations in liver and kidney. In conclusion, LM-based diets were found to be most suitable without any adverse effects on the histopathological disorders in experimental fish.

1. Introduction

Artificial feeding plays an important role in intensive and semi-intensive fish culture systems. It offers the best means of fish production within the shortest possible time in earthen ponds. The use of supplementary feed in carp culture has become inevitable for the success of fish culture (Shahzadi et al. 2006). Exotic carps also performed best in ponds supplemented with artificial feed as compared to major carps (Alam et al. 1996). 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. Aquaculturists and nutritionists are looking for various alternatives to be used as aquafeed production to replace costly feed ingredients such as fish meal, soy bean meal and oil seed meals.

Water hyacinth (Eichhornia crassipes Martius) is a monocotyledonous freshwater aquatic plant belonging to the family Pontederiaceae, and is native to the Brazil and Ecuador region. In the developing countries, this plant is used in traditional medicine and also used to remove toxic elements from polluted water bodies (Center et al. 1999). They reproduce both asexually and sexually through seeds, which remain viable for up to 20 years and, therefore, are difficult to control (Center et al. 1999). It can double its biomass within 8–10 days and one plant can produce 3000 offspring in 50 days because of its capacity for exponential increase in the biomass (Singh 1999). Some uses are reported for this weed such as compost making, paper industry, biogas plants, cattle feed, furniture making, and waste water treatments. Water hyacinth is available in many parts of Punjab and Sindh as well. Thousands of acres of water-logged areas can be used to grow water hyacinth as per need (Verma et al. 2003). Recently, considerable attention has been given to its harvesting for practical uses, as an alternative plant protein source in livestock feed including fish (Daddy 2000; Sotolu 2008; Aderolu & Akinremi 2009). Some studies regarding the utilization of water hyacinth as feed for different fish species including Indian major carps are also available (Edwards et al. 1985; Hasan et al. 1990; Ray & Das 1994). However, information on the use of processed meals of aquatic weeds in aquafeeds is scanty (Bairagi et al. 2002; El-Sayed & Abdel-Fattah 2003).

Grass carp (Ctenopharyngodon idella) is primarily a herbivorous fish and prefers to feed on plant materials (Jhingran 1997). This species has been introduced into more than 50 countries throughout the world for aquatic weed control and aquaculture. The reason for C. idella introduction to some western European countries was almost exclusively for weed control (Van Zon 1977; Müller 1995). C. idella have also been successfully introduced for weed control in the USA (Guillory & Gasaway 1978) and South America, and other parts of Asia, Africa and Australia. Keeping in view the above-mentioned facts, the present study was planned with the aim to evaluate the potential of aquatic plant ‘water hyacinth’ as aquafeed for grass carp and its effects on growth, body composition and histology.

CONTACT Khalid Javed Iqbal khalidjavediqbal@gmail.com Department of Life Sciences, The Islamia University of Bahawalpur, Baghdad Campus, Bahawalpur, Punjab 63100, Pakistan

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2. Materials and methods

The study was conducted in glass aquaria having dimensions of $3' \times 1.5' \times 2' \ (L \times W \times D)$ in the Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki. Grass carp was used as the candidate species for this study and was stocked at 20 juveniles per aquarium. Water level was maintained at 1.5' in each aquarium and 20% water was replaced through siphoning from each aquarium daily in the morning. Aeration was supplied constantly with the help of aerators through air stones. The photo period of 12 hours was kept constant throughout the experiment. Water hyacinth was collected from natural ponds near Gujranwala District, Punjab, Pakistan. The plants were sun-dried and after drying, leaves and roots were separated from the plants. Three different meals were prepared, that is, whole plant meal diet (WPM), leaf meal diet (LM), root meal diet (RM). Ingredients composition and level of inclusion in the experimental diets are given in Table 1. All the ingredients were well mixed along with the addition of Cr$_2$O$_3$ and vitamin premix at the rate of 1% each. Dough was prepared and passed through the local pelleting machine to make pellets of 1.0 mm size. The pellets were sun-dried, kept in zipped plastic bags, stored in refrigerator and then used for the current study.

