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Investigations on growth performance of grass carp *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844) under forage crops and aquatic macrophyte feeding regimes

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Abstract

The objective of the study was to assess the growth, survival, and production performance of grass carp *Ctenopharyngodon Idella* (Cuvier and Valenciennes, 1844) under different feeding regimes of forage crops and aquatic macrophytes. In the present experiment, 650 fingerlings (average length, 11.13±0.29 cm and average weight, 13.89±0.81 g) of grass carp were stocked separately in two earthen ponds (40 x 25 x 2.0 m). Fishes in pond one (T1) were fed with forage crops, chopped Napier grass (*Pennisetum purpureum*) from June to November 2017 and chopped Berseem (*Trifolium alexandrinum*) from January to May 2018. Similarly, fishes in pond 2 (T2) were fed with Azolla (*Azolla pinnata*) and Lemna (*Lemna minor*) during the period June to November 2017 and January to May 2018, respectively. Linear trend analysis for the length of grass carp showed a significant ($p<0.05$) increase in size in both treatments (T1 and T2) and r^2 was found to be 0.94 and 0.95, while increasing trend of weight of grass carp showed significantly ($p<0.05$) high r^2 for T1 (0.88) and T2 (0.84). It is observed that the application of forage (T1) and aquatic macrophytes (T2) had no significant ($p>0.05$) effect on the water quality parameters of each pond. The overall specific growth rate, average daily weight gain and feed conversion ratio of grass carp were significantly ($p<0.05$) higher in T1 compared to T2, while the survival rate was significantly ($p<0.05$) high in T2.

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Introduction

Fish growth and its attainment of market size within the shortest possible time depend on a high-quality, nutritionally balanced diet (Gabriel *et al*., 2007). The adoption of semi-intensive and intensive fish culture practices for enhancing fish production depends on the availability and quality of supplementary feed (Azim and Wahab, 2003) and the common supplementary feedstuffs in India are rice bran, wheat bran, oil cake and some other agricultural wastes (Bhaskar *et al*., 2015). In aquaculture, fish feed accounts for 50-70% of the total costs based on types of culture systems, feeding intensity and cost of feed ingredients (Henry *et al*., 2015; Daniel, 2018; Hossain *et al*., 2020). The increase in cost and demand of conventional protein sources such as fish meal, fish oil and soybean meal forced poor fish farmers in developing countries to look for cheap and locally available ingredients for fish diets (Mosha, 2018). Furthermore, due to intense competition for agricultural wastes among livestock farmers, aquaculturists and biofuel producers, possibilities of utilising alternative locally available sources like plant leaves and aquatic vascular plants either directly for use in fish feed (Azim and Wahab, 2003) or as a substrate to develop biofilm have been increasing (Mridula *et al*., 2003; Pandey *et al*., 2014; Bharti *et al*., 2016). Supplementing plant ingredients to the fish diet also promoted growth performance by reducing the high cost of fishmeal and fish oil (Hossain *et al*., 2020). Duckweed (*Lemna minor*) is a natural protein source with high-quality essential amino acids and closely resembles animal proteins (Aslam and Zuberi, 2017). It is also used as a preferred feed item for fish in polyculture with grass carp (Talukdar *et al*., 2012). A meagre amount of fibre in Duckweed leaves and a small quantity of lignin in its cell wall make a palatable food for herbivorous fish (Aslam and Zuberi, 2017). Azolla (*Azolla pinnata*) is rich in protein, fat and its essential amino acids are superior to those in wheat bran and maize (Basak *et al*., 2002; Cherryl *et al*., 2014; Das *et al*., 2018). The presence of Azolla in fish diets reduces the fat content in fish muscles (Datta, 2011). A study suggested that Berseem (*Trifolium alexandrinum*) leaf meal and leaf protein concentrate could be incorporated into aqua-feed because of their respective crude protein content of 23.98 and 41.67% (Singh *et al.,* 2019). It has been found that a mixture of compound feed and Napier grass promotes the growth of grass carp and controls the development of fatty livers (Yu *et al*., 2018). The grass carp *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1844) is a herbivore cyprinid fish that feeds voraciously on aquatic weeds (Dasgupta, 2009). It is native to the Amur River in eastern Asia (Hossain *et al*., 2020). It was introduced to India in 1959 to control weeds in natural water bodies. However, it became an important species in composite fish culture (Singh and Lakra, 2011) due to its fast growth and compatibility with other carp for food and space (Hemlata *et al*., 2016). Grass carp can survive on various green grasses (Shrestha and Yadav, 1998) and aquatic vegetation such as *Azolla* sp. and *Lemna* sp. (Majhi *et al*., 2006; Ferdoushi *et al*., 2008) can be cultured without supplemental feed (Halver and Hardy, 2002). The ability to utilise macro vegetation and regular supplies of weeds to feed the cultured grass carp may act as complementary feed and could help to lower input cost (Hemlata *et al.,* 2016). In the present study, four macro vegetation such as Azolla (*A. pinnata*), Lemna (*L. minor*), Berseem (*T. alexandrinum*), and Napier grass (*Pennisetum purpureum),* were used directly as fish feed. Azolla and Lemna are aquatic macrophytes, and Berseem and Napier grass are forage (terrestrial) crops. In the present experiment, an attempt was made to record the comparative performance of forage plants *vs.* aquatic macrophytes on the growth performance of grass carp in a pond culture system.

