Performance of Asian Watergrass, *Hygroryza Aristata* as Supplementary Fish Feed in Aquaculture

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ABSTRACT

The quality and high price of fish feed are important issues for the development of sustainable aquaculture and food security globally. Present study was investigated for a period of 153 days using Asian watergrass as fish feed to reduce reliance on fishmeal in aquaculture. In treatment one, no commercial feed used and designated as T_0 (Asian watergrass was planted at pond bottom and grown as 100% fish feed); in treatment two, 50% commercial feed was applied and designated as T_{50} (grass was planted as 50% fish feed and applied 50% commercial feed); and in treatment three, 100% commercial feed was supplied as control and designated as T_{100} (no grass was applied). Grass carp, common carp, tilapia, mrigal and rohu were stocked at 6:2:2:1:1 ratio with similar stocking densities of 15000 fish ha^{-1} in all treatments. Important water quality parameters were monitored those were suitable range for fish culture. The survival of stocked fishes varied from 76.67 ± 5.77 % to 96.67 ± 3.33 % irrespective to the species. The total production was significantly highest in T_{100} (5579.04 ± 238.74 kg ha^{-1}) followed by T_{50} (4553.24 ± 124.05 kg ha^{-1}) and T_0 (4448.37± 237.26 kg ha^{-1}). Calculation of benefit cost ratio showed the highest net benefit in T_{50} (USD: 5936.67) followed by T_0 (USD: 5436.53) and T_{100} (USD: 4130.62). The present findings indicate that 50% commercial feed along with 50% Asian watergrass as supplementary feed practiced is economically viable and more congenial with getting more net benefit. To improve the productivity with congenial environment and searching more benefit, this technique should be applied in haor, baor, beels, canals, and other water logged areas pervaded by climate changes.

Keywords: Aquaculture; supplementary feed; Asian watergrass; Fish production cost, benefit.

INTRODUCTION

The Asian watergrass (*Hygroryza aristata*) naturally grows in all the freshwater bodies and it grows vigorously in the inundated coastal wetlands of Bangladesh (Hossain et al., 2021). This grass has ability to improve the water quality by absorbing nitrogenous compounds with their effective root system and they actively contribute to the promotion and maintenance of food webs and services in freshwater ecosystems. This grass contains requisite amount of protein, lipid and carbohydrate and whole body of the grass is very soft and preferred food of grass carp that was used as fish feed in polyculture (Hossain et al., 2020b; Hossain et al., 2021). Grass carp have herbivorous appetites and consume large quantities of higher aquatic plants and it can be cultured only by fertilized the water body without artificial feeds (Halver & Hardy, 2002; Pillay, 2004). Pipalova, (2006) reported that the grass carp prefers soft-tissue aquatic plants, filamentous algae and duckweeds, and consumes all parts of preferred plants. Once more, common carp is an omnivorous bottom-dweller species and survives mainly on benthic fauna and decaying floral matters' of grass carp. It growth depends upon the underneath fauna, stocking density and the rate of added feed. Mrigal is also a bottom dweller fish and prefers feed on detritus and benthic fauna. The omnivorous tilapia preferred the roots and leaves of Asian watergras and various types of plankton and other available natural foods.

Currently, the price of fish feed is being increased due to the increase of the fishmeal price all over the world. FAO (2020) mentioned about the increase of price of fish meal and fish oil throughout the world due to the decline of world capture fisheries. On the other hand, the reduction of the environmental impact from aquaculture is a prime issue to produce quantity and quality fish through improving culture systems (Robinson et al., 2018). Accordingly, the development of sustainable aquaculture depends considerably on the utilization of alternative plant protein ingredients to fish meal (Olukayode & Emmanuel, 2012; Daniel, 2018). Aquatic plants are very important source of fish feed and have positive effects on improvement of water quality and remediation of aquaculture effluents (Sipauba-Tavares et al., 2002; Henry-Silva & Camargo, 2006; Ferdoushi et al., 2008; Carlozzi & Padovani, 2016), and reduce the fish production cost in aquaculture system noticeably (Hossain et al., 2020b).

