Biological and Applied Environmental Research

Vol. 4 (2): 167-176, 2020

ISSN: 2002-6153
Biol. Appl. Environ. Res.
www.baerj.com
editor@baerj.com

Partial Replacement of Fish Meal with *Azolla filiculoides* Meal in the Grass Carp *Ctenopharyngodon idella* Feed

Adel Y. Al-Dubakel[1], Majid M. Taher[1]* & Jennan N. Abdullah[2]

[1]Aquaculture Unit, College of Agriculture, University of Basrah, Basrah, Iraq
[2]Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq

*Corresponding author: Maj61ae@yahoo.com

Abstract: Laboratory experiment was conducted to investigate the replacement of fish meal (FM) with *Azolla filiculoides* meal (AM) in the formulated diet of grass carp, *Ctenopharyngodon idella*. Eight aquaria, two for each treatment, were used for the current experiment which was conducted at the Laboratory of Live Food- Aquaculture Unit, College of Agriculture during 9 December 2019. A total of four treatments: Control (0% AM), T1 (5% AM), T2 (10% AM) and T3 (15% AM) were used in feeding the grass carp. Fishes were fed five or six days a week at 3% of fish weights as feeding ratio for 55 days. Results showed that the highest final weight was recorded by T1, while lowest final weight was recorded by T3. Weight increments were 2.30, 1.55, 2.50 and 1.60 g for C, T1, T2 and T3, respectively, while average values of daily growth rate were 0.042, 0.028, 0.050 and 0.030 g/day for C, T1, T2 and T3, respectively. Average values of specific growth rate were 0.090, 0.100, 0.099 and 0.062 %/day for C, T1, T2 and T3, respectively. Statistical analysis of growth criteria proved that there were no significant differences (P>0.05) in growth criteria among all treatment groups.

Keywords: *Azolla filiculoides*, Fish meal, Grass carp, Growth, Partial replacement

Introduction

Farming operations almost based on the use of fish feed, are highly dependent on available fishery resources for the production of fish meal, thus becoming a reducing activity rather than an activity producing fishery resources, and hence nutritionists have been searching worldwide for effective fish meal substitutes, and several attempts have been made to partially or totally replace fish meal with less expensive protein sources (Velásquez, 2014). Around the world, many efforts have concentrated on replacing animal protein (especially fish meal) with cheaper plant protein in fish diets (Pouomogne et al., 1997; Fasakin et al., 1999, 2001; Adebayo et al., 2004).

Because of high costs and demand of conventional protein sources necessitates fish farmers and hatcheries manager to incorporate cheap locally available

Received Mar. 12, 2020, accepted June 3, 2020

ingredients in fish diets such as *Azolla* which is considered as a good replacer of protein from expensive sources (Mosha, 2018). Costa et al. (2009) stated that *Azolla* is a genus of aquatic ferns and small leafed floating plants native to the tropical, subtropical and warm temperate regions of Africa, Asia and America. Radhakrishnan et al. (2014) pointed out that floating freshwater *Azolla pinnata* is aquatic plant with high biomass and protein production which can be used directly to feed fishes or as a diet ingredient of an alternative protein source. *Azolla* plants gained their importance in aquaculture due to their high crude protein content (13% to 30%) and essential amino acid composition (rich in lysine) comparing with most green forage crops and other aquatic macrophytes (Panigrahi et al., 2014). Anitha et al. (2016) stated that sundried *Azolla* contains 22.48 % crude protein, 4.5% oil, 14.7 % crude fiber, 17.34 % ash and 40.98 % nitrogen free extract. Yadav et al. (2014) pointed that *Azolla* can fix atmospheric nitrogen due to the presence of a symbiotic cyanobacterium *Anabaena azollae*, and because of this property, it has been widely exploited as bio fertilizer for rice plants. In addition, it has several other uses such as food, feed, biogas producer and hyper accumulator of heavy metals.