2.1. Feeding protocol

Diets were offered to experimental fish at the rate of 4% fish wet body weight twice a day at 9:00 am and 4:00 pm. Mostly feed is offered twice a day to fish and the most convenient time is about 8:30–9:00am and 3:30–4:00 pm (Ahmed et al. 2011) to reduce feed waste and maximize feed intake.

2.2. Fish growth studies

Fish growth was monitored at the time of stocking and then fortnightly through the measurement of weight and length, and feed was adjusted as per weight gain. The gain in weight, percent weight gain, specific growth rate percentage (SGR%) and feed conversion ratio (FCR) were determined with following formulas:

- Net weight gain (NWG) = Average final weight (g) – Average initial weight (g)
- Percent weight gain = Final weight (g) – Initial weight (g) × 100/Initial weight (g)
- SGR% = ln (Final wet body weight) – ln (Initial wet body weight) × 100/No. of days
- FCR = Feed intake (g)/Wet weight gain (g)

2.3. Proximate composition

Fish feed ingredients and experimental fish were analysed for proximate analysis using Association of Official Analytical Chemists (2002) for dry matter, crude protein (CP), ether extract (EE), ash and gross energy (GE). Dry matter was determined with oven drying at 105°C, CP in micro-Kjeldahl, ether extract with Soxtec apparatus using diethyl ether as solvent, ash in muffle furnace by burning 1 g of sample at 600°C overnight and GE with oxygen bomb calorimeter.

2.4. Digestibility

Faecal matter was collected from each tank and kept in the freezer for storage and final analysis. Feed digestibility was determined by following Furukawa and Tsukahara (1966) using Chromic oxide analysis as the marker. Finely ground faeces were weighed up to 50–500 mg and placed in a 100 ml kjeldahl flask. Then 10 ml HNO$_3$ was added to the flask and digested by gently boiling for at least 30 minutes or until yellowish vapours stop rising. The sample was cooled to room temperature and added 5 ml perchloric acid. The flask was placed in the digester again and boiling was continued until the solution turned from green to lemon yellow, and then cooled again. There was a reddish ring around the edge of the liquid. Then the cooled liquid was transferred to a 25 ml volumetric flask and diluted with distilled water. The spectrophotometer was adjusted to ‘0’ with a blank reagent and read at 350 nm. The amount of chromic oxide (mg) present in the