Materials and methods

Experimental fish and design

Fingerlings of grass carp were obtained from the fish hatchery of ICAR-Research Complex for Eastern Region (ICAR-RCER), Patna. Before starting the experiment, collected fingerlings were acclimatised in circular Fiber Reinforced Plastic (FRP) tanks for seven days. During acclimatisation, the fish were fed once daily at 5% of their body weight with a balanced formulated feed (22% protein). The experiment was conducted in two earthen ponds with 40 x 25 x 2.0-m dimensions. For pre-stocking pond management, both ponds were sun-dried for a few days, filled with groundwater and fertilised with agricultural grade urea, single super phosphate and potassium sulphate (Azim *et al*., 2001). Each pond was stocked with 650 fingerlings (11.13±0.29 cm and 13.89±0.81 g). Fish in one pond (T1) were fed with chopped Napier grass from June to November 2017 and the same fish were fed chopped Berseem from January to May 2018. Similarly, fish from pond 2 (T2) were fed with Azolla and Lemna from June to November 2017 and January to May 2018, respectively. The feeding was performed daily in the morning, and the feeding rate and quantity are given in Table 1.

Source of plants

The ICAR-RCER, Patna has a large herd of livestock population and to feed them, seasonal forage crops like Berseem and Napier grass are grown in the fodder field regularly and fresh fodder was collected for feeding grass carp. Similarly, Lemna was grown naturally in the institute's wallowing tank (81 m^2). Daily, a sufficient quantity of Lemna was harvested from the wallowing tank through a hand net, washed in running freshwater, hung in a hand net (10-15 min) to remove excess moisture and then applied in the fish pond to feed grass carp. Azolla was grown in 7 cement tanks (5 x 4 x 0.2 m). Each tank's bed was prepared with 10 cm of soil, 6 kg of cow dung was applied and the tank was filled with groundwater. Each tank was fertilised with 30 g of single super phosphate and inoculated with 100 g of Azolla. After ten to fifteen days of growing, the daily required quantities of Azolla were harvested from the tanks, hung in a hand net for 10 to 15 min and then applied to the fish pond.

Growth parameters

Growth parameters were calculated on a monthly basis to ascertain survival, weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) of the fish using the following formulae:

$$\text{Survival (\%)} = \frac{\text{No. of fish survived at the end of the experiment}}{\text{No. of fish stocked}} \times 100$$

