Vol. 2, No. 4, pp. 249-258, (December 2021)
DOI: 10.21608/aujes.2021.91897.1038
Aswan University Journal of Environmental Studies (AUJES)
Online ISSN: 2735-4237, Print ISSN: 2735-4229
Journal homepage: https://aujes.journals.ekb.eg/ E-mail: AUJES@aswu.edu.eg

Original research

Effect of Azolla pinnata on growth performance and survival rate of fingerlings of grass carp fish Ctenopharyngodon idellus (Valenciennes, 1844)

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Received: 21/8/2021 Accepted: 3/12/2021

Abstract:

Grass carp fishes were caught approximately in the same size (initial weight: 10 grams) from earthen ponds and maintained in the laboratory in 15 glass aquaria (35 cm x 70 cm x 40 cm) containing 98L of filtered Nile water in 5 treatments (3 replicates per treatment) control, T1, T2, T3, T4 with stocking densities (10 fish/aquarium). The fish diet provided was a commercial floating pellets diet containing (30% protein, 6% lipids, fibers 4.5%, total energy not less than 4100-kilo calories / Kg) and Azolla plant which was cultivated. Fish fed at 5% of their body weight in the control group and replaced with Azolla plant in the T1, T2, T3, T4 with percentages 25,50,75,100 % respectively. The current study showed that replacing the artificial feed with Azolla pinnata positively enhanced the growth performance, haemato-biochemical values, of grass carp. These results indicated that Azolla pinnata with a rate of 25-50% can be considered a beneficial dietary supplement for grass carp.

Keywords: Grass carp, Azolla pinnata, Growth performance,Blood biochemical, floating pellets

1- INTRODUCTION

Due to the fact that the quality and efficiency of agriculture depends on the efficiency of the irrigation and drainage networks, the weeds and aquatic plants are the biggest problems that impede the flow of water, which causes the loss of a large amount of water due to the evaporation process (Ahmed et al, 2012). The increase in the spread of pathogenic snails harmful to public health is closely linked to the increase in the growth and spread of weeds and aquatic plants. On the contrary, it can be considered that biological control of the proliferation of aquatic plants is the most efficient, least expensive, and safest for the environment (Van and Van 2002).

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Studies and researches have proven that the application of manual, mechanical, and even chemical methods to resist the growth of water weeds leads to high costs in addition to damaging the ecosystem, so resorting to biological or natural resistance was the best solution to maintain the ecological balance on the one hand and to reduce the cost on the other hand (Ahmed et al, 2012).

Fish used to control aquatic vegetation include several species of tilapia (Tilapia spp.), Silver carp (Hypophthalmichthys molitrix), and grass carp (Ctenopharyngodon idella). Only grass carp can consume large quantities of large aquatic plants (Zweerde 1990).

Therefore, the use of weed carp to control the spread of aquatic plants is the best option to get rid of the problems of vegetation sweeping as it is a safe, economical, and long-term option (Mitchell and Kelly, 2006). Grass carp is a herbivorous fish native to China (Eskinazi-Sant'Anna & Pace, 2018). In the early 1960s, weed carp were originally imported into Egypt as a "biological control" option for bothersome aquatic plants.

The large rivers of East Asia are considered the original habitat of grass carp, living in the tropical and subtropical range. Grass carp prefer habitats in continuous lakes, lower and middle reaches of rivers. Large leafy plants, as well as submerged plants, can be considered the food of choice for grass carp (Bain et al. 1990, Pine and Anderson 1991). Stevanovski (2010) determined that grass carp prefer to also live in the presence of plankton and large plants in non-flowing or low-flow water.

The main and important potential in aquaculture on which the cost depends is the feed, and supplementary diets are the main determinants of the economic achievements of aquaculture (Omosowone and Ogunrinde, 2018). El-Shalal hatchery in Aswan aims to produce grass carp for biological control of canals and agricultural drains in Upper Egypt.