Aquaculture products play a vital role in providing affordable high quality protein all around the world. The sources of nutrients and micronutrients are fish, which play an important role in human nutrition and the global food supply (FAO, 2018; Tacon & Metian, 2018; Hicks et al., 2019). Additionally, fish is a dietary sources of health-promoting omega-3 or n-3 long-chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), essential minerals (calcium, phosphorus, zinc, iron, selenium, and iodine), and vitamins (A, B, and D) (FAO, 2018). Fish production from aquaculture must be continued to maintain the levels of these fatty acids and micronutrients for healthy and nutritious human diets with increasing its production and prevalence of alternative ingredients in aqua feeds (NRC, 2011; FAO, 2018). But, the increasing price of fish meal creates hazardous situation in recent years throughout the world (FAO, 2020) that had been used in the formulation of aqua feed development. Currently, the insufficiency of fish meal protein is an issue worldwide. As a result, the cost of commercial fish feed beyond the capacity of the farmers. Fish meal is the main protein source in aqua feed industries due to its balanced amino acid profiles, high protein content, and good source of essential fatty acids (Abdelghany, 2003), but due to its high cost, variable quality as well as uncertain supply emphasis has been given to the development of cost-effective, high quality, alternative protein sources for complete or partial replacement of fish meal (Gallagher, 1994). At present, the aqua feed industries have faced severe crisis to develop inexpensive and quality fish feed due to insufficient and irregular supply of fish meal and fish oils. The average annual price of fish meal was the lowest in 1994 and 1999 at 403 and 433 USD/ton, respectively. It had continued to increase reaching 1230 USD/ton in 2009 and thereafter with 1687 USD/ton in 2010 and a peak of 1747 USD/ton in 2013 (Sofia, 2018). Increasing expenses and uncertain supplies of fish meal have led scientists to search for less expensive but compatible alternative protein sources for fish feed, most of which may be plant protein sources (Ayadi & Kurt, 2012; Hossain et al., 2021). To make aquaculture more profitable there is an urgent need to identify and incorporate the qualitative and quantitative requirement of dietary protein among the various ingredients used for the preparation of fish feeds. The increasing price and scarcity of diet components have created a crucial situation, which driven for inexpensive and abundantly available substitutes (Bureau & Meeker, 2011; Liland et al., 2012; Ghosh & Ray, 2017), particularly non-fishmeal protein ingredients. Therefore, Aquatic plant, especially Asian watergrass may be an alternative appropriate source of commercial feed in pond aquaculture, because Asian watergrass contains requisite amount of nutrients and used directly for grass carp (*Ctenopharyngodon idella*) culture and obtained satisfactory growth and production in tidally flooded coastal wetlands, with reducing production cost 2.95 times in comparison with commercial feed (Hossain et al., 2020a). Hossain et al. (2020b) reported higher growth and production performance of fishes in polyculture using the Asian watergrass in natural condition as fish feed and obtained economic efficiency of 4.52 in comparison with commercial feed. Therefore, the aim of this study is to use Asian watergrass as supplementary fish feed in pond aquaculture to reduce reliance on fishmeal and enhance the benefit of marginal fish farmers in the southern coastal region of Bangladesh.

MATERIALS AND METHODS

Study area

The study was conducted in 9 experimental ponds in the research field under the Faculty of Fisheries, Patuakhali Science and Technology University, Dumki, Patuakhali for a period of 153 days from July to November 2023.

The experimental site is situated in the coastal region of Bangladesh close to the Bay of Bengal (latitude 22°45´ to 22°55´ north and longitude 90°00´ to 90°15´ east) (Figure 1).

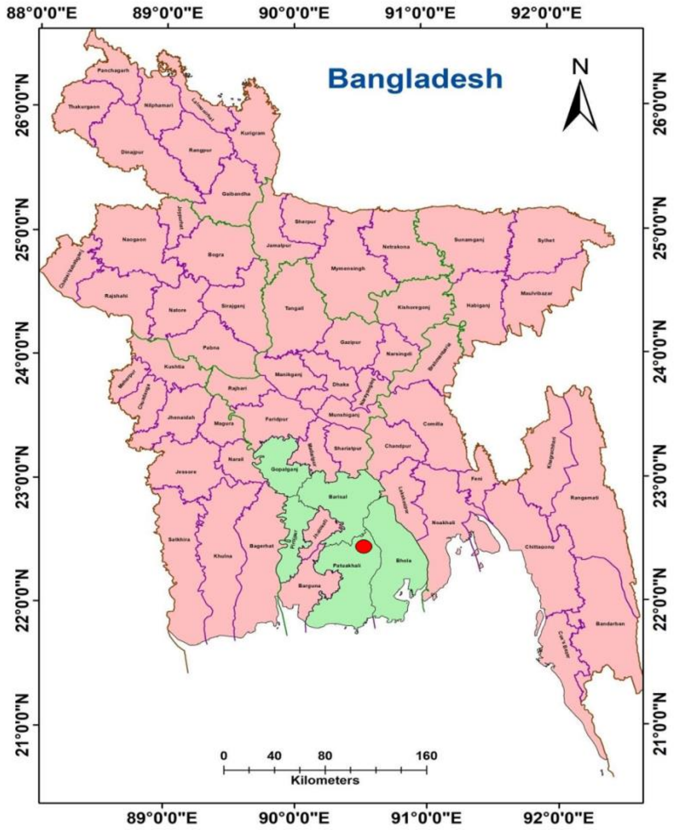

Figure 1. Map of Bangladesh showing the location of the study area. The paste color indicates the Asian watergrass growing zone and red color indicate the experimental site in the coastal region of Bangladesh

Experimental design

The selected ponds were similar sized and adjacent to each other. The experiment was carried out in three treatments each with three replications. In treatment one, no commercial feed used and designated as T_0 (Asian watergrass was planted at pond bottom of 80% area and grown as 100% fish feed); in treatment two, 50% commercial feed was applied and designated as T_{50} (grass was planted on 50% bottom area as 50% fish feed and applied 50% commercial feed); and in treatment three, 100% commercial feed was supplied as control and designated as T_{100} (no grass was applied). Homogeneity of Asian watergrass growth and production were maintained and confirmed in respected treatments.

