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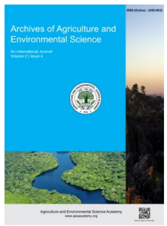

ORIGINAL RESEARCH ARTICLE

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Optimization of dietary protein level for good aquaculture practice based carp fattening in ponds under drought prone area of Bangladesh

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ABSTRACT

Good aquaculture practice (GAP) based carp fattening is a potential technique to obtain higher and safe fish production within shorter period in ponds of drought prone area. Sustainability of this technique, however, is constrained by high feed cost and poor water quality. Therefore, as an overcoming effort, three diets (protein content of 20%, 25% and 30%) under three different treatments (T_1, T_2 and T_3) were tested during January-June, 2020 in fattening ponds of carps (Catla, *Gibelion catla;* silver carp, *Hypophthalmichthys molitrix;* rohu, *Labeo rohita*; mrigal, *Cirrhinus cirrhosis*; and carpio, *Cyprinus carpio var. specularis*) under Rajshahi district, Bangladesh. Variation in protein level had no significant effect on environmental parameters of pond water. Combined fish yield was found to vary significantly (*P<0.05*) among the treatments, while feed conversion ratio did not vary significantly. Although second degree polynomial regression analysis identified 28.50% dietary protein for optimal growth of carps but no significant difference between T_2 and T_3 was found for the total fish yield. However, significantly (*P<0.05*) highest cost-benefit ratio obtained with the diet containing 25% protein suggested this protein level in diet was profitable for carp fattening in pond.

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INTRODUCTION

Importance of fattening technique under good aquaculture practices (GAP) to promote safe carp production within shorter culture period is well documented (Hossain *et al.*, 2020). Carp contributes 52.06% of the total pond production in Bangladesh (FRSS, 2020). Aquaculture, especially carp culture, is essential for supplying nutrition, income and employment in Bangladesh (Mohsin *et al.*, 2012). It has the advantages of easy access and developed market demands over the other aquaculture practices. Moreover, low cost fish production through carp culture can contribute significantly to food security in developing countries (Mataka and Kangombe, 2007). Although aquaculture has grown significantly over the years in Bangladesh, its full potential is yet to be realized (Jahan *et al.*, 2010), and the major constraints in sustainable carp production in Bangladesh is feed cost, as it accounts for more than 40-60% of total fish production cost (Craig and Helfrich, 2002). The main goal of supplementary feeding in aquaculture is to triumph maximum protein deposition and growth with least input and feed. Therefore, efficient management of feeding can play an important role in successful culture of carps in ponds (Liu *et al.*, 2011). Insufficient supply and high production cost of fish feed hinders the sustainability in aquaculture production. Success in commercial aquaculture is solely depending on the diet which can provide essential nutrients for efficient digestion and optimal growth in fish (Mokolensang *et al.*, 2003). Essential macro nutrients are also needed in large quantities to meet the energy balance and as a growth material for fish (Abowei and Ekubo, 2011). Protein is one of the most important macro nutrients and in commercial feed; protein determines the overall production cost of fish feed. Protein requirements of fish are varied depending on food habit and types of species (Goddard, 1996). Protein deficient diet can hinders fish growth and fish become vulnerable to utilize its own body proteins to meet the energy demands, which cause a reduction in net profit from aquaculture. Metabolic process and tissue repair in fish require regular intake of necessary protein feed (Deng *et al.*, 2011; Singh *et al.*, 2008; Sayed *et al.*, 2020). On the other hand, excess protein in the diet cannot be utilized properly as because only part of it would be used to make new proteins while the excess become engaged in the direction of amino acid's deamination and results in ammonia production (Wilson, 2002). The excess protein in diet also limits protein deposition or retention of the amino acids in muscles and forces the deamination and catabolism of amino acids (National Research Council, 2011). As protein is not only the most important nutrient in fish growth but also the major factor determining the production cost of fish feed, determination of optimal protein level in diet can help to reduce the operational feed costs and maximize the feeding efficiency by fish (Yadav *et al.*, 2019; Ahmad *et al.*, 2008). Quality of nutritional composition, ingredients and the formulation process of the diets affect the protein level in feed (Siddiqui and Khan, 2009). Protein is the most costly ingredient in fish feed and a factor which determines the overall production cost of supplementary feed. Therefore, it is essential to determine the optimal nutrient requirement of the targeted fish species to get desirable fish growth and to reduce feed production cost. The studies about feeding the fish with the required and balanced protein have been widely reported (Yadav *et al.*, 2019; Jayant *et al.*, 2018; Mansour *et al.*, 2017; Ahmed and Maqbool, 2017; Suharmili *et al.*, 2015; Gandotra *et al.*, 2014; Al-Saraji and Nasir, 2013; Sardar *et al.*, 2011). There are also evidences to produce safe fish through maintaining good aquaculture practices (GAP) (Hossain *et al.*, 2020; Mondal *et al.*, 2013), unfortunately there are no comprehensive studies about protein requirement by carps under GAP based fattening in ponds. Thus, the present study aims at determining the best protein level in diet for the growth, production and economics of carp fattening in polyculture pond. The specific objectives included in this study were to monitor the important water quality parameters; to assess the GAP aspects (in terms of determining the heavy metals and pathogenic microbial load in pond environment); to evaluate the growth, yield and economics of carp fattening using varied levels of dietary protein levels; and finally to recommend suitable protein level for carp fattening.