Grass carp, *Ctenopharyngodon idella* is native to southeastern Russia and northwestern China, and it has been deliberately introduced into many countries for vegetation control, while this fish escapes from aquaculture facilities in some countries (Cudmore & Mandrak, 2004). Grass carp is now considered as belonging to the family Xenocypridinae instead of Cyprinidae according to a recent phylogenetic study (Tan & Armbruster, 2018). Feeding activity of grass carp is decreased when water temperature exceeded 30 ^{0}C in summer and again when the temperature dropped below 26 ^{0}C during fall (Pfeiffer & Lovell, 1990).

Many researchers claimed that aquatic fern *Azolla* is a widely successful component of fish diets in both aquaria and tanks (El-Sayed, 1992; Leonard et al., 1998; El-Sayed, 1999; Fiogbé et al., 2004). Little studies in Iraq on grass carp were achieved. Al-Dubakel et al. (2011) studied growth performance and implication of grass carp larvae reared in recirculation system. Taher (2017) conducted some laboratory experiments on the grass carp. The aim of the current experiment was to investigate effects of replacement fish meal with *Azolla* meal in the formulated diet of grass carp on growth criteria.

Materials and Methods

The current experiment was conducted in Fish Laboratory of Aquaculture Unit, College of Agriculture, University of Basrah during December, 2019. As indicated in Table 1, pelleted feed manufactured in the laboratory by using raw materials with changing the ratio of fish meal and *Azolla filiculoides* meal (AM) was applied. Grass carp (Average weight 46.34±7.4 g) were brought from the earthen ponds of the Aquaculture Unit located in Al-Hartha Station for Agricultural Researches, north Basrah. Eight aquaria were used during the current experiment with ten fishes each, two for each treatment, with dimensions of 60×40×30 cm provided with pumping aeration and heaters (water temperature 25-26 ^{o}C). Four treatments i.e. control (0% AM), T1 (5% AM), T2 (10% AM) and T3 (15% AM) were used in

feeding grass carp during the experiment trail. The experiment was lasted for 55 days and fishes were fed five or six days a week 3% of body mass. Fishes were weighed periodically to alter daily feed. Throughout this period, five sampling data were collected to calculate the following performances:

Weight increments (WI, g) = FW - IW

Daily growth rate (DGR, g/day) = FW - IW/days

Specific growth rate (SGR, %/day) = 100 * [(ln FW) - (ln IW)] days

Where: FW = Final fish weight (g); IW = Initial fish weight (g)

By application of SPSS (version 22), the data were subjected to one-way analysis of variance (ANOVA) to determine the difference between the means and the significant differences which were tested by LSD Test.

Table 1: Feed ingredients with their ratios of different treatment.

Treatments	Feed ingredients (%)					
	Fish meal	*Azolla* meal	Soybean meal	Wheat flour	Wheat bran	Premix (vitamins and minerals)
Control	20	0	20	35	23	2
T1	15	5	20	35	23	2
T2	10	10	20	35	23	2
T3	5	15	20	35	23	2

Results

Table 2 shows the measurements of fish weight with standard deviation during the experiment. The highest final weight (52.3 g) was reached by fishes fed on T1 feed in pond 4, while the lowest final weight (46.8 g) was reached by fishes fed on T3 and control fishes in pond 7 and 2. Table 3 shows the growth criteria of different treatments in the experiment. Weight increments were 2.30, 1.55, 2.50 and 1.60 g for C, T1, T2 and T3, respectively (Figure 1). Statistical analysis of weight increments proved that there were no significant differences ($P>0.05$) between all treatments.

Results indicated that average values of daily growth rate were 0.042, 0.028, 0.050 and 0.030 g/day for C, T1, T2 and T3, respectively (Figure 2). Statistical analysis of weight increments proved that there were no significant differences ($P>0.05$) between all treatments. Average values of specific growth rate were 0.090, 0.100, 0.099 and 0.062 %/day for C, T1, T2 and T3, respectively (Figure 3). Statistical analysis of weight increments proved that there were no significant differences ($P>0.05$) among all treatments.