Table 1. Feeding rate and quantity of feed given from June 2017 to May 2018

Month	Feeding rate (%)	Feed			
		T_1 (kg day^{-1})		T_2 (kg day^{-1})	
		Hybrid Napier grass	Berseem grass	Azolla	Lemna
June 2017	10	2.15	0	1.57	0
July	10	4.17	0	3.02	0
August	10	7.03	0	6.51	0
September	10	10.27	0	8.62	0
October	08	12.26	0	10.91	0
November	08	15.72	0	11.62	0
January 2018	05	0	5.64	0	4.85
February	06	0	9.79	0	8.35
March	06	0	21.33	0	13.93
April	08	0	32.08	0	22.05
May	10	0	41.23	0	28.87

$$\text{Weight gain (\%)} = \frac{\text{(Final weight - Initial weight)}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\text{(Ln Final weight - Ln initial weight)}}{\text{Experimental days}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed by the fish}}{\text{(Final weight of fish - Initial weight of fish)}} \times 100$$

Water quality parameters

Water quality parameters were recorded during morning hours at monthly intervals. A portable digital instrument (Eutech Instruments, India) was used to measure water temperature, pH and dissolved oxygen (DO) content in water samples. Parameters such as alkalinity and hardness were estimated by titrimetric method and nitrite, nitrate and ammonia were estimated using Kit (Spetroquant, Merck KGaA).

Digestibility study

For the digestibility study, eight grass carps (Initial weight, 9.91±0.36 g) each were stocked in aquarium tanks (0.9 x 0.3 x 0.3 m) in triplicate and 40 l of water was maintained throughout the experiment. Fish were fed on the grasses as mentioned above in the early morning (09.00 hrs). Feeding was done at 5% of the body weight, with dried and powdered materials and the faecal matter was collected each evening by siphoning and dried at 60^{0}C in a hot air oven and used for analysis.

Proximate composition of feed

Proximate compositions of Berseem, Napier grass, Azolla, Duckweed and faecal matter were analysed as per AOAC (1995). Protein was determined by measuring nitrogen using the Kjeldahl method; Crude fat was determined using petroleum ether (40-60°C boiling point) extraction method with Soxhlet apparatus and ash by combustion at 550°C (AOAC,1995).

Statistical analysis

All statistical analyses were carried out with the help of software R 3.4.4 for Windows (R Core Team, 2013). Results were analysed using the student's t-test to determine significant differences. Linear regression analysis was performed to know and compare the growth performance of grass carp. Pearson correlation was used to measure the relationship between all water quality parameters and fish growth.

Results

Water quality parameters

Table 2 shows the range and average values for pH, temperature, dissolved oxygen (DO), alkalinity, hardness, ammonia, nitrite, phosphate, gross primary productivity (GPP), net primary productivity (NPP) and respiration quotient (RQ). Both the treatments had no statistically significant ($p>0.05$) differences in the above-mentioned water quality parameters.

Growth performance of fish

Linear trend analysis (LTA) for the length and weight of grass carp under treatments T1 (Napier and Berseem grass) and T2 (Azolla and Lemna) are illustrated in Fig. 1a and b and the weight gain against the time curve of this species is illustrated in Fig. 2. The LTA for the length of grass carp showed a significant ($p<0.05$) increase in size in both treatments and r^2 was found to be 0.94 and 0.95 for T1 and T2, respectively (Fig. 1a). The increasing trend of weight of grass carp showed significantly ($p<0.05$) high r^2 for T1 (0.88) and T2 (0.84) (Fig. 1b). Overall weight gain of grass carp was slightly higher in T1 than T2 from 93rd day onwards (Fig. 2).