The increase in feed prices and known protein sources has led many hatchery managers to attempt replacing the expensive feed ingredients with other cheaper sources such as Azolla, which is a good and identifiable substitute for protein (Mosha, 2018). Costa et al. (2009) found that Azolla belongs to the floating aquatic ferns. They have small leaves and grow in tropical, semi-tropical, warm, and temperate regions as well as in Africa, Asia, and America.

Radhakrishnan et al. (2014) reported that Azolla contains a high protein content that can be safely used in fish as a direct protein substitute. The importance of Azolla and the popularity of its use in aquaculture is due to its high content of crude protein, which is about 13: 30%. It is also rich in essential amino acids when compared to other forage plants (Panigrahi et al., 2014).

Thus, this study aims to reduce the cost of feed provided to grass carp in El-Shalall hatchery Aswan and to use the Azolla plant as an alternative to forage and evaluate its effect on growth performance, survival, food utilization, and blood composition.

2- MATERIALS AND METHODS

Study Area and Period

This study took place in El-shalal fish hatchery of the General Directorate of Water Resources and Irrigation in Aswan, during the period from June to September 2020.

Fish and Laboratory Condition

The fingerlings grass carb fishes were caught approximately in the same size (initial weight: 10 grams) from earthen ponds and maintained in the laboratory in 15 glass aquaria (35 cm x 70 cm x 40 cm) containing 98L of filtered Nile water in 5 treatments (3 replicates per treatment) control, T1, T2, T3, T4 with stocking densities (10 fish/aquarium).

Diet

The fish diet provided was a commercial floating pellets diet from Aller Aqua Egypt company containing (30% protein, 6% lipids, fibers 4.5%, total energy not less than 4100-kilo calories / Kg) and Azolla plant which was cultivated. The Essential Amino Acid (EAA) composition (g/100 g feed) of *Azolla filiculoides* was maintained as shown in Table (1).

Table (1): Essential amino acid (EAA) composition (g/100 g feed) of *Azolla filiculoides* from Youssouf, A. et al (2011).

Cystine	0.22
Methionine	0.39
Valine	1.49
Isoleucine	1.24
Leucine	2.12
Tyrosine	0.87
Phenylalanine	1.68
Histidine	0.57
Lysine	1.31
Arginine	1.51
Threonine	1.34
Tryptophan	0.41

Table 2: Composition (%) of the experimental diets for Grass Carp

Ingredients	Percent
Soybean	46%
Fish meal	60%
Corn gluten	60%
Protein	30%
Fiber	4,5 %
Total energy	4100 kcal / kgm Diet

Cultivation of A.Pinnata

About 2 weeks before the start of the experiment, the Azolla plant was cultivated by digging a pit of 3 x 2 x 0.25 m, where fertile soil was uniformly spread over the plastic sheet which was placed at the bottom of the pit. Slurry, made of 2 kg poultry manure and 30 g of superphosphate mixed in 10 liters of water, is poured onto the sheet. More water is poured on to raise the water level to about 15 cm, and about 0.5 – 1 kg of fresh and pure culture of Azolla is placed in the water. This grows rapidly and fills the pit within 10 – 15 days. From then on, 500 – 600 g of Azolla can be harvested daily. Collected Azolla was washed to remove dirt and sun-dried before used in feeding.

Water Quality

Every aquarium was provided with aeration and running water. To keep water in good quality, the aquarium was cleaned and the water was changed weekly. Through the experiment. To calculate the growth parameters of fish based on biometry of grass carb, fish samples were taken at the beginning and end of the experiment for initial and final body weight.

Feed Analyze

The feed ingredients used were analyzed chemically, where crude fat was determined by the Soxhlet apparatus. Also, the protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method and ash by combustion at 550°C.

Feeding Rate

The feeding of fish was from a commercial pellets diet (30 % protein) at 5% of their body weight in the control group and replaced with Azolla plant in the T1, T2, T3, T4 with percentages 25,50,75,100 %.