Best feed conversion rate (25.0) was achieved by fishes fed on feed with 10% *Azolla* meal (T3), while worst feed conversion rate (40.6) was achieved by fishes fed on feed with 5% *Azolla* meal (T1) as indicated in Figure 4. Statistical analysis of feed conversion rate showed that there were no significant differences ($P>0.05$) between all treatments. So, partial replacement of fish meal by *Azolla* meal had no any effects on growth criteria of grass carp.

Table 2: Measurements of average fish weight during the experiment.

Date	Average fish weight (g)±SD							
	CP1	CP2	T1P3	T1P4	T2P5	T2P6	T3P7	T3P8
9/12/2019	45.4±6.5	45.2±4.1	45.4±6.4	50.6±6.6	44.4±8.8	45.8±9.9	45.6±3.0	47.0±7.0
22/12	44.2±5.8	44.2±4.5	45.4±6.7	50.4±6.4	45.0±9.3	46.2±11.6	45.4±3.4	47.6±7.2
8/1/2020	46.0±5.6	45.4±5.8	44.0±4.5	50.8±5.9	46.0±9.7	47.0±12.3	45.0±3.2	47.4±5.8
22/1	49.4±4.8	46.8±7.2	46.6±5.1	51.5±6.4	48.4±11.0	48.4±14.7	45.9±2.8	49.2±5.8
2/2	48.4±4.8	46.8±7.2	46.8±4.4	52.3±7.4	47.4±11.0	47.8±14.2	46.8±2.3	49.0±5.8

Table 3: Growth criteria of different treatments in the experiment.

Growth criteria	Treatments							
	C		T1		T2		T3	
	P1	P2	P3	P4	P5	P6	P7	P8
WI (g)	3	1.6	1.4	1.7	3	2	1.2	2
Average	2.30^{a}		1.55^{a}		2.50^{a}		1.60^{a}	
DGR (g/day)	0.055	0.029	0.025	0.031	0.055	0.036	0.022	0.036
Average	0.042^{a}		0.028^{a}		0.050^{a}		0.030^{a}	
SGR (%/day)	0.116	0.063	0.055	0.060	0.119	0.078	0.047	0.076
Average	0.090^{a}		0.100^{a}		0.099^{a}		0.062^{a}	
FCR	20.3	37.4	43.1	38.1	20.1	30.8	48.1	31.5
Average	28.9^{a}		40.6^{a}		25^{a}		39.8^{a}	

Different letters in one rows are significantly different ($P<0.05$).

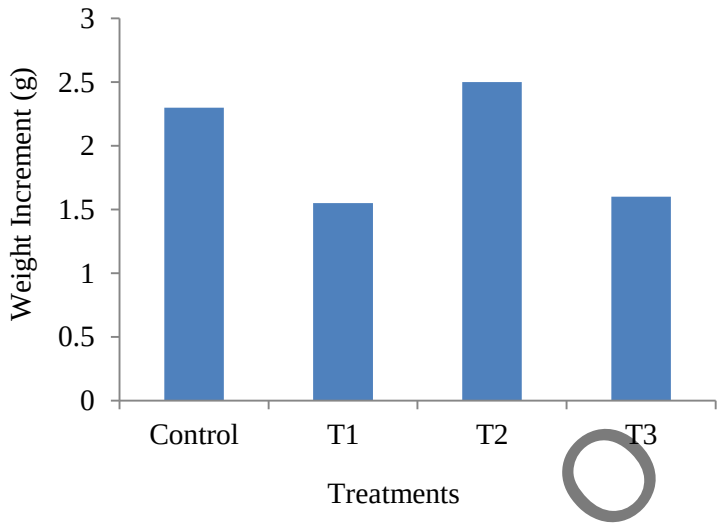

Figure 1: Weight increments for fish during experiment.