Pond preparation and Asian watergrass plantation

The selected ponds were rectangular shaped having a surface area of each 40 m^2 (8m × 5m) with an average depth of 1.5 m. At the beginning of the works, all types of unwanted weeds were cleaned and dewatered using submergible pump. Subsequently, the heights of the embankments were increased (where necessary) using bottom soil. Finally, Asian watergrass was planted on the bottom soil according to Hossain et al. (2020b).

Fish stocking

On the 01 July, large sized fingerlings of grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*), mrigal (*Cirrhinus cirrhosus*) and rohu (*Labeo rohita*) were stocked at the similar ratio of 6:2:2:1:1 with stocking density of 15000 fish ha^{-1} in all treatments.

Fertilization

Urea, TSP (Triple Supper Phosphate) and MP (Muriate of Potash) fertilizers were applied at similar ratio of

2:2:1 with 312.5 kg ha^{-1} at monthly intervals from March to July in T_0 and T_{50} for increasing growth and production of Asian watergrass whereas those fertilizers were applied in T_{100} from July to November for better growth and production of plankton during culture period.

Feed supply

Commercial floating fish feed (Paragoan Fish Feed Ltd, Bangladesh) was applied twice a day between 9.00 - 10.00 a.m. and 16.00 - 17.00 p.m. in T_{50} and T_{100} according to the design. Feed was applied at the rate of 5% of the fish body weight for first two months then reduce to 4% of the fish body weight for rest of the culture period.

Monitoring of fish growth and health status

Considerable number of stocked fishes was sampled at monthly intervals from T_{50} and T_{100}. The weighed of sampled fishes were measured to monitor the growth, health status and adjust the feeding ration throughout the study period.

Monitoring of water quality parameters

Important water quality parameters such as water temperature (°C), dissolved oxygen (mg L^{-1}), pH and salinity (‰) were measured at monthly intervals using a thermometer, a dissolved oxygen meter (DO-5509, AF.11581, Taiwan), a portable pH meter (pHep, HANNA Instruments, Romania) and a digital refractometer (Brix HI 96801) in the spots throughout the study period. At the same time, water samples were collected in the 250 ml plastic bottle to determine the total alkalinity, ammonia, nitrate-nitrogen and phosphate-phosphorus in the laboratory. The total alkalinity was measured by titrimetric method using 0.02N H_2SO_4 titrant and methyl orange indicator. Ammonia, nitrate-nitrogen and phosphate-phosphorus were measured using a spectrophotometer (DR 1900, HACH, USA). Mineral stabilizer, polyvinyl alcohol dispersing agent and nessler reagent were used for the determination of ammonia, whereas NitraVer®6 nitrate reagent, NitriVer@3 nitrite reagent and PhosVer@3 phosphate reagent were used for the determination of nitrate-nitrogen and phosphate-phosphorus, respectively.

Harvesting of fish

On the 30 November, all the stocked fishes were harvested by dewatering pond water using submergible pump. The harvested fishes were counted and weighed of each species separately from each treatment. In addition, 25% of grass carp and 100% of other harvested fishes from each treatment were weighed to determine the growth parameters. Thereafter, the final weight, weight gain, specific growth rate (SGR), survival and the total production were calculated by using the following formulae:

(a) Weight gain = Final weight (g) – Initial weight (g);

(b) Specific growth rate (% body weight day^{-1}) = [{ln (final weight) – ln (initial weight)} /culture period (days)] × 100;

(c) Survival (%) = [No. of harvested fishes/Initial no. of stocked fish] ×100; and

(d) Production (kg·ha^{-1}) = [{No. of total harvested fishes × average individual weight of fish (g)/cultured area} × 10000] /1000.

Data analysis

Values of all the measured variables were expressed as means ± SD. The normality and homogeneity of variance tests were done before statistical analyses in all groups of data. The data of water quality parameters and growth parameters were analyzed following one-way analysis of variance (ANOVA). The paired comparison was made following Duncan's Multiple Range Test (DMRT). Significant was assigned at the 5% level ($p<0.05$). A tabular financial analysis was done to assess the economic viability of the culture technique according to Shang (1990).