MATERIALS AND METHODS

Location and duration of the study

The present experiment was conduction for a period of 6 months from January to June, 2020 in ponds (Mean area and depth of 1.38±0.01 ha, 1.61±0.01 m, respectively) of Paba upazila (Sub-district) under Rajshahi district, Bangladesh (Figure 1). Experimental ponds (latitude between 24°27.080´´ and 24° 23.611´´ E and longitude between 88°32.556´´ and 88°40.973´´; and elevation between 09 to 60 m) were well exposed to sunlight and facilitated well through inlets and outlets for water drainage. Embankments of the ponds were high enough to protect the run off.

Experimental design

Three diets (protein content of 20%, 25% and 30%) under three different treatments (T_1, T_2 and T_3) were tested in the present experiment. Each treatment had three replications. Two surface feeders (*G. catla* 75% and *H. molitrix* 25%), one column feeder *L. rohita*); and two bottom feeders *C. cirrhosus* 65% and *C. carpio var. specularis* 35%) were used in the present experiment. Combination of surface (40%), column (30%) and bottom (30%) feeding carps; and total stocking density (2470 fishes ha^{-1}) were same for all the treatments (Table 1).

Figure 1. *Location of study area indicated with dot circle at Paba upazila (Sub-district) of Rajshahi district, Bangladesh.*

Table 1. Experimental layout for carp fattening under varied protein levels in diet.

Parameters	Treatments								
	T_1 (20% protein level)			T_2 (25% protein level)			T_3 (30% protein level)		
	T_1R_1	T_1R_2	T_1R_3	T_2R_1	T_2R_2	T_2R_3	T_3R_1	T_3R_2	T_3R_3
Pond area (ha)	1.43	1.36	1.35	1.33	1.42	1.36	1.37	1.42	1.34
Pond depth (m)	1.6	1.63	1.60	1.64	1.62	1.58	1.59	1.58	1.62
Stocked carps (nos. pond^{-1})	3532	3359	3334	3285	3507	3359	3384	3507	3310

Pond management

Experimental ponds were subjected to the removal of aquatic weeds manually. Predatory and unwanted fishes were removed through repeated netting. Ground water was used for all the ponds to maintain the water depth of around 2.0 m throughout the culture period. Both wild (Indian major carps) and hatchery (exotic carps) seeds reared through overwintering were used for stocking into the ponds under different treatments. Regular liming was done (Basal dose 250 kg ha^{-1} and periodic dose 50 kg ha^{-1} fortnight^{-1}) for experimental ponds under the different treatments. Guideline for GAP aspects were followed after DoF (2012) and no organic manure was used except limited use of inorganic fertilizers (urea: basal dose of 40 kg ha^{-1} and periodic dose of 1.0 kg ha^{-1}day^{-1}; triple super phosphate, TSP: basal dose of 40 kg ha^{-1} and periodic dose of 1.0 kg ha^{-1}day^{-1}) to enhance the natural feed. Basal dose of inorganic fertilizers was given after seven days of liming. Fishes were fed with supplementary feed twice daily at 2-5% of body weight.