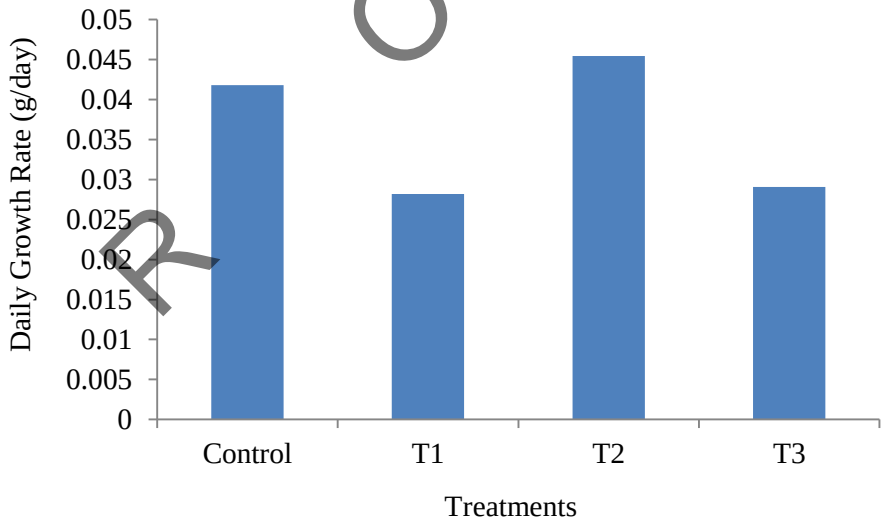

Figure 2: Daily growth rate for fish during experiment.

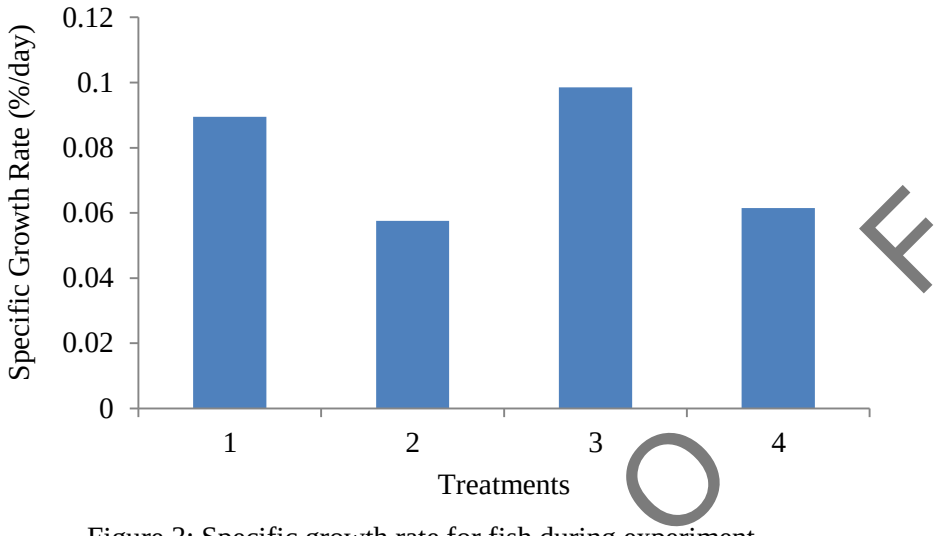

Figure 3: Specific growth rate for fish during experiment.

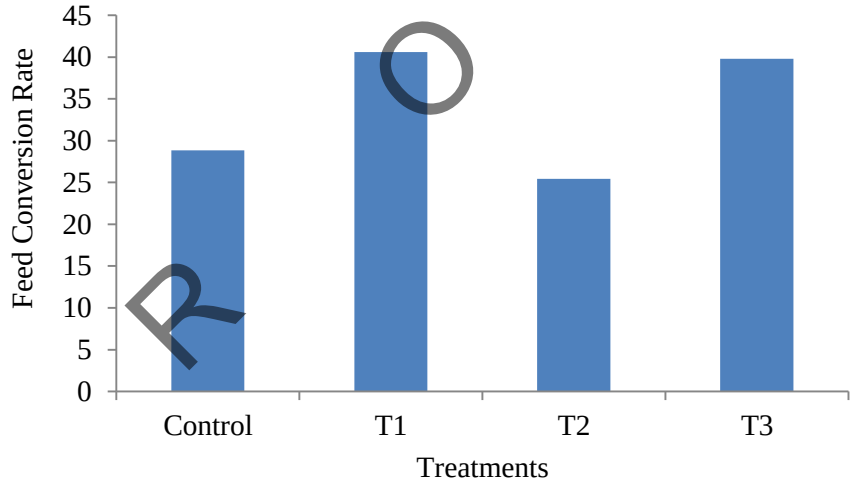

Figure 4: Feed conversion rate for fish during experiment.