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RESULT

Water quality parameters

The accomplished water quality parameters showed no marked variation among the treatments accepts total alkalinity and nitrate-nitrogen are presented in Table 1. The nitrate-nitrogen and total alkalinity were found to be significantly ($p<0.05$) higher in T_0 followed by T_{50} and T_{100} respectively. However, the temperature showed monthly variations (data not shown) with the change of the season. The highest water temperature was found to be 34.9°C in T_{50} in July, and the lowest temperature was recorded 29°C in T_0 and T_{100} in November. The dissolved oxygen and pH were found to be more or less similar in all treatments, whereas the salinity was found to be 1‰ throughout the study period. The phosphate-phosphorus level was moderate, but ammonia was found comparatively lower levels during the study period.

Table 1. Water quality parameters (mean ± SD, n = 21) in three different three treatments

Parameters	Treatments		
	T_0	T_{50}	T_{100}
Temperature (^{0}C)	31.11 ± 1.63 (29.00 - 34.10)	31.85 ± 1.68 (29.50 - 34.90)	31.77 ± 1.11 (29.00 - 32.70)
DO (Mg L^{-1})	4.54 ± 0.24 (4.10 - 4.90)	4.68 ± 0.16 (4.40 - 4.90)	4.89 ± 0.15 (4.50 - 4.90)
pH	7.92 ± 0.16 (7.70 - 8.20)	7.87 ± 0.15 (7.60 - 8.10)	7.05 ± 0.10 (7.40 - 8.10)
Salinity (‰)	1.0±0.0	1.00±0.0	1.00 ± 0.0
Total alkalinity (mg L^{-1})	221.60 ± 37.30^a (150 - 280)	178.27 ± 21.34^b (144 - 216)	163.60 ± 22.24^c (132 - 202)
Ammonia (mg L^{-1})	0.09 ± 0.09 (0.01 - 0.25)	0.06 ± 0.08 (0.01 - 0.31)	0.06 ± 0.07 (0.01 - 0.20)
Nitrate-nitrogen (Mg L^{-1})	0.41 ± 0.22^a (0.95 - 0.17)	0.31 ± 0.28^b (0.90 - 0.10)	0.19 ± 0.18^c (0.80 - 0.06)
Phosphate-phosphorus (Mg L^{-1})	0.52 ± 0.14 (0.33-0.79)	0.49 ± 0.11 (0.29-0.74)	0.54 ± 0.15 (0.34-0.94)

SD = Standard deviation, n = Number, M = Milligram, ‰ = Parts per thousand, L = Liter, DO = Dissolved oxygen, C = Celsius. Values within the parentheses indicate the ranges.

Growth performance

The growth performance (initial weight, final weight, weight gain, SGR), survival and total production of the fishes are presented in Table 2. The survival of the stocked fishes varied from 76.67 ± 5.77 % to 96.67 ± 3.33 % irrespective to the fish species. The final weight, weight gain, SGR and production of grass carp, common and tilapia were found significantly higher ($p< 0.05$) in T_{100} followed by T_{50} and T_0, respectively.

Table 2. The growth and the production performance of cultured fish (mean ± SD, n = 15 for grass carp, common and tilapia; and n = 9 for mrigal and rohu) in three different treatments

Species	Treatments	Weight (g)			Survival (%)	SGR (% day^{-1})	FCR	Production (kg·ha^{-1}·5 month^{-1}).
		Initial weight (g)	Final weight (g)	Weight gain (g)				
Grass carp	T_0	32.60±4.21	251.87±26.59^{c}	219.27±26.45^{c}	92.22±5.09	1.34±0.10^{b}	-	1741.37±189.79^{c}
	T_{50}	33.07±3.96	331.07±40.28^{b}	298.00±39.20^{b}	96.67±3.33	1.51±0.10^{a}	1.05	2400.30±305.04^{b}
	T_{100}	33.20±3.69	368.60±42.85^{a}	335.40±42.86^{a}	86.67±3.33	1.57±0.10^{a}	2.72	2676.02±356.84^{a}
Common carp	T_0	35.73±4.53	243.87±49.21^{c}	208.13±49.76^{c}	86.67±5.77	1.25±0.17^{c}	-	529.78±117.98^{c}
	T_{50}	35.93±5.59	328.00±53.53^{b}	292.07±52.65^{b}	93.33±5.77	1.44±0.14^{b}	1.05	763.07±119.70^{b}
	T_{100}	35.27±6.30	509.80±53.63^{a}	474.53±54.69^{a}	90.00±10.00	1.75±0.14^{a}	2.72	1148.65±170.06^{a}
Tilapia	T_0	35.27±5.00	210.53±47.15^{b}	175.27±49.15^{b}	76.67±5.77^{b}	1.16±0.19^{b}	-	404.78±100.72^{b}
	T_{50}	36.47±4.87	313.00±44.09^{a}	276.53±43.54^{a}	90.00±10.10^{ab}	1.40±0.12^{a}	1.05	703.15±110.98^{a}
	T_{100}	33.67±5.39	320.33±60.51^{a}	286.67±61.18^{a}	93.33±5.77^{a}	1.47±0.17^{a}	2.72	747.23±147.49^{a}
Mrigal	T_0	37.00±6.29	221.67±30.25^{b}	184.67±27.13^{b}	86.67±11.55	1.17±0.10	-	240.22±43.52^{b}
	T_{50}	37.78±5.31	288.89±33.08^{a}	251.11±37.11^{a}	93.33±11.55	1.33±0.16	1.05	337.56±56.58^{a}
	T_{100}	38.44±5.15	279.89±74.66^{a}	241.44±77.23^{a}	91.11±10.54	1.28±0.23	2.72	326.64±92.89^{a}
Rohu	T_0	40.44±4.69	245.67±41.66^{b}	205.22±41.12^{b}	80.0±0.00	1.17±0.13^{b}	-	245.67±41.66^{b}
	T_{50}	41.67±3.64	323.11±59.98^{a}	281.44±60.83^{a}	86.67±11.55	1.33±0.14^{a}	1.05	349.17±68.87^{a}
	T_{100}	38.78±4.27	313.00±64.74^{a}	274.22±65.46^{a}	86.67±11.55	1.36±0.16^{a}	2.72	336.50±62.24^{a}