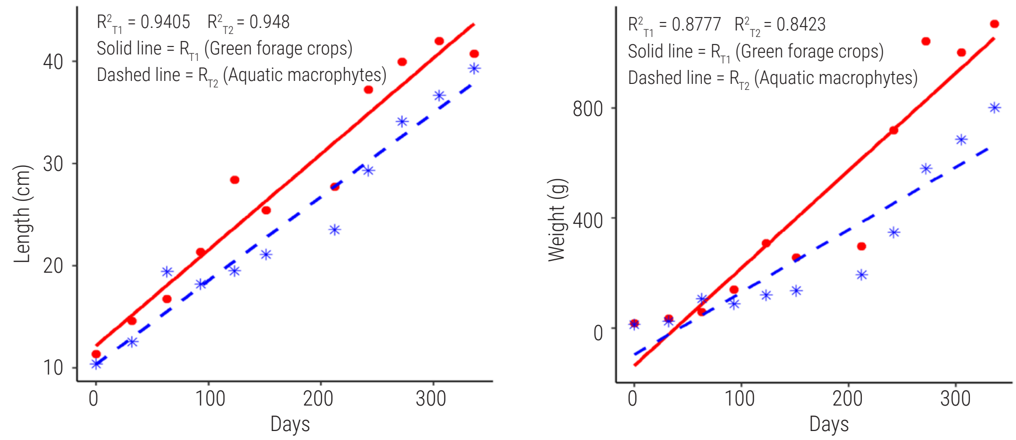
In contrast to weight gain, SGR of grass carp showed a decreasing trend during the study period for both treatments and SGR was consistently higher for T_1 from the 93rd day onwards than T_2 (Fig. 3), however, the rate was very slow compared to the SGR achieved till 93 days of rearing.

The overall specific growth rate (SGR%), average daily weight gain (ADG), feed conversion ratio (FCR), average fish body weight, harvested biomass and estimated fish production were significantly ($p<0.05$) higher in treatment T1, while the survival rate was significantly ($p<0.05$) high in treatment T2 (Table 3).

Table 2. Water quality parameters of different experimental treatments

Parameters	T_1		T_2		p-Value
	Range	Mean	Range	Mean	
pH	6.99-7.72	7.32	7.01-7.64	7.38	0.5693
Temperature (^{0}C)	26.9-36.6	32.4	24.9-36.7	31.8	0.7117
Dissolved oxygen (mg l^{-1})	02-6.1	4.1	2.4-6.2	4.5	0.5537
Alkalinity ($CaCO_3$ mg l^{-1})	110-135	121.9	104-136	121.9	1.00
Hardness ($CaCO_3$ mg l^{-1})	162-188	174.3	165-181	175.0	0.8224
Ammonia (mg l^{-1})	0.32-0.55	0.44	0.27-0.59	0.43	0.9476
Nitrite (mg l^{-1})	0.01-0.05	0.014	0.01-0.02	0.011	0.4833
Phosphate (mg l^{-1})	0.15-0.39	0.27	0.14-0.39	0.29	0.536
Gross primary productivity (g C m^{-3} h^{-1})	0.042-0.280	0.140	0.031-0.188	0.124	0.6745
Net primary productivity (g C m^{-3} h^{-1})	0.029-0.176	0.08	0.021-0.799	0.148	0.4131
Respiratory quotient (g C m^{-3} h^{-1})	0.010-0.104	0.058	0.018-0.399	0.091	0.4105

T_1: Fish fed with Napier and Berseem grass; T_2: Fish fed with Azolla and Lemna



(a) (b)

Fig. 1. Linear trend analysis of length (a) with p-value for R^2_{T1} and R^2_{T2} as 0.0000005049 and 0.0000002732 and weight (b) with p-value for R^2_{T1} and R^2_{T2} as 0.00001321 and 0.00004241 for grass carp fed with forage crops and aquatic macrophytes

