Food preference and growth of grass carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844) fed some aquatic and terrestrial plants

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Abstract

The present study was carried out to examine 6 plants including aquatic and terrestrial plants on food preference and growth of grass carp. 7 experimental treatments with three replicates were considered. The experimental treatments were ponds containing T_1: *Myriophyllum spicatum*, T_2: *Ceratophyllum demersum*, T_3: *Azolla filiculoides*, T_4: *Lemna minor*, T_5: *Cynodon dactylon*, T_6: *Medicago sativa* and T_7: *M. Spicatum* + *C. demersum* + *A. filiculoides* + *L. minor* + *C. dactylon* + *M. sativa*. 12 grass carps (20 g) were added to each experimental pond. After 5 months, the fish body composition was assessed. According to results, the higher values of fish weight gain rate were obtained in treatments T_4 (3.13 g), T_6 (2.93 g) and T_1 (2.95 g) compared to other experimental groups (p<0.05). Also, the highest percentage of body protein and lipid content were observed in T_4 and T_1. In addition, the biomass and percentage of examined plants decreased after delivery of grass carps to each pond over the course of the experiment, but higher decreases were recorded for T_4 and T_1. The mean values of relative growth rate were higher in T_4 and mixture of all plants (p<0.05). Also, the highest fish biomass was recorded in T_1 and T_4 (p<0.05). In conclusion, our results showed that T_4 and T_1 have higher adaptability compared to other examined plants in the present study and these two plants could be used for feeding grass carp in aquaculture.

Keywords: Food preference, Aquatic plant, Growth, Grass carp

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Introduction

The grass carp is a good candidate species for aquaculture due to rapid growth, herbivorous feeding behavior and ability to feed on a variety of plant materials (Lin, 1935; Bailey, 1972). This species is a large cyprind native to eastern Asia from northern Vietnam to the Amur River on the Siberia-China border. The grass carp has been introduced to many countries around the world including Iran for food and sometimes for controlling aquatic weed (Sills, 1970; Stott and Robson, 1970; Shireman and Maceina, 1981; Pierce, 1983; Leslie et al., 1987; Shireman, 1984; Wiley et al., 1984, 1987). An appropriate food with acceptable quality is essential for the economical production of healthy, high quality fish in all aquaculture systems (Zambonino Infante and Cahu, 1999; Bahrami Babaheydari *et al*., 2015). During the last decade, various commercial and artificial diets were developed for cultured fish species (Kanazawa and Teshima, 1988; Person Le Ruyet et al., 1989; Guillaume et al., 1999; Zambonino Infante and Cahu, 1999; Yufera et al., 1999; Mazurkiewicz *et al*., 2017). Nevertheless, the production of formulated feeds is costly and increases costs of fish production. As an alternative, use of natural feeds with low cost and high performance was considered only or in combination with artificial diets in aquaculture. Plant materials are inexpensive food items for herbivorous fish including grass carp (Cassani *et al.,* 1981; De-Silva and Weerakoon, 1981; Riemens, 1982; Sutton, 1985; Pieterse and Murphy, 1990). Nevertheless, selection of plants with high nutritional and palatability can enhance growth and nutritional values of grass carp. To this end, in the present study, we examined the effects of 4 aquatic plants including two submerged plants (Eurasian watermilfoil, *Myriophyllum spicatum*; hornwort, *Ceratophyllum demersum*) and two floating plants (Water Fern, *Azolla filiculoides*; common duckweed, *Lemna minor)* and also two terrestrial plants (Bermuda grass, *Cynodon dactylon*; Lucerne, *Medicago sativa*) on food preference, growth and body protein and lipid content of grass carp.

Materials and methods

This study was carried out in a fish farm situated in Karaj, Iran. 21 concrete ponds ($6 \times 2 \times 1.5$ m^3) were considered for the experiment. Experimental aquatic and terrestrial plants were collected from the Anzali Wetland and farms around the Anzali Wetland, respectively. For collection of submerged aquatic plants i.e. *Myriophyllum spicatum* and *Ceratophyllum demersum*, firstly the meristic and fresh parts of plants with 10-15 cm length were cut, transported in plastic bags containing water to the farm in Karaj and maintained at 5 ºC until the beginning of the experiment. Also, the floating aquatic plants i.e. *A. filiculoides* and *Lemna minor* were collected from water surface of the Anzali Wetland by purse seine net, transported in plastic bags containing water to the farm and maintained in

small ponds (1 × 1× 1 m^3) until the beginning of the experiment. After cleaning and disinfecting experimental ponds, 7 experimental treatments with three replicates were designed as presented in Table 1. The amount of plant used in each treatment is also presented in Table 1.

Four weeks after planting of floating and submerged aquatic plants, 12 grass carps (20±5 g) were added to each experimental pond. Also, on the same day, terrestrial plants were added to ponds of T_5 and T_6 groups daily. Water temperature was 23-25ºC over the course of the experiment.

Table 1: Experimental groups designed and amount of used plant in each group in the present study.