Sampling and Analytical Methods

Blood samples were collected at the end of the experiment (90 days) by inserting a syringe needle along the dorsal midline of fish and drawing blood from the vein, then the sample was sent to the laboratory on ice. Blood was placed in non-heparinized tubes and left to clot at 4°C for 15 min., Afterwards, tubes were centrifuged at 3000 rpm using an Eppendorf centrifuge for 10 min to obtain serum. The sera were separated into aliquots and were frozen and stored at -80°C until metabolite analyses. All samples were immediately immersed in liquid nitrogen and then transferred to a -80°C freezer until analysis. The quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran (1 500 0178), at 546 nm and 37°C by the glucose oxidase method according to Trinder (20). Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard at 546 nm and 37°C.

Analytical Methods Body Composition

At the end of the experiment, 15 fish from every replicate were selected randomly for taken tissue samples, rapidly frozen and stored at -20°C for composition analysis.

The growth parameters were calculated as follows: final body weight (WG)=final body weight (g)–initial body weight (g). Specific growth rate (SGR) (%BW day-1)=(Ln final body weight (g)-Ln initial weight (g))/ (experimental period)×100. Feed conversion ratio (FCR) (%)=(total fed/body weight increase (g))×100. Daily growth rate (DGR)=(final body weight (g)–initial weight (g))×(100)/(experimental period×initial weight (g)).

Statistical Analysis

To determine significant differences, the data were analyzed statistically by one-way ANOVA, and an LSD0.05 test was applied in the case of significant differences.
The following calculations have been conducted:
Weight gain (g) = Mean final weight (g) − Mean initial weight (g)
Specific growth rate (SGR) (% day−1) = (Ln final weight (g) −
Ln initial weight (g)/(Period in days) ×100
Feed conversion ratio (FCR) = Feed intake (g)/Live weight gain (g)
Condition Factor (K) = Fish weight (g)/(Fish length)3 (cm) × 100
Protein efficiency ratio (PER) = Weight gain (g)/Protein intake (g)
Survival rate % = (No. of harvested fish/No. of initial stocked fish) × 100

3- Results and Discussion

Due to the nature of grass carp that it is herbivorous and without a stomach compared to omnivorous predatory fish, it is necessary to work on improving the efficiency of digestion and absorption so that the passage of food in the intestine is short, as the natural food of grass carp is plants with low protein content and low energy, of which Azolla is a good example in a study. Lin (1991) determined that the intestines of grass carp are longer than that of carnivorous fish by 2.29–2.54. In this study, Azolla was used as a substitute for artificial feed at different levels with the addition of probiotics to the culture water to maintain water efficiency, as well as to improve digestion and food utilization. The T25 and T50 treatments were the highest in growth performance. Growth performance indicators are shown in Table (3). The results showed that the addition of Azolla to the grass carp feed led to significant differences in the final body weight, as the fish that got 50% Azolla achieved the highest body weight without significant differences compared to the fish that got 25% and the control diet, while the fish that consumed Azolla, at a rate of 75%, achieved the significant lowest body weight, followed by a diet of 100% Azolla.

This result is consistent with Ayapan, (1992) where it was found that common carp and grass carp achieved the highest significant increase in final weight when using Azolla by 30%. Also, the result of this study is consistent with Nekoubin and Sudagar, (2013) which noted that for (A. fliculoids) the feed conversion rate of Azolla was significantly better compared to other treatments. Many studies also indicated an improvement in the utilization of food and an improvement and increase in the growth performance when adding Azolla in the feed provided to Rohu fish at rates of 10-50% (Panigrahi et al., (2014), Datta., (2011), Tuladhar., (2003)). While other studies showed that a level ranging from 10-25% of Azolla on feed was most appropriate in feeding grass carp Majhi., (2006), Orange fin labeo Gangadhar., et al (2017), silver carp and mrigal Tuladhar., (2003).