### Table 1. Feed composition and ingredients analysis.

| Ingredients                              | CP % | Fat % | Fibre % | Inclusion % | CP % | Fat % | Fibre % |
|------------------------------------------|------|-------|---------|-------------|------|-------|---------|
| **Whole plant diet (WP)**                |      |       |         |             |      |       |         |
| Fishmeal                                 | 56.72| 8.73  | 1.06    | 25          | 14.18| 2.18  | 0.27    |
| Guar meal                                | 40.04| 0.85  | 11.46   | 21          | 8.41 | 0.18  | 2.41    |
| Canola meal                              | 31.96| 3.01  | 13.28   | 13          | 4.15 | 0.39  | 1.73    |
| Whole plant meal                         | 6.85 | 2.55  | 11.34   | 15          | 1.03 | 0.38  | 1.70    |
| Rice polish                              | 6.06 | 3.41  | 20.17   | 12          | 0.73 | 0.41  | 2.42    |
| Wheat bran                               | 15.14| 3.36  | 6.14    | 12          | 1.82 | 0.40  | 0.74    |
| Vitamin premix                           | 1    |       |         |             | 1    |       |         |
| Total                                    | 100  | 30.31 | 3.95    | 9.26        |      |       |         |
| **Leaf meal diet (LM)**                  |      |       |         |             |      |       |         |
| Fishmeal                                 | 56.72| 8.73  | 1.06    | 22          | 12.48| 1.92  | 0.23    |
| Guar meal                                | 40.04| 0.85  | 11.46   | 20          | 8.01 | 0.17  | 2.29    |
| Canola meal                              | 31.96| 3.01  | 13.28   | 17          | 5.43 | 0.51  | 2.26    |
| Water hyacinth leaf meal                  | 11.85| 1.16  | 13.73   | 15          | 1.78 | 0.17  | 2.06    |
| Rice polish                              | 6.06 | 3.41  | 20.17   | 12          | 0.73 | 0.41  | 2.42    |
| Wheat bran                               | 15.14| 3.36  | 6.14    | 12          | 1.82 | 0.40  | 0.74    |
| Vitamin premix                           | 1    |       |         |             | 1    |       |         |
| Total                                    | 100  | 30.24 | 3.59    | 10.00       |      |       |         |
| **Root meal diet (RM)**                  |      |       |         |             |      |       |         |
| Fishmeal                                 | 56.72| 8.73  | 1.06    | 25          | 14.18| 2.18  | 0.27    |
| Guar meal                                | 40.04| 0.85  | 11.46   | 20          | 8.01 | 0.17  | 2.29    |
| Canola meal                              | 31.96| 3.01  | 13.28   | 18          | 5.75 | 0.54  | 2.39    |
| Water hyacinth root meal                 | 2.56 | 4.01  | 8.79    | 15          | 0.38 | 0.60  | 1.32    |
| Rice polish                              | 6.06 | 3.41  | 20.17   | 10          | 0.61 | 0.34  | 2.02    |
| Wheat bran                               | 15.14| 3.36  | 6.14    | 10          | 1.51 | 0.34  | 0.61    |
| Vitamin premix                           | 1    |       |         |             | 1    |       |         |
| Total                                    | 100  | 30.44 | 4.17    | 8.90        |      |       |         |
| **Control diet**                         |      |       |         |             |      |       |         |
| Fishmeal                                 | 56.72| 8.73  | 1.06    | 20          | 11.34| 1.75  | 0.21    |
| Guar meal                                | 40.04| 0.85  | 11.46   | 21          | 8.41 | 0.18  | 2.41    |
| Canola meal                              | 31.96| 3.01  | 13.28   | 21          | 6.71 | 0.63  | 2.79    |
| Rice polish                              | 6.06 | 3.41  | 20.17   | 18          | 1.09 | 0.61  | 3.63    |
| Wheat bran                               | 15.14| 3.36  | 6.14    | 18          | 2.73 | 0.60  | 1.11    |
| Vitamin premix                           | 1    |       |         |             | 1    |       |         |
| Cr$_2$O$_3$                               | 1    |       |         |             | 1    |       |         |
| Total                                    | 100  | 30.28 | 3.78    | 10.14       |      |       |         |
sample was calculated as follows:

\[ X = \frac{1}{4} Y - 0.0032/0.2089, \]  

where \( Y = \) absorption 0.0032 and 0.2089 are constants.

0.2089 are constants.

% of chromic oxide in the sample: % chromic oxide \[ = 100 \frac{X}{A}, \]

where \( X = \) weight of chromic oxide, \( A = \) weight of the sample.

Apparent nutrient digestibility:

The apparent nutrient digestibility (%) of the reference diet, experimental diet and faecal matter was estimated by using the following formula:

\[
\text{App. nutrient digestibility(%) = } 100 - \left\{ 100 \times \left( \frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in diet}} \right) \times \left( \frac{\text{Cr}_2O_3 \text{in feed}}{\text{Cr}_2O_3 \text{in feces}} \right) \right\}.\]

2.5. Histology

After the termination of the feeding trial, five fish out of each treatment were killed in clove oil and then dissected to extract kidneys and livers, which were preserved in 10% buffered formalin; the samples were then dehydrated in graded ethanol series and were embedded in paraffin blocks. The samples were cut into 5 µm sections with a rotary microtome and stained with haematoxylin and eosin (Martoja et al. 1970).