Experimental groups	T1	T2	T3	T4	T5	T6	T7
Examined plant	*Myriophyllum spicatum* (n=28)	*Ceratophyllum demersum* (n=28)	*Azolla filiculoides* (2 kg)	*Lemna minor* (2 kg)	*Cynodon dactylon*	*Medicago sativa*	*Myriophyllum spicatum* (n=28) + *Ceratophyllum demersum* (n=28) + *Azolla filiculoides* (2 kg) + *Lemna minor* (2 kg) + *Cynodon dactylon* + *Medicago sativa*

For planting of submerged aquatic plants, the 10-15 cm prepared parts were planted in plastic vases (four 10-15 cm parts per vase) containing sediments collected from the Anzali Wetland. Then, 7 plastic vases were placed in the bottom of each pond of T_1 and T_2 groups. Immediately, the water surface increased to 75 cm and then *A. filiculoides*, *L. minor* and *A. filiculoides* + *L. minor* were added to ponds of T_3, T_4 and T_7 groups, respectively. After 15 days the water surface of each pond increased to 100 cm. Four weeks after introducing of submerged and plants to ponds, 12 grass carps (20 g mean weight) were added to each experimental pond. Also, on the same day, terrestrial plants were added to ponds of T_5 (*Cynodon dactylon*), T_6 (*Medicago sativa*) and T_7 (*C. dactylon* + *M. sativa* in equal amounts) daily till the end of the experimental period. During the 5 month experiment fish growth, fish body composition, and also the biomass and percentage of plants were measured. The growth parameters of fish including relative growth rate (RGR), fish biomass and weight gain rate (WGR) were calculated on a monthly basis through biometry of 3 grass carps captured from each pond as follows:

$WGR= BWF- BW_I / t_2-t_1$ (Rueda-Jasso *et al.*, 2004)

Where BW_F and BW_I refer to the final weight and initial weight of grass carps, respectively. Also, t_2-t_1 refers to time difference between the two samplings i.e. 30 days.

$RGR= \ln BW_F- \ln BW_I / t_2-t_1$ (Rueda-Jasso *et al*., 2004)

Where BW_F and BW_I refer to the final weight and initial weight of grass

carps, respectively. Also, t_2-t_1 refers to time difference between the two samplings i.e. 30 days.

After biometry of examined fish sample from each pond, fish were returned to the ponds immediately. Before biometry, fish were anaesthetized in 100 ppm of MS222 (tricaine methane sulphonate).

After the end of the experiments, 2 grass carps from each pond were considered for analysis of body protein and lipid content and transported to the tissue analysis lab. Muscle tissue was used for this analysis. All analyses were conducted according to standard methods of ISO SPRS. The crude protein was measured by Kjeldahl set on the basis of calculation of total nitrogen and regarding conversion coefficient of 6.25 according to Ćirković *et al*. (2011). Total lipids were extracted by accelerated solvent extraction (ASE 200; Dionex, Sunnyvale, CA). Homogenate of sample mixed with diatomaceous earth was extracted with a mixture of n-hexane and isopropanol (60:40, v/v) in a 33-mL extraction cell at 100ºC and nitrogen pressure of 10.3 MPa (Spiric´ *et al*., 2009; Trbovic´ *et al*., 2009). Then, the solvent was removed under a stream of nitrogen using a Dionex Solvent Evaporator 500 at 50ºC until dryness. The fat extract was further used for fatty acid determination.

All data were analysed by SPSS software (Version 16). Normality of non-parametric and parametric data was examined by Shapiro–Wilk test and Kolmogorov–Smirnov, respectively. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different.

Results

The higher mean values of fish WGR obtained in treatments containing *Lemna minor* (T_4: 3.13 ± 1.13), *Medicago sativa* (T_6: 2.93±1.06) and *Myriophyllum spicatum* (T_1: 2.95±1.08) compared to other experimental groups (Table 2) (p<0.05). The mean values of RGR were higher in fish fed by *L. minor* (T_4: 0.024±0.013), (T_1: 0.024±0.011) and mixture of all plants (T_7: 0.023±0.010) (Table 3) (p<0.05). Also, the highest fish biomass was yielded in T_1 (135±119.5) and T_4 (144±128.9) (Table 4) (p<0.05). The highest percentage of body protein and lipid content were observed in fish fed *L. minor* (T_4: 16.38), and *M. spicatum* (T_1: 7.27), respectively (Table 5) (p<0.05). Also, the biomass and percentage of examined plants decreased after delivery of grass carps to each pond over the course of the experiment, but higher decreases were recorded for *L. minor* (T_4: 91.5%) and *M. spicatum* (T_4: 89.2%) (Table 6) (p<0.05).