Protein productive value (PPV) was significantly affected by the addition of the Azolla plant to the feed, as the fish fed with the addition of 100% Azolla was the least significant in PPV than the other groups, and there were no significant differences between the fish fed on the control diet, 25%, 50%, and 75%.

Protein efficiency ratio (PER) of fish that consumed the control diet achieved the significantly highest, followed by fish that ate 25%, then fish that ate 50%, then fish that ate 75%, while fish that ate 100% achieved the lowest significantly protein efficiency ratio.

At the end of the experiment, there were no significant differences in the energy retention (ER) for each of the fish that consumed the control diet, 25%, and 50%, while the significantly least ER was for the fish that fed at the diet 75% and 100%.

The specific growth rate SGR was significantly higher for the groups that fed control diets, 25% and 50% /, while the significantly lowest value for the specific growth rate was for fish fed 100%.

At the end of the study, it was clear from the results that the feed conversion ratio was very high significantly in the group of fish that got the diet 75 and 100, while there was an improvement and a significant difference between it and the fish that got the control diet, 25%, and 50%.

Table 3: Growth response and feed utilization (mean ± SD) of grass carb, after 90 days of feeding on experimental diets containing different levels of Azolla pinnata

	Control	**T25**	**T50**	**T75**	**T100**
Survival	76.67 ± 3.33^{a}	66.67 ± 3.33^{b}	56.67 ± 3.33^{c}	63.33 ± 3.33^{bc}	70.00 ± 0.0^{ab}
IBW	10	10	10	10	10
FBW	47.50 ± 0.53^{a}	48.17 ± 0.67^{a}	48.75 ± 0.60^{a}	21.70 ± 0.27^{b}	12.12 ± 0.16^{c}
PPV	25.99 ± 6.73^{a}	29.04 ± 0.53^{a}	22.90 ± 0.45^{a}	11.78 ± 0.28^{b}	4.73 ± 0.90^{b}
PER	2.80 ± 0.03^{a}	2.50 ± 0.03^{b}	1.97 ± 0.02^{c}	0.95 ± 0.01^{d}	0.22 ± 0.02^{e}
ER	7.79 ± 1.58^{a}	10.21 ± 0.20^{a}	9.32 ± 0.18^{a}	5.11 ± 0.19^{b}	3.45 ± 0.71^{b}
SGR	2.60 ± 0.02^{a}	2.62 ± 0.02^{a}	2.64 ± 0.02^{a}	1.29 ± 0.02^{b}	0.32 ± 0.02^{c}
FCR	1.98 ± 0.02^{c}	1.81 ± 0.03^{c}	1.95 ± 0.02^{c}	4.04 ± 0.04^{b}	17.40 ± 1.45^{a}
FI	74.30 ± 0.90	68.92 ± 0.97	75.37 ± 0.89	47.25 ± 0.69	36.42 ± 0.12

Values within columns with the same superscript are not significantly different ($P > 0.05$).

The body composition results showed that using Azolla – as an alternative to compound feed – did not significantly affect the quality of whole body values such as protein, moisture, and ash. However, the highest protein content was obtained in the control treatment. Yudu., et al (2009) found that the combination diet containing two levels of 23 and 35% protein did not differ significantly from the protein content and had no significant difference in the whole body fat value of juvenile grass carp. This result is in agreement with the results obtained during this study.

Table (5): Body composition of grass carb, after 90 days of feeding on experimental diets containing different levels of Azolla pinnata (mean ± SD)

	Control	T25	T50	T75	T100
CP	16.62 ± 0.58^a	15.72 ± 0.03^b	15.68 ± 0.02^b	15.68 ± 0.03^b	15.67 ± 0.01^b
CF	2.99 ± 0.58^a	1.52 ± 0.05^b	1.93 ± 0.02^b	1.92 ± 0.10^b	1.99 ± 0.05^b
CM	79.73 ± 0.00^a	79.73 ± 0.68^a	79.93 ± 0.30^a	80.80 ± 0.31^a	80.83 ± 0.26^a
Ash	1.72 ± 0.00^b	2.28 ± 0.08^a	2.39 ± 0.07^a	2.38 ± 0.09^a	2.48 ± 0.25^a

Values within columns with the same superscript are not significantly different ($P > 0.05$).