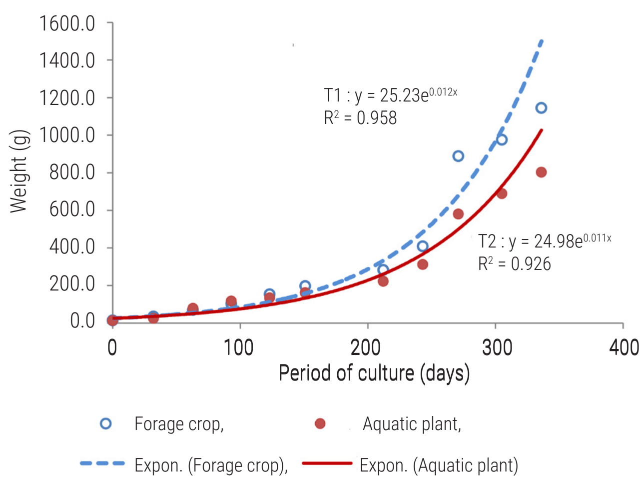


Fig. 2. Weight gain of grass carp, fed with forage crops and aquatic macrophytes

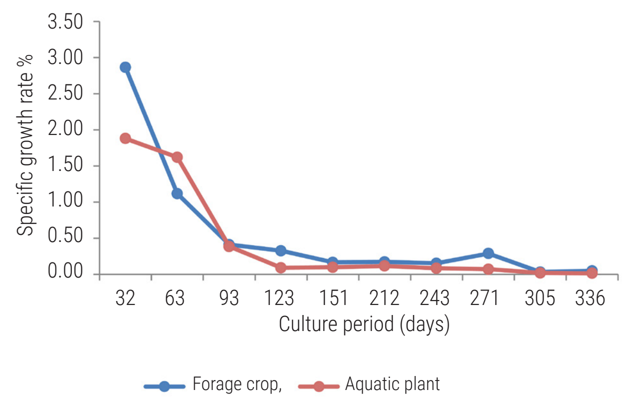


Fig. 3. Specific growth rate of grass carp fed with forage crops and aquatic macrophytes

Table 3. Growth parameters and survival rate of grass carp fed with forage crops and aquatic macrophytes

Treatment	Initial weight	Final body weight (g)	Survival rate (%)	SGR%	ADG	FCR	Harvested biomass (kg)	Estimated net production (t ha^{-1})
T_1	13.89±0.81	1145.23±87.10	54.00	1.31	3.37	12.21	401.98	6.18
T_2	13.11±1.70	801.88±33.68	63.00	0.37	2.35	11.52	327.96	5.04
p-value	0.687	0.001	0.0004	0.041	0.040	0.009	0.047	0.005

T_1 : Fish fed with Napier and Berseem; T_2 : Fish fed with Azolla and Lemna

Chemical composition of grass and macrophytes

The chemical composition of green grass and aquatic macrophytes are depicted in Table 4. The crude protein (CP) content of Hybrid Napier (non-leguminous) was 7.29±0.19, while Berseem grass, a leguminous plant, had 18.27±0.23% protein on dry matter (DM) basis. The protein content of aquatic macrophytes varied from 22 to 28%, with the highest content in Lemna. Ether extract (EE) content in forage grasses ranged from 1.2 to 2.18%, while in aquatic macrophytes, it was more than 4%. Similarly, both the grasses contained higher crude fibre (CF) and organic matter (OM) than the aquatic macrophytes. Between two aquatic macrophytes, Lemna contained higher CP, EE, OM and energy but lower CF than Azolla. The gross energy (GE) content of grasses and macrophytes varied from 16000 to 17000 J g^{-1}.

DM intake and digestibility of nutrients

From Table 5, it is evident that the DM intake (DMI) of grass carp varied significantly (p<0.05) depending on the species of grass or macrophyte. The highest DMI (g^{-1} d^{-1} replicate^{-1}) was recorded in Lemna fed group (43.74±0.84 g d^{-1}) followed by Berseem

Table 4. Proximate composition of grass and macrophytes (% dry weight basis)

Macrophyte	CP	EE	CF	OM	GE (J g^{-1})
Napier	7.29±0.19	2.18±0.08	25.22±0.27	88.27±0.21	16356±196.32
Berseem	18.27±0.23	1.26±0.06	17.55±0.27	90.62±0.27	16177±83.61
Azolla	22.44±0.24	4.23±0.20	14.51±0.35	82.20±0.21	16093±114.09
Lemna	28.26±0.68	4.86±0.25	9.74±0.41	85.69±0.52	17029±134.23

Table 5. Digestibility of grass carp fed with forage crops and aquatic macrophytes