Table 2: Comparison of WGR (g per day) values between experimental fish groups fed by various ratios of plants. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

Experimental groups	April	May	June	July	August	Mean ±SD
T_1	1.56	3.45	4.44	2.43	2.91	2.95 ± 1.08
T_2	1.17	2.42	3.02	2.14	2.15	2.18 ± 0.66
T_3	1.02	1.89	2.75	2.10	2.07	1.96 ± 0.62
T_4	1.79	3.48	4.78	2.41	3.2	3.13 ± 1.13
T_5	0.89	1.51	2.3	1.9	1.79	1.67 ± 0.52
T_6	1.55	3.39	4.41	2.44	2.87	2.93 ± 1.06
T_7	1.57	2.38	3.09	2.24	2.33	2.32 ± 0.53

Table 3: Comparison of monthly RGR (%) values between experimental fish groups fed by various ratios of plants. Bars (Mean±SD) with different letters are significantly different (p<0.05).

Experimental groups	April	May	June	July	August	Mean ± SD
T_1	0.0366	0.0337	0.0187	0.00766	0.0242	0.024±0.011
T_2	0.0367	0.0273	0.0180	0.00880	0.022	0.022±0.010
T_3	0.0305	0.0262	0.0199	0.00937	0.015	0.020±0.008
T_4	0.044	0.028	0.0184	0.00678	0.0245	0.024±0.013
T_5	0.0285	0.0249	0.0178	0.00799	0.012	0.018±0.008
T_6	0.0349	0.0319	0.0178	0.00757	0.0224	0.022±0.011
T_7	0.0392	0.0243	0.0211	0.00902	0.022	0.023±0.010

Table 4: Comparison of monthly fish growth (g) values between experimental fish groups fed by various ratios of plants. Bars (Mean±SD) with different letters are significantly different (p<0.05).

Experimental groups	April	May	June	July	Mean ± SD
T_1	20±2.5	66±4.5	165±7.5	290±8	135 ± 119.5
T_2	20±2.5	52±4	125±8	215±10	103 ± 86.6
T_3	20±2.5	49±3.25	110±6.5	200±7	94.7 ± 79.5
T_4	20±2.5	68±3.75	178±9	310±11	144 ± 128.9
T_5	20±2.5	40±2.5	98±9	178±8	84 ± 70.8
T_6	20±2.5	63±3	156±7	267±11	126.5 ± 109.5
T_7	20±2.5	65±4	135±6	225±6.5	111.2 ± 89.38

Table 5: Comparison of body protein and lipid content (%) between experimental fish groups fed by various ratios of plants. Bars (Mean±SD) with different letters are significantly different (p<0.05).

Experimental groups	Lipid	Protein
T_1	7.28	15.92
T_2	6.39	13.91
T_3	6.68	14.33
T_4	7.19	16.38
T_5	7.2	15.43
T_6	6.12	13.55
T_7	6.58	15.03

Table 6: Changes of plant percentage and biomass in fish groups fed by various ratios of aquatic plants. Bars (Mean±SD) with different letters are significantly different (p<0.05).

Plant percentage	T1	T2	T3	T4
April	85	90	90	90
May	65	75	75	50
June	30	55	60	25
July	12	40	45	10
Plant biomass (g)				
April	25000	2700	10000	10000
May	15500	18500	8900	5800
June	8000	13500	6400	2300
July	2700	9000	4300	850
Total decrease percent of plant biomass	89.2	67	57	91.5

Discussion

Aquatic plants play an important role in the nutrition of herbivorous fishes including grass carp. Our results showed that the palatability of examined plants is different for grass carp. In this regard, the plants utilized more by fish were *Lemna minor* (with 91.5% decrease) and *Myriophyllum spicatum* (with 89.2% decrease). Some studies reported that plants with rigid structure and high cellulose content have low palatability for herbivorous fishes (Buckly and Stott, 1977; Fowler and Robson, 1978; George, 1982; Hart and Hamrin, 1988; Filizadeh, 1996). In the present study, more palatability of *L. minor* and *M. spicatum* return probably to softer structure and low cellulose content compared to other examined plants especially *A. filiculoides* and *Ceratophyllum demersum* (Van Zon, 1973; Sutton, 1974, 1978; Van Zon *et al*., 1977; Shireman *et al*., 1979; Van Schayck, 1986; Filizadeh, 1996). Also, with regard to food preference of grass carp in this study, it seems that this species is more suitable for controlling floating aquatic plants than submerged types (Cassani *et al*, 1981; Ewell and Fontaine, 1982). The growth of fish in culture conditions is dependent on several parameters such as food quality, fish stocking rate, water quality parameters (i.e. dissolved oxygen, salinity, temperature and etc.) (Reviewed by Ekubo and Abowei, 2011). In our study, the values of WGR and RGR were lower in the early months of the experiment than in the later months that are could be due to the low temperatures during the early months. Our results confirm that growth parameters of examined grass carps are related to food quality i.e. the use of experimental plants since other cues such as water quality were stable during the experiment. In this regard, the highest growth (WGR and RGR) and also fish biomass was obtained in treatments fed by *L. minor and M. spicatum*. This result may be due to the higher palatability and nutritional quality of these plants compared to other examined plants as fish fed on *L. minor* and *M. spicatum* had more protein and lipid content compared to

other groups. In conclusion, our results showed that *L. minor* and *M. spicatum* have higher adaptability and nutritional value compared to the other plants used in the present study and these two plants could be used to feed grass carp in aquaculture.

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