Through the experiment and during the study period, water quality parameters (Do, PH and temperature) were within the acceptable ranges for C. idellus health protection and growth. However, these parameters did not show any statistical differences during the study period.

Table (4): Water quality parameters (mean ± SD) after 90 days of feeding grass carb on experimental diets containing different levels of Azolla pinnata

	DO (mg/L)	PH	Temperature C^o
Control	4.50 ± 0.25^a	7.67 ± 0.03^a	28.50 ± 0.15^a
T25	4.50 ± 0.17^a	7.63 ± 0.09^a	29.07 ± 0.23^a
T50	5.93 ± 0.30^a	7.80 ± 0.06^a	28.17 ± 0.17^a
T75	5.57 ± 0.23^a	7.70 ± 0.06^a	28.37 ± 0.12^a
T100	4.93 ± 0.30^a	7.67 ± 0.09^a	29.07 ± 0.22^a

Values within columns with the same superscript are not significantly different ($P > 0.05$).

The results of amylase levels may be an important determinant of efficient digestive system functions as well as the ability of fish to benefit from diets containing high levels of carbohydrates (Lazzari et al., 2010). In the current study, the results indicated an increase in amylase in meal 75, followed by meal 50 and then 25 compared to meal 100, and this is evidence that these percentages of feed replacement achieved high benefit from meals with percentages of easily digestible carbohydrates. Whereas Fountoulaki et al. (2005) reported that the sea bream fish is affected by the level of amylase by changing the level of fat in the diet.

The fact that a diet supplemented with a vegetable protein source may influence growth performance and digestion efficiency in carp and needs more long-term research. In previous studies, there are strong indications of an increase in the ability of fish to benefit from changing feed compositions by increasing body growth in different stages of growth and then stabilizing with changes in temperature and acidity (Jun-sheng et al., 2006).

As the temperature decreases, the ability to digest food in fish decreases. Jun-Jun-sheng et al. (2006) changes in tilapia protein enzyme, including the change in tilapia growth rates, where the maximum activity of amylase was at pH 6-7 to 7.5, and the highest amylase activity was determined at 25-35 °C. The lipase activity was at pH 6.0-9.0 and temperatures between 25 and 35 °C (Ugwumba, 1993; Tocher and Sargent, 1984; Jun-sheng et al., 2006). It was also found (Steffens, 1987) in trout that with the increase in feed consumption and the increase in temperature and salinity, the activity of amylase increases. In this study, the value of lipase enzyme was the lowest significantly in the control group, and the T25 group achieved the highest value significantly in lipase.

water quality measurements were estimated by increasing the oxygen level, and the rest of the water parameters were within the range necessary for the activity of digestive enzymes.

Hematological values are determined in different cultured fish species for a variety of purposes, including diagnosis of diseases, assessment of culture environment conditions, measurement of physiological changes in fish (Kori-Siakpere et al. 2005), or determination of standard rates for blood parameters (Etim et al. 1999, Baghizadeh and Khara. 2015), in our current study, some blood parameters were measured in order to identify the effect of grass carp feeding on Azolla plants in different proportions. At the end of the study, it was noted that the total protein decreased in grass carp fed on Azolla plants compared to those fed on a controlled diet. The albumin values were significantly decreased in fish-fed Azolla plants compared to those fed on a controlled diet (C). In addition, a significant increase in globulin concentration was observed in the control diet. Statistical comparison between the different treatments did not reveal significant differences in the values of RBC, WBC, Hct, Hb, MCV, MCH, and MCHC, ($P < 0.05$) (Table 6).