2.6. Statically analysis

Data collected were subjected to the analysis of variance (ANOVA) test using SAS version 9.1. Means were compared by Duncan’s Multiple Range test.

3. Results and discussion

After the 90 days feeding trial, maximum weight gain was observed in LM (7.14 g) and followed by WP (6.87 g), control (5.81 g) and RM (2.10 g) (Table 2). Igbinosun and Amako (1988) reported similar results when they formulated water hyacinth supplemented feed for tilapia. Mean weight gain was highest in fish fed leaf meal-based diet with minimum feed intake. Significantly low weight gains and SGRs in fish fed diets containing water hyacinth root meal may be due to the high fibre content present in the plant (Table 2). This observation is in line with the reports of Nwanna et al. (2008) when he was working on high crude fibre content in the Eichhornia-based fish feed. The results are also in line with the reports of Nwanna and Ajani (2005) who studied the growth and blood parameters of catfish fed dietary water hyacinth LM. Significant variations were observed between MWG, SGR and FCR due to the high fibre content of whole water hyacinth plant meal (WLM) compared with that of the leaf meal (WPM), even though both diets contained crude fibre greater (Table 2) than the limit suggested by Nwanna et al. (2008). This observation seems to corroborate with the report of Alceste and Jory (2000) on the effect of the presence of certain substances in feed ingredients such as crude fibre content that limit diets utilization. Based on several reports on the utilization of water hyacinth, Nwanna et al. (2008) recommended further processing of hyacinth meal in order to bring its crude fibre content to the lowest possible level and consequently improve its digestibility. Our findings about the LM diet (maximum growth: 7.14 g) (Table 2) are similar to those of Saha and Ray (2011) who prepared diet from raw Eichhornia leaf meal and two fermented diets. They concluded that Labeo rohita fed diets containing 30% leaf meal fermented with B. megaterium CI3 strain performed better in terms of growth response, FCR, protein efficiency ratio and apparent net protein utilization, followed by diets containing 20% CI3-fermented leaf meal and raw Eichhornia leaf meal. Our results are little different from those of Sotolu and Sule (2011). Their findings show that fish fed traditional diet (soybean meal (SBM)-based diet) had the highest MWG (23.08 g), while the lowest MWG value recorded (14.79 g) was for fish fed Eichhornia LM.

| Parameters Initial Final | Whole plant diet | Leaf meal diet | Root meal diet | Control diet |
|-------------------------|------------------|----------------|----------------|--------------|
| Growth of fish on different treatments |                  |                |                |              |
| Initial body weight (g) | 1.618 ± 0.138    | 1.465 ± 0.246  | 1.346 ± 0.072  | 1.263 ± 0.043 |
| Final body weight (g)   | 8.490 ± 0.124    | 8.602 ± 0.050  | 3.445 ± 0.268  | 7.069 ± 0.120 |
| Weight gain (g)         | 6.871 ± 0.013    | 7.136 ± 0.196  | 2.098 ± 0.341  | 5.806 ± 0.076 |
| Initial length (mm)     | 54               | 58             | 55             | 53           |
| Final length (mm)       | 104              | 104            | 75             | 91           |
| Length gain (mm)        | 50               | 46             | 20             | 38           |
| ANOVA of weight and length |                |                |                |              |
| Weight (g)              | 4.606 ± 0.585    | 4.636 ± 0.605  | 2.465 ± 0.178  | 3.676 ± 0.500 |
| Length (mm)             | 78.506 ± 4.131a  | 78.757 ± 3.995b| 65.405 ± 1.877a| 71.224 ± 3.386ab|
| SGR%                    | 0.735 ± 0.022a   | 0.787 ± 0.031a | 0.415 ± 0.021c | 0.763 ± 0.005a |
| FCR                     | 2.223 ± 0.031a   | 2.113 ± 0.020b | 5.142 ± 0.212a | 2.188 ± 0.025b |
| Body composition of fish|                  |                |                |              |
| Parameters              | Initial          | Final          |                |              |
| Protein                 | 65.364 ± 1.152a  | 69.650 ± 0.098a| 66.640 ± 0.212b| 66.635 ± 0.247c| 67.185 ± 0.289c|
| Fat                     | 8.045 ± 0.210a   | 8.400 ± 0.197a | 9.260 ± 0.197b | 10.705 ± 0.190a| 9.815 ± 0.247b|
| Ash                     | 64.130 ± 0.179a  | 72.460 ± 1.626a| 45.535 ± 4.772b| 45.535 ± 4.772b| 67.660 ± 5.062a|
| Fat%                    | 32.895 ± 1.817d  | 53.495 ± 0.106b| 81.375 ± 0.346a| 84.805 ± 0.756c|