Monitoring of environmental parameters

Water quality parameters of the experimental ponds were monitored between 09:00 am and 10:00 am in each month. Water temperature was recorded with the help of a Celsius thermometer at 20-30 cm below the water surface. Water transparency (cm) was measured by a Secchi disk. Alkalinity (mg L^{-1}) and ammonia-nitrogen (NH_3-N) (mg L^{-1}) were determined by the help of a HACH kit (FF2, USA). Dissolved oxygen (DO) (mg L^{-1}), pH and total dissolved solids (TDS) (mg L^{-1}) were determined by a multimeter (HQ 40 D, HACH, USA). Plankton was identified using the key after Ward and Whipple (1959), Prescott (1962) and Bellinger (1992) and their concentration in water was determined by the help of a microscope. Concentration of heavy metals and pathogenic bacteria in pond water was also determined monthly to monitor the GAP aspects. For heavy metal analysis, water samples from all studied ponds were collected at a depth of about 0.3 m below water surface into 500 ml plastic bottles. Prior to sampling, the bottles were cleaned with 10% nitric acid and rinsed with distilled water. The bottles were rinsed three times with the pond water at the time of sampling. Immediately after sample collection, 2 mL nitric acid was added to the water samples to reduce adsorption of metals onto the walls of the plastic bottles. Sample bottles were then labeled to indicate date of sampling and the sampling pond. Samples were transported in an ice-box to the laboratory and stored at 4°C awaiting analysis. Samples were digested through concentrated HNO_3 and hydrogen peroxide acid (5 mL) and the determination of Cadmium (Cd) and Lead (Pb) concentrations were carried out by the use of Flame Atomic Absorption Spectrometer (Shimadzu, AA-6800) in central lab of University of Rajshahi, Rajshshi. For the bacteriological analysis, 500 ml of water samples were sampled from each studied ponds. The sample was labeled and stored in the dark to prevent the entry of light and photolysis. The sample was delivered to the laboratory of Department of Fisheries, University of Rajshahi, Bangladesh as quickly as possible i.e. the time gap between sampling and analysis was maintained below 3 h. In the laboratory, total heterotrophic bacterial (THB) count was done in plate count agar and *Pseudomonas* and *Aeromonas* in GSP Agar (*Pseudomonas Aeromonas* Selective Agar Base), *Salmonella* and *Shigella* in SS Agar plate (Hi Media). Two steps were followed to analyze *Escherichia coli* in pond water samples. Firstly, total and fecal coliform were detected in MacConkey Agar plate and secondly, the colony of bacterial cells appeared as green metallic sheen in MacConkey Agar plate were tested for *E. coli* after the confirmation with biochemical test (iMVIC test). Colony counts were made from plates with a digital colony counter and expressed as colony forming units (cfu mL^{-1}) of the sample.

Fish growth monitoring

Fortnightly sampling of fishes were done to monitor growth performance and adjustment of the feeding ration. In each sampling date, 10% of the stocked fishes of each species were caught from each pond with the help of a seine net for the study of growth performances of fishes. The examined fishes were then immediately released into the ponds without any harm. Growth, survival and production performances of fishes were analyzed after Brett and Groves (1979) as follows:

Initial weight (g) = Weight of fish at stock (1)

Final weight (g) = Weight of fish at harvest (2)

Daily weight gain (g) = Mean final weight (g) - Mean initial weight (g) (3)

$$\text{Specific Growth Rate SGR } (\%, \text{bwd}-^{1}) = \frac{\text{L}_n \text{ final weight} - \text{L}_n \text{ initial weight}}{\text{Culture period}} \times 100 \quad (4)$$

$$\text{Survival rate } (\%) = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100 \quad (5)$$

Fish yield (kg ha^{-1}) = Fish biomass at harvest – Fish biomass at stock (6)

Economic analysis

Cost-benefit analysis of different treatments was calculated on the basis of the cost of lime, ash, fertilizer, fish seed and labor used; and the income from the sale of fishes. The prices are expressed in Bangladesh currency (BDT; 83.45 BDT = 1 USD in June 2020).