SD = Standard deviation, n = Number, g = Gram, SGR = Specific growth rate, % = Percent, FCR = Food conversion ratio, kg = Kilogram, ha = Hectare. Figures in a column having different superscript in different treatments are significantly different ($p < 0.05$)

But significantly higher ($p< 0.05$) growth and production performance of mrigal and rohu were observed in T_{50} followed by T_{100} and T_0, though T_{50} and T_{100} did not show any significant difference (Table 2). The total production was found to be significantly higher in T_{100} followed by T_{50} and T_0 (Figure 2).

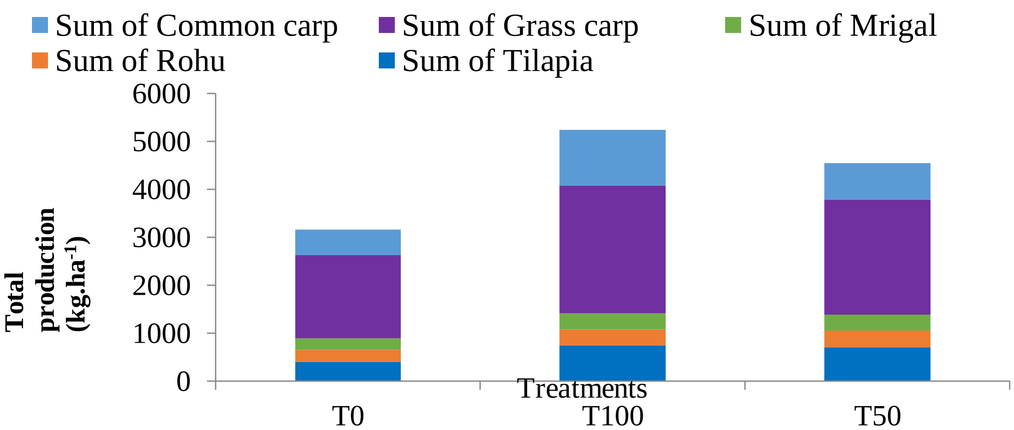

Figure 2. Total production performance (kg ha^{-1}) of cultured fish in three different treatments. In T_0: No commercial feed used, only Asian watergrass as fish feed; in T_{50}: 50% commercial feed and 50% Asian watergrass used as fish feed; in T_{100}: 100% commercial feed applied as control.

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Cost-benefit analysis

The composition of installation cost, operational cost and return from the aquaculture production (considering the market price of the fish), net benefit and benefit-cost ratio (BCR) were considered during calculation of economic efficiency (Table 3).

Table 3. The production performance and economic efficiency of cultured fish in three different treatments **1USD = 80 BDT**