Discussion

The probable causes of considering non-conventional plant feedstuffs not suitable as feed ingredient for fishes include poor nutritive value in terms of poor digestibility, low availability of nutrients and presence of anti-nutritional factors (De Silva, 1991). Velásquez (2014) pointed out that aquatic plants are important nutritional sources for herbivorous-omnivorous fishes and could replace 25% of formulated diets and 50% of commercial feeds (35% protein) without any adverse effects on growth and body composition of fishes.

Results of the present experiment revealed that *Azolla* meal is a good substitute for fish meal, where the replacement up till 75% of fish meal has no effect on growth criteria of grass carp cultivated in aquaria. Majhi et al. (2006) stated that grass carp preferred *Azolla caroliniana* and statistical analysis indicated that final weight gain was significantly higher than that of the control pond without this plant.

Datta (2011) recorded highest weight gain with a diet containing 25% *Azolla* when added a mixture of six species of *Azolla* as a feeding ingredient in the diet of *Labeo rohita.* Nile tilapia, *Oreochromis niloticus* fed on diets with aquatic plants (*Spirodela polyrhiza*, *Lemna minor* and *A. filiculoides*) replaced fish meal showed no significant differences in the growth parameters. On the contrary, significant differences in the growth parameters were noticed for the species *Piaractus brachypomus* by Velásquez (2014). Zolfinejad et al. (2017) pointed out that among six aquatic and terrestrial plants fed to grass carp, *A. filiculoides* had higher values of fish weight gain rate.

El-Sayed (1992) stated that growth rates and utilization efficiency of Nile tilapia were better when fed on diet with fish meal comparing with that of fish fed on diet with substituting *A. pinnata* for fish meal at rates of 25, 50, 75 and 100%. Weight gain of Nile tilapia was decreased compared with the control, when fed diet containing 20.7, 34.4 and 48.2% of the total weight of dried *Azolla* as indicated by Shiomi & Kitoh (2001). All Nile tilapia fed on diet levels with incorporated *Azolla* meal (0, 15, 20, 30, 40 and 45%) exhibited weight gain. Also, the *Azolla*-free diet and the diet containing 15% *Azolla* produced the same growth performance (Fiogbé et al., 2004). Abou et al. (2007) found that there were no significant differences between growth parameters and production of Nile tilapia fed on diets containing 30, 35 and 40% *Azolla* mixing with locally by-products of Porto-Novo suburb, Benin, West Africa. Abdel-Tawwab (2008) stated that growth retardation and weight loss in *Tilapia zillii* (a synonym of *Coptodon zillii*) were observed in all fish sizes fed fresh *A. pinnata*, and also growth of these fishes was reduced when the level of dry *Azolla* meal in the diet exceeds 25%. Abou et al. (2012) pointed out that specific growth rate of Nile tilapia was higher with the control diet (without *Azolla* meal) and the lower values being obtained in fishes fed on 50% *Azolla* meal as partial fishmeal substitutes. Das et al. (2018) stated that there were significant reduction of growth of silver barb *Barbonymus gonionotus* in the case where more than 25% commercial fish feed was substituted with *A. pinnata*.

It is concluded from results of current experiment that partial replacement of fish meal by *Azolla* meal had no any effects on growth criteria, so it is recommended to replace 15% of fish meal with *Azolla* meal when manufacturing commercial feed for grass carp.