Feeding	DMD (%)	OMD (%)	CPD (%)	CFD (%)	DMI (g day^{-1} $fish^{-1}$)	GEI (MJ d^{-1})	CPI (g)
Napier	49.81^{c}±0.92	47.96^{d}±0.95	52.46^{c}±0.93	29.06^{bc}±1.31	24.47^{c}±0.35	3.67^{c}±0.05	1.78^{c}±0.03
Berseem	57.68^{b}±0.36	57.92^{c}±0.37	74.30^{a}±0.22	34.48^{b}±0.60	34.39^{b}±0.87	4.57^{b}±0.12	6.28^{a}±0.16
Azolla	61.97^{b}±1.54	63.69^{b}±0.59	64.22^{b}±0.61	26.46^{c}±1.16	26.41^{c}±0.33	4.19^{b}±0.05	5.92^{a}±0.07
Lemna	73.27^{a}±0.85	72.99^{a}±0.86	76.52^{a}±0.79	52.66^{a}±1.52	43.74^{a}±0.84	7.10^{a}±0.14	12.36^{b}±0.06

Means with different superscripts in a column differ significantly (p<0.05). (DMD: Dry matter digestibility, OMD: Organic matter digestibility, CPD: Crude protein digestibility, CFD: Crude fiber digestibility, DMI: Dry matter intake, GEI: Gross energy intake, CPI: Crude protein intake)

(34.39±0.87 g d^{-1}). However, the lowest DMI was recorded in Napier (24.47±0.35 g d^{-1}) and Azolla (26.46±1.16 g d^{-1}) fed group. The DMI was non-significant (p<0.05) between the Napier and Azolla fed groups. Similarly, crude protein intake (CPI) was found significantly higher(p<0.05) in grass carp fed Lemna (12.36±0.06 g d^{-1}) followed by Berseem (6.28±0.16 g d^{-1}) and Azolla (5.92±0.07 g d^{-1}). The lowest CPI (g d^{-1}) in fish was observed in Napier fed group. Similar trend was observed in gross energy intake (GEI). The highest GEI was observed in Lemna fed (7.44±0.14 MJ d^{-1}) and the lowest in Napier fed groups (3.67±0.05 MJ d^{-1}). Similar to CPI, GEI in Berseem and Azolla fed groups did not differ significantly.

In respect of the digestibility of nutrients, it was observed that DM digestibility (DMD) varied significantly (p<0.05) among the groups. Significantly high DMD was observed in Lemna fed groups followed by Azolla and Berseem fed groups. There was no significant difference between Azolla and Berseem fed groups. The organic matter digestibility (OMD) was found significantly higher in Lemna fed group (p<0.05) followed by Azolla and lowest in Napier fed groups. However, crude protein digestibility (CPD) was significantly (p<0.05) highest in Lemna fed group followed by Berseem fed groups. In respect of crude fibre digestibility (CFD), it was observed that Lemna fed group showed significantly higher value (52.66±0.84%) followed by Berseem (34.39±0.87%) and Azolla (26.41±0.33%).

Discussion

The present study attempted to record the performance of grass carp reared in earthen ponds and fed with forage crops *vs.* aquatic macrophytes. Water quality parameters estimated from both treatments were not significantly different (p<0.05). Similarly, both treatments did not substantially alter GPP, NPP and RQ (p<0.05). Hence, the application of forage (T1) and aquatic macrophytes (T2) had no significant effect on the fluctuations in the water quality. Das *et al*. (2004) suggested a desirable range of a few water quality parameters for carp production and comparable ranges were also observed in the present study. In the current investigation, total ammonia and nitrite, typically produced by the deposition of fish waste and the mineralisation of organic manure, were likewise and within the permitted range. Though, the average value of DO level in both treatments was above 4 ppm but relatively lower than the permissible limit (>5 ppm) for aquaculture purposes. It has been reported that endogenous sources of nutrients, such as residual feeds, faeces and other organic waste, are deposited at the bottom and re-released into the water due to environmental biodegradation may influence the DO level in the fish pond (Lazzari and Baldisserotto, 2008). Fresh forage and aquatic macrophytes used as feed ingredients for culturing grass carp might have increased residual feeds and faeces deposited at the bottom and this may be one of the possible reasons for the comparatively lower DO in both treatments.