Inputs/items/procedure	Amount (meter/no/kg)	Unit price (USD)	T_0 cost ha^{-1} (USD))	T_{50} cost ha^{-1} (USD)	T_{100} cost ha^{-1} (USD)
A. Structural cost					
i. Knotless net	220 m	0.63	137.50	137.50	137.50
ii. Bamboo	15p	5.00	75.00	75.00	75.00
iii. Hapa	2 p	18.75	75.00	75.00	75.00
iv. Rope	2 kg	4.37	8.75	8.75	8.75
v. Setting cost	20 days	6.25	62.50	62.50	62.50
Total structural cost	-	-	358.75	358.75	358.75
Per year depreciation cost (3 years longevity)	-	-	119.58	119.58	119.58
Structural cost per year	-	-	**119.58**	**119.58**	**119.58**
B. **Operational cost**					
i. Renovation and cleaning	LS	-	62.50	62.50	62.50
ii. Asian watergrass plantation	7 days	6.25	43.75	21.88	-
iii. Amount & cost of fish seeds					
a. Grass carp	7500	0.13	937.50	937.5	937.50
b. Common carp	2500	0.10	250	250	250
c. Tilapia	2500	0.06	156.25	156.25	156.25
d. Mrigal	1250	0.13	156.25	156.25	156.25
e. Rohu	1260	0.13	156.25	156.25	156.25
iv. Amount of feeds (kg ha^{-1})	-	-	-	4799.90	10452.33
v. Fish feed cost	-	0.62	-	2999.94	6532.71
vi. Fertilizers					
a. Urea	625	0.25	156.25	156.25	156.25

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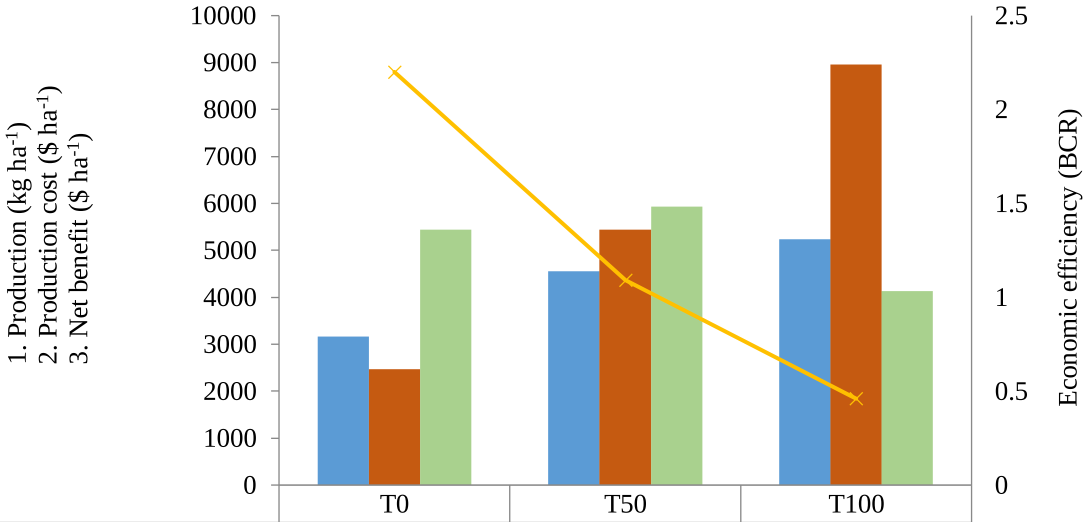
b. TSP	625	0.38	234.38	234.38	234.38
c. MP	312.5	0.63	195.31	195.31	195.31
Total operational cost (i+ii+iii+v+vi)	-	-	**2348.44**	**5326.50**	**8837.39**
Ratio of operational cost			**1.00**	**2.27**	**3.76**
C. Total input cost (A+B)	-	-	**2468.02**	**5446.43**	**8956.98**
Ratio of total production cost			-		
D. Return Total gross revenue (Considering market price of fish)	-	2.50	7904.55	11383.10	13087.60
E. Net benefit ha^{-1} (D-C)	-	-	**5436.53**	**5936.67**	**4130.62**
F. Economic efficiency **Benefit cost ratio (BCR)=E/C**	-	-	**2.20**	**1.09**	**0.46**

The total installation costs were same in all treatments, but the total operational costs were highest in T_{100} (3.76 times higher compare to T_0) and lowest in T_0. The total input cost (installation cost and operational cost) was highest in T_{100} and lowest in T_0. The highest net benefit and benefit-cost ratio (BCR) were found of USD 5936.67 ha^{-1} and 2.20 in T_{50} and T_0 respectively. The production cost was found to be 1.66 times lower and the net benefit was 1.44 times higher in T_{50} in comparison with commercial feed supplied in T_{100} (Figure 3).



	T0	T50	T100
Total fish production (kg·ha-1)	3161.82	4553.24	5235.04
Production cost ($ ha-1)	2468.020833	5446.426219	8956.977083
Net benefit ($ ha-1)	5436.53	5936.67	4130.62
Economic efficiency (BCR)	2.2	1.09	0.46

Figure 3. Relationship among total fish production (kg ha^{-1}), production cost ($ ha^{-1}), net benefit ($ ha^{-1}) and economic efficiency (BCR) in three different treatments