References

Abdel-Tawwab, M. (2008). The preference of the omnivorous-macrophagous, *Tilapia zillii* (Gervais), to consume a natural free-floating fern, *Azolla pinnata.* J. World Aquac. Soc., 39 (1): 104-112. Doi:10.1111/j.1749-7345.2007.00131.x.

Abou, Y.; Fiogbé, E.D. & Micha, J.-C. (2007). Effects of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus* L., fed *Azolla* diet, in earthen ponds. Aquac. Res., 38 (6): 595-604. Doi:10.1111/j.1365-2109.2007.01700.x.

Abou, Y.; Aliou, S.; Daouda, M.; Fiogbé, E.D. & Micha, J.-C. (2012). Evaluation of nitrogen and phosphorus wastes produced by Nile tilapia (*Oreochromis niloticus* L.) fed *Azolla*-diets in earthen ponds. J. Environ. Prot., 3: 502-507. Doi:10.4236/jep.2012.36060.

Adebayo, O.T.; Fagbenro, O.A. & Jegede, T. (2004). Evaluation of *Cassia fistula* meal as a replacement for soybean meal in practical diet of *Oreochromis niloticus* fingerlings. Aquac. Nutr., 10 (2): 99-104. Doi:10.1111/j.1365-2095.2003.00286.x.

Al-Dubakel, A.Y.; Jabir, A.A. & Al-Hamadany, Q.H. (2011). Growth performance and implication of a thermal-unit growth coefficient of grass carp *Ctenopharyngodon idella* and silver carp *Hypophthalmichthys molitrix* larvae reared in recirculation system. J. King Abdulaziz Univ. Mar. Sci., 22 (2): 33-43. Doi:10.4197/Mar.22-2.3.

Anitha, K.C.; Rajeshwari, Y.B.; Prasanna, S.B. & Shree, J.S. (2016). Nutritive evaluation of *Azolla* as livestock feed. J. Exp. Biol. Agric. Sci., 4 (6): 670-674. Doi: 10.18006/2016.4(issue6).670.674.

Costa, M.L.M.C.; Santos, M.C.R.; Carrapico, F. & Pereira, A.L. (2009). *Azolla*-Anabaena's behaviour in urban wastewater and artificial media-influence of combined nitrogen. Water Res., 43 (15): 3743-3750. Doi:10.1016/ j.watres.2009.05.038.

Cudmore, B. & Mandrak, N.E. (2004). Biological synopsis of grass carp (*Ctenopharyngodon della*). Can. MS Rep. Fish. Aquat. Sci., 2705: v + 44 pp.

Das, M.; Rahim, F.I. & Hossain, M. A. (2018). Evaluation of fresh *Azolla pinnata* as a low-cost supplemental feed for Thai silver barb *Barbonymus gonionotus.* Fishes, 3 (15): 1-11. Doi:10.3390/fishes3010015.

Datta, S.N. (2011). Culture of *Azolla* and its efficacy in diet of *Labeo rohita*. Aquaculture, 310 (3-4): 376-379. Doi:10.1016/j.aquaculture.2010.11.008.

De Silva, S.S. (Ed.) (1991). Fish nutrition research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society in association with the Int. Develop. Res. Cent. Can.: 205 pp.

El-Sayed, A.-F.M. (1992). Effects of substituting fish meal with *Azolla pinnata* in practical diets for fingerling and adult Nile tilapia, *Oreochromis niloticus* (L). Aquac. Fish. Manag., 23: 167-173.

El-Sayed, A.-F.M. (1999). Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture, 179 (1/4): 149-168. Doi:10.1016/s0044-8486(99)00159-3.

Fasakin, E.A.; Balogun, A.M. & Fagbenro, O.A. (2001). Evaluation of sun-dried water fern, *Azolla africana*, and duckweed, *Spirodela polyrrhiza* in practical diets for Nile tilapia, *Oreochromis niloticus*, fingerlings. J. Appl. Aquac., 11 (4): 83-92. Doi:10.1300/J028v11n04_09.