As for the group that was treated with Azolla at a rate of 50%, the results showed that the lowest cholesterol values were achieved. While T100 group achieved the lowest values for triglycerides and amylase, and it was the highest reported in glucose levels

Table (6): Changes in haemato-biochemical parameters of grass carb (M±SE) fed diets supplemented with various levels of Azolla for 90 days.

	control	**T25**	**T50**	**T75**	**T100**
TP g/dl	3.30 ± 0.05^{a}	2.77 ± 0.32^{b}	2.77 ± 0.09^{b}	2.80 ± 0.06^{b}	2.67 ± 0.07^{b}
cholesterol mg/dl	302.83 ± 0.90^{a}	214.97 ± 2.80^{c}	157.63 ± 3.24^{e}	194.97 ± 3.55^{d}	255.40 ± 3.10^{b}
trigly	228.00 ± 1.70^{a}	221.53 ± 1.16^{a}	182.40 ± 2.42^{b}	146.43 ± 2.44^{c}	152.23 ± 2.79^{c}
Amaylease	209.87 ± 2.09^{c}	560.90 ± 4.02^{b}	736.63 ± 4.44^{a}	730.67 ± 2.40^{a}	220.03±4.07c
lipase	153.77 ± 2.70^{d}	241.93 ± 3.61^{b}	212.17 ± 3.61^{b}	269.20 ± 1.76^{a}	173.33 ± 2.33^{c}
albumin g/dl	2.00 ± 0.00^{a}	1.53 ± 0.28^{b}	1.97 ± 0.03^{b}	1.87 ± 0.03^{b}	1.70 ± 0.06^{b}
globulin g/dl	1.30 ± 0.06^{a}	1.23 ± 0.03^{a}	0.80 ± 0.06^{b}	0.93 ± 0.09^{b}	0.97 ± 0.03^{b}
WBC	7.43±0.09a	7.40 ± 0.10^{ab}	7.57 ± 0.09^{a}	7.51±0.06a	7.50 ± 0.06^{a}
RBC	1.82 ± 0.01^{a}	1.86 ± 0.02^{a}	1.88 ± 0.02^{a}	1.88 ± 0.01^{a}	1.81 ± 0.05^{a}

	control	T25	T50	T75	T100
MCV	173.22 ± 2.79^{a}	169.27 ± 6.10^{a}	164.81 ± 3.33^{a}	164.30 ± 4.07^{a}	176.25 ± 3.19^{a}
MCHC	26.87 ± 0.68^{a}	27.07 ± 1.44^{a}	26.67 ± 0.50^{a}	27.23 ± 1.16^{a}	25.42 ± 0.29^{a}
MCH	46.51 ± 0.63^{a}	45.64 ± 0.88^{a}	43.93 ± 0.11^{a}	44.65 ± 0.94^{a}	44.82 ± 1.31^{a}
HCT	31.47 ± 0.58^{a}	31.48 ± 1.13^{a}	31.03 ± 0.38^{a}	30.95 ± 0.91^{a}	31.93 ± 0.36^{a}
HB	8.45 ± 0.06^{ab}	8.49 ± 0.20^{a}	8.27 ± 0.08^{ab}	8.41 ± 0.12^{ab}	8.11 ± 0.05^{b}
Glucose	99.47 ± 0.52^{b}	82.47 ± 1.15^{c}	82.94 ± 0.95^{c}	96.00 ± 2.68^{b}	111.05 ± 6.04^{a}

Values within columns with the same superscript are not significantly different ($P < 0.05$).

Conclusions

The current study showed that replacing the artificial feed with Azolla pinnata positively enhanced the growth performance, haemato-biochemical values, of grass carp. These results indicated that Azolla pinnata with a rate of 25-50% can be considered a beneficial dietary supplement for grass carp.

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