DISCUSSION

Water quality parameters

In the present study, all the accomplished water quality factors were found suitable and within the acceptable range for fish culture (Jena et al., 2007; Rahman et al., 2012; Mamun & Mahamud, 2014; Haque et al., 2015; Hossain et al. 2020b). The water temperature was observed reasonably higher (29.0-32.9ºC) throughout the study period that might be suitable for the growth of both grass and fishes. Sun & Chen (2014) reported that fish growth, food consumption and energy utilization increased with increasing water temperature at 27.0–33.0°C. Salinity was found 1.0 ‰ in all treatments throughout the study period that is supported by Hossain et al. (2021) for the growth of Asian water grass. The total alkalinity and nitrate-nitrogen were found significantly higher in T_0 followed by T_{50} and T_{100} that might be due to the presence of Asian water grass as feed at bottom area of 80% and 40% in T_0 and T_{50} respectively. Comparatively low level of phosphate-phosphorus and ammonia were observed in all treatments that are supported by many other authors (Haque et al., 2015; Chowdhury & Hasan, 2015; Hossain et al, 2020a) that indicate that Asian watergrass has ability to absorb phosphorus compounds from pond water and keep the quality of water in good condition.

Growth and production performance

In the present investigation, grass carp was stocked to consume Asian watergrass directly for nutrient and grow like cattle and fertilized the system by releasing feces, enriching other natural foods including plankton and benthic invertebrates, whereas other species of fishes rely on them. Accordingly, the common carp and mrigal were stocked to feed the defecated materials of grass carp, detritus and the benthic food organisms of the bottom mud. Tilapia was also stocked for omnivorous feeding habit and the ability to feed on the lowest trophic level while planktivorous rohu was stocked for consuming plankton and other available natural food in the pond.

In the present study, the growth (final weight, weight gain, SGR) and production of grass carp, common carp and tilapia were found highest in $T_{100,}$ because of supplying 100% commercial feed. But, mrigal and rohu showed highest growth and production performance applying 50% commercial feed along with 50% Asian watergrass as feed in T_{50} which indicate suitable environmental condition and sufficient preferred food like plankton, benthic fauna, detritus and defecated materials of grass carp for mrigal and rohu was available in that treatment. Moreover, the growth and production performance of all stocked fishes were highly appreciable in T_{50} and T_0 (Table 2) considering the applied feed and cost of commercial feed, because benefit and benefit cost ratio were found higher in T_{50} and T_0 in comparison with T_{100}. Grass carp have herbivorous appetites and consume large quantities of higher aquatic plants and it can be cultured by providing fish edible leaves and whole soft plants either aquatic or terrestrial in the water body without costly artificial feeds (Halver & Hardy, 2002; Pillay, 2004). The Asian watergrass contains 17.49 ± 0.21% of protein in leaves and 12.17 ± 0.65% in roots whereas its carbohydrate content is also high (50.96 ± 0.65% in leaves and 56.97 ± 0.97% in roots), and the whole body of it is very soft, preferred food of grass carp and is very efficient for their higher growth (Hossain et al., 2020b). Feedstuffs with high carbohydrate can preferably be used to activate the protein-sparing effect which makes the feed more cost effective (Hidalgo et al., 1993). Pipalova (2006) reported that the grass carp prefers soft-tissue of aquatic plants, filamentous algae and duckweeds, and consumes all parts of preferred plants. The edible aquatic plants that grow in the water body and used directly as feed by the fishes can be transformed into suitable feed (Edwards et al., 1992; Gavina, 1994). Dibble & Kovalenko (2009) reported that the submerged aquatic plants, algae and pond weeds (*Potamogeton sp.*) are the regular food items eaten by grass carp. *Hydrocharis dubia* floating aquatic plant is used as a good feed for grass carp in China, and it is collected and cultivated for feeding of carp fingerlings (Hasan and Rina, 2009). Many authors reported the grass carp as a good species for aquaculture considering its fast growth and herbivorous feeding habit (Pipalova, 2006; Dibble & Kovalenko, 2009; Hossain et al., 2020a) that strongly supported our findings. The omnivorous common carp is a bottom living fish and mainly feeds the benthic fauna and decaying floral matter. Its growth generally depends upon the availability of benthic macro-invertebrates, quality and quantity of added supplemental feed and its own stocking density. Common carp exposes exceptional growth rate, omnivorous feeding habit and easy adaptation to diversified feeds. The common carp was found as a suitable species in the polyculture system with grass carp using Asian watergrass as feed (Hossain et al., 2020b). Many studies reported that common carp cultured alone or in combination with other fish species provided high growth, production performance and economic benefit

(Ibrahim, 2011; Noman et al., 2011; Abbas et al., 2014; Khan et al., 2016) that strongly supported our findings. Mrigal is also a bottom dweller fish and prefers to feed on detritus and benthic fauna. In the present study, mrigal and rohu showed highest growth and production performance using 50% commercial feed along with 50% grass as supplementary feed indicates that these species are suitable as co-species in pond polyculture of grass carp using grass as supplementary fish feed.