Fasakin, E.A.; Balogun, A.M. & Fasuru, B.E. (1999). Use of duckweed, *Spirodela polyrrhiza* L. Schleiden, as a protein feedstuff in practical diets for tilapia, *Oreochromis niloticus* L. Aquac. Res., 30 (5): 313-318. Doi:10.1046/j.1365-2109.1999.00318.x.

Fiogbé, E.D.; Micha, J.-C. & Van Hove, C. (2004). Use of a natural aquatic fern, *Azolla microphylla*, as a main component in food for the omnivorous–phytoplanktonophagous tilapia, *Oreochromis niloticus* L. J. Appl. Ichthyol., 20 (6): 517-520. Doi:10.1111/j.1439-0426.2004.00562.x.

Leonard V.; Breyne, C.; Micha, J.-C. & Larondella, Y. (1998). Digestibility and transit time of *Azolla filiculoides* Lamarck in *Oreochromis aureus* (Steindachner). Aquac. Res., 29 (3): 159-165.

Majhi, S.K.; Das, A. & Mandal, B.K. (2006). Growth performance and production of organically cultured grass carp *Ctenopharyngodon idella* (Val.) under mid-hill conditions of Meghalaya; North Eastern India. Turk. J. Fish. Aquat. Sci., 6: 105-108.

Mosha, S.S. (2018). A review on significance of *Azolla* meal as a protein plant source in finfish culture. J. Aquac. Res. Develop., 9 (7): 1-7. Doi:10.4172/2155-9546.1000544.

Panigrahi, S.; Choudhury, D.; Sahoo, J.K.; Das, S.S. & Rath, R.K. (2014). Effect of dietary supplementation of *Azolla* on growth and survibility of *Labeo rohita* fingerlings. Asian J. Anim. Sci., 9 (1): 33-37.

Pfeiffer, T.J. & Lovell, R.T. (1990). Responses of grass carp, stocked intensively in earthen ponds, to various supplemental feeding regimes. Prog. Fish. Cult., 52: 213-217.

Pouomogne, V.; Takam, G. & Pouemegne, J.-B. (1997). A preliminary evaluation of cacao husks in practical diets for juvenile Nile tilapia (*Oreochromis niloticus*). Aquaculture, 156: 211-219.

Radhakrishnan, S.; Bhavam, P.S.; Seenivasan, C.; Shanthi, R. & Muralisankar, T. (2014). Replacement of fishmeal with *Spirulina platensis*, *Chlorella vulgaris* and *Azolla pinnata* on non-enzymatic and enzymatic antioxidant activities of *Macrobrachium rosenbergii*. J. Basic Appl. Zool., 67 (2): 25-33. Doi:10.1016/j.jobaz.2013.12.003.

Shiomi, N. & Kitoh, S. (2001). Culture of *Azolla* in a pond, nutrient composition, and use as fish feed. Soil Sci. Plant Nutr., 47 (1): 27-34. Doi:10.1080/00380768.2001.10408365.

Taher, M.M. (2017). Laboratory experiments on cultivation of grass carp *Ctenopharyngodon idella* (Valenciennes, 1844). Basrah J. Agric. Sci., 30 (2): 91-98.

Tan, M. & Armbruster, J.W. (2018). Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi). Zootaxa, 4476 (1): 6-39. Doi:10.11646/zootaxa.4476.1.4.

Velásquez, Y.C.C. (2014). Study on the locally available aquatic macrophytes as fish feed for rural aquaculture purposes in South America. Ph. D. Diss., Fac. Agric. Hortic., Humboldt-Univ. zu Berlin: 141 pp. Doi:10.18452/17510.

Yadav, R.K.; Abraham, G.; Singh, Y.V. & Singh, P.K. (2014). Advancements in the utilization of *Azolla-Anabaena* system in relation to sustainable agricultural practices. Proc. Ind. Natn. Sci. Acad., 80 (2): 301-316.

Zolfinejad, K.; Khara, H. & Filizadeh, Y. (2017). Food preference and growth of grass carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844) fed some aquatic and terrestrial plants. Iran. J. Fish. Sci., 16 (4): 1278-1286.