In the present study, forage crops (Napier and Berseem) and aquatic macrophytes (Azolla and Lemna) have significantly increased the length and weight of grass carp in both treatments. It indicates that all those plants can be used as feed or feed supplements for grass carp, which will considerably reduce the cost of production. The main reason for an increase in length and weight is that these plants are rich in protein sources (Kabir *et al*., 2005; Or and Joy, 2018; Khursheed *et al*., 2019; Singh *et al*., 2019). A continuously increasing weight gain in grass carp is also supported by Khan *et al*. (2004), where they have observed that weight gain in grass carp increased with an increase in dietary protein by up to 35%. A further increase in dietary protein reduces weight gain due to reduced dietary energy available for growth, as extra energy is required to excrete excess amino acids (Drummen *et al*., 2018). The present result also revealed that fish survival is not much affected by these sets of plants as a feed material for grass carp. However, a slightly higher survival rate was recorded in T2, but the overall survival rate in both treatments was similar to Das *et al*. (2004), with a 52.5-58.0% range. Marginal variations in the survival rate of grass carp may be attributed to low DO in the experimental ponds. In the present study, SGR was also progressively decreasing in both treatments with increase in the culture period, which was similar to the observations made by Venkatachalam *et al.* (2018). The response of SGR in fish depends on various factors like feeding rate (Mizanur *et al*., 2014) and nutritional quality (Taipale *et al*., 2022). Tan *et al*. (2013) demonstrated that an overload of rapeseed meal in the diet would depress SGR in grass carp. Moreover, initially, the feeding rate was 10%, when the SGR of grass carp was found increasing, but later, the SGR was found to show a decreasing trend when the feeding rate was decreased as mentioned in Table 1.

The decrease in SGR could also be attributed to lower feeding rate, mainly due to the reduced availability of required nutrients for grass carp. Singh *et al*. (2019) observed that though Berseem leaf meal is a potential resource for the aqua feed industry, it has lower nutritional quality than Berseem leaf protein concentrate for preparing fish feed. According to Zolfinejad *et al*. (2017), growth parameters of grass carp, including relative growth rate, fish biomass and weight gain, were higher for plants with higher palatability and nutritional quality than other plants tested for use as fish feed. They also opined that due to the higher palatability and nutritional quality, *L. minor* promotes growth of grass carp and helps to reduce application of artificially formulated feed in aquaculture (Zolfinejad *et al*., 2017). In the present study, CP, CF and OM content values in the Napier grass were similar to those reported by NDDB (2012) as well as Venkatesh and Shetty (1978). However, the gross energy value was higher than the value (1.9 M cal kg^{-1}) reported by NDDB (2012) which could be attributed to different geographical locations, the variety used, the fertiliser applied and the stage of maturity at the time of cutting. Similarly, CP, EE and OM content of Berseem grass corroborated with the values reported by NDDB (2012) and Dey *et al*. (2014); however, GE content was higher than the value (2.3 Mcal kg^{-1}) reported by NDDB (2012). In the case of Azolla, values of CP, EE, CF and OM were similar to those reported by NDDB (2012). CP, EE and OM values in Lemna were higher than those reported by Yilmaz *et al*. (2004). However, the values were similar to those reported by Hasan and Edwards (1992) as well as Bairagi *et al*. (2002). Van Dyke and Sutton (1977) reported that apparent digestibility of duckweed for crude protein was 80%, and for gross energy was 61%. The CP digestibility of 76.52%, estimated for duckweed, in the current study is nearly identical to that reported by Van Dyke and Sutton (1977). In grass carp, Gilca (2010) reported fodder digestibility as 92, 91, 60 and 91% respectively for CP, EE, CF and OM. In the current investigation, the corresponding values were found lower. It may be attributed to the species and variety of forage crops used, stage of maturity, fish species, fish age and variation in DM intake by fish. Rajadevan and Schramm (1989) reported the digestibility of Kikuyu grass in grass carp as 66% for crude protein and 36% for crude fibre, similar to the observations in the present study.

The preliminary results indicate that forage crops and aquatic macrophytes support the growth and survival of grass carp. However, despite a comparatively low survival rate and high FCR, the finding of this study illustrates that aquaculture of grass carp with Napier and Berseem grass at 10% of feeding rate delivers better production than similar use of Azolla and Lemna. However, more systematic study may be required to identify the performance of individual components and supplementation of other potential terrestrial plants in fish feed preparation.

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