The growth and production of tilapia was reasonably excellent in T_{50} considering the feed because similar growth and production was found in T_{100} providing 100% commercial feed. In natural condition tilapia consume large quantity of plant materials dominated by detritus, algae and the associated bacteria (Getachew, 1987; Diana et al., 1991). Hossain et al. (2020b) reported that tilapia can be a suitable species for polyculture with grass carp using Asian watergrass as feed. Tilapia can tolerate higher dietary fiber and carbohydrate concentrations than many other cultured species of fishes (El-Sayed & Teshima, 1992). The utilization of Asian watergrass directly as feed in polyculture in the present study is coherent with the findings of Santiago et al. (1988), Wahab et al. (2001), Uddin et al. (2007), Chowdhury et al. (2008) and Agbo et al. (2011), who used fresh duckweed as feed for tilapia and silver barb. Tilapia farmers should consider the alternative dietary sources to reduce production cost because replacement of fishmeal in the supplemental diet of tilapia is experimentally proved and would be economic (Ogello et al., 2014), and our findings may provide valuable information for using aquatic plant directly as supplementary fish feed as well as in the formulation of aqua feed.

Generally in the aquaculture system, huge amount of feeds and fertilizers are used all over the world including Bangladesh and uneaten portion of applying feeds and biomass metabolic wastes release nutrients in water and sediments through microbial decomposition which create water body hyper-nutrified (Hossain et al. 2005). These excess nutrients in the water body create phytoplankton blooms and cause mass mortality of cultured fish in some tropical countries. Asian watergrass is very interesting source of supplementary fish feed and it has capacity to improve the water quality by absorbing excess nutrients with their effective root system. At the same time, they actively contribute to the promotion and maintenance of food webs and service in freshwater ecosystems (Scheffer & Jeppesen 2007; Smith 2011). Many authors reported that aquatic plants are important source of fish feed and have positive effects for the improvement of water quality and remediation of aquaculture effluents (Sipauba-Tavares et al. 2002; Henry-Silva & Camargo 2006; Carlozzi & Padovani 2016) that strongly supported our findings (Table 1). These two eco-services are beneficial for increasing the sustainability of small-scale fish farming all over the world.

Cost-benefit analysis

To reduce the fish feed cost in aquaculture system, we designed a technique to develop pond aquaculture using Asian watergass as supplementary fish feed. In the present study, the highest net benefit was found in T_{50} (USD 5936.67) followed by T_0 (USD 5436.53) and T_{100} (USD 4130.62) those were supported by many authors (Osman et al., 1996; Rahman et al., 2014; Saokat *et al.*, 2017; Ali et al., 2018; Hossain et al., 2018; Samad & Imteazzaman, 2019; Hossain et al., 2020a; 2020b). Although, the total fish production was found highest in T_{100} with highest production cost, but the fish production cost reduced 1.66 times applying 50% Asian watergrass as supplementary feed along with 50% commercial feed in T_{50} compare to T_{100}. These findings indicate that applying of commercial feed is the prime issue for higher production cost and less benefit in aquaculture system (Figure 3). Utilization of commercial feed in aquaculture system is inversely related to the economic benefit of the farmers. Therefore, the economic benefit depends on how much amount of commercial feed deduction is possible replacing by low-cost supplementary feed in the system. The local farmers should be encouraged to apply this technique in aquaculture system to get more benefit that enhance their livelihood and increase the total fish production of the country.

CONCLUSION

Polyculture is very important in contrast of developing countries like Bangladesh to increase the fish production of the rural poor levels that contribute the blue economy of the country, but high fish feed cost is the main obstacle for sustainable aquaculture development. Moreover, suitable fish species selection is very important, which assemble the farmers more benefited from their efforts and enhance their livelihood. Considering the fish feed cost, Asian watergrass utilization in the polyculture system is more practical to produce good quality fish

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with the maintenance of friendly environment. The present findings indicate that 100% Asian watergrass is more cost effective, but increasing the amount of total fish production and net benefit, 50% commercial feed along with 50% Asian watergrass as supplementary feed may be more congenial for getting more benefit with minimal cost. To improve the productivity with friendly environment and getting more benefit, this technique should be developed in haor, baor, beels, canals, and other water logged areas affected by climate changes. More research requires on using of this grass as supplementary fish feed and uses as formulated feed may enrich the feed industry.

Ethical Statement

Not applicable.

Author Contribution

Md. Moazzem Hossain: conceived, designed, performed the experiments, analyzed data, and wrote the manuscript.

Md. Amanullah parosh: performed the experiments, data collection and input.

Afiya Jahan: assisted in data analysis and drafting the manuscript.

Md. Shahjahan: assisted in the experimental design, edited the manuscript.

Saleha Khan: edited the manuscript.

Newton Saha: analyzed and interpreted the data; wrote the paper.

All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional or non-professional conflict that would have appeared to influence the work reported in this paper.

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