Note: a,b,c,d absignificance at \( P \leq 0.05 \).
Apparent digestibility of protein (72.460 ± 1.626%) was the highest in LM diet among other treatments. Digestibility of the protein content in Control and WPM treatment was noted as 67.660 ± 5.062% and 64.130 ± 0.179%, respectively. In RM treatment, protein digestibility was lowest (45.535 ± 4.772%) (Table 2). These results are similar to the results of the digestibility study conducted by Sotolu and Sule (2011). In their experiment, Sotolu and Sule (2011) found that the apparent digestibility coefficient (ADC) of protein and energy was the highest in traditional diet (76.14 and 73.02) followed by LM (71.28 and 67.30), while whole plant diet had the least ADC of 65.44 and 63.16 for protein and energy, respectively. Fat digestibility was significantly high in RM treatment. Fat deposition in fish led to its slower growth and resulted in mortality. Our results contradict those of Saha and Ray (2011) who found that apparent protein digestibility was better in fish fed diets containing fermented leaf meal and digestibility was low in raw *Eichhornia* leaf meal. In our studies, maximum protein digestibility was observed in LM diet (72.460 ± 1.626%) (Table 2). Sotolu and Sule (2011) made similar findings when he conducted a digestibility study on the protein of LM (71.28 ± 1.02%).

Post-trial analysis of fish body composition showed that fish fed with WPM diet gained more protein (69.650 ± 0.098%); fish fed with RM diet gained more fat (10.705 ± 0.190%); fish fed with WP diet gained more fibre content (11.145 ± 0.176%) and fish fed with control diet gained more mineral content (ash) (Table 2). Our findings slightly differ from those of Sotolu and Sule (2011). They found that African catfish gained more protein (64.77 ± 0.04%), fat (9.52 ± 0.03%) and ash (8.22 ± 0.02%) in SMB diet, followed by fish fed with WLM diet.

Histological studies of the liver and kidney of fish in experimental treatments revealed that the liver of fish fed on the control diet without water hyacinth showed a normal pattern of hepatic lobules, normal central vein and blood sinusoid with regular arrangement of the hepatocytes. Degeneration of cells, necrosis of epithelial cells, infiltration of lipid vacuoles and dilation of veins were among the significant changes. Histological examination of experimental treatments shows that the kidneys of fish fed with treatment diets contain disintegration of renal tubules, necrotic damage in tubular epithelial cells and tubular lysis. Our histological studies are in line with those of Abdelhamid et al. (2010) who included water hyacinth in fish feed for a feeding trial on Nile tilapia. Their results indicated abnormal effects proportionate to the increasing level of water hyacinth. In the liver of fish fed diet containing 20% water hyacinth, slight effects were found in the hepatic central vein and hepatocytes. However, the liver of fish fed diet containing 30% water hyacinth showed monocytes aggregation and mild necrosis of the hepatocytes within the hepatic lobules and small vacuoles were present in the hepatic lobules.

There were slight variations observed in water quality parameters of the different diet treatments. Further water quality parameters were within the range for the optimum growth of the present specie.

4. Conclusion

It has been concluded from the present study that *E. crassipes* meal has an optimistic nutrient utilization effect on fish growth. Farmers can use water hyacinth to formulate cost-effective fish feeds. Water hyacinth leaf meal is more appropriate for fish production than the whole plant meal.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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