The following simple equation was used according to Asaduzzaman *et al.* (2010) to find out net return: R = I-(FC + VC + Ii)
Where, R = net return, I = income from fish sale, FC = fixed/ common costs, VC = variable costs and Ii = interest on inputs

The benefit-cost ratio was determined as:
Cost-benefit ratio (CBR) = Net benefit/Total input cost

Statistical analysis

Environmental parameters; fish growth and yield; and economics of carp fattening under different treatments were analyzed by one-way analysis of variance (ANOVA). When a mean effect was significant, the ANOVA was followed by Duncan New Multiple Range Test (Duncan, 1995) at 5% level of significance (Gomez and Gomez, 1984). The percentages and ratio data were analyzed using arcsine transformed data. All analyses were performed using SPSS (Statistical Package for Social Science) version 20.0 (IBM Corporation, Armonk, NY, USA). The study also conducted second degree polynomial regression analysis between fish yield and protein levels in diets.

RESULTS AND DISCUSSION

Water quality parameters

Water quality parameters recorded during the study period are shown in Table 2. There was no significant effect of the protein level on water quality parameters. Study conducted by Ara *et al.* (2018) and Dulic *et al.* (2010) also reported no significant effect of feed types on water quality parameters in semi-intensive carp culture pond. Quite the reverse, Bechara *et al.* (2005) reported that ponds receiving higher protein levels showed significantly higher values of alkalinity, conductivity and nitrites. However, the ranges of water quality parameters were all suitable for fish culture in aquaculture pond (Alikunhi, 1957; Swingle, 1967; Boyd, 1998; WHO, 2008). Variation in total plankton cell density among the treatment was also insignificant (*P>0.05*). However, numerically higher cell density of plankton was visible at treatment T_3 indicating the influence of increasing amount of nitrogen comes from the breakdown of higher level of protein in feed. As because, catabolism of higher level of protein creates a condition to produce higher amount of ammonia in water, which are converted into nitrogen and are easily accessible by plankton for their growth (Zeb and Javed, 2018; Kim and Lee, 2005). However, the water quality parameters obtained in the current study are similar to other carp polyculture ponds reported by Hossain *et al.* (2020), Talukder *et al.* (2017) and Talukder *et al.* (2018). Study on good aquaculture practice (GAP) aspects and subsequent heavy metal parameters (Cadmium and lead) showed no significant difference among the treatments (Table 3). Difference in total heterotrophic bacterial count (THB) was also insignificant. However, presence of other pathogenic bacteria (*Pseudomonas* spp., *Aeromonas* spp., *Salmonella* spp., *Shigella* spp. and *Escherichia coli*) was not detected during the study period (Table 3).

Growth performance and FCR

Growth performance of different carp species is shown in Table 4. Final weight, weight gain and SGR of *G. catla* were varied significantly (*P<0.05*) among the treatments, whereas the highest value of these growth parameters was obtained from fish fed with 30% protein containing diet at treatment T_3. No significant effect of protein level was observed in survival rate of *G. catla*. On the contrary, variation in protein level in diet had significant effect on final weight of *H. molitrix*. However, numerically higher value of growth parameters of *H. molitrix* was observed at treatment T_3. Growth performance of column feeder, *L. rohita* was found to be affected significantly (*P<0.05*) by the incorporation of different protein ratios in different diets, and higher level of protein diet favoured significantly higher growth performance (final weight and weight gain) of *L. rohita* at treatment T_3. Two other bottom feeder fish species *C. cirrhosus* and *C. carpio* were also found to response insignificantly (*P>0.05*) on different protein diets, but numerically higher growth performance of these two species was also recorded at treatment T_3. Study conducted by Bechara *et al.* (2005) also reported no significant effect of protein level on the improvement of final weight of several carp species. Zeb and Javed (2018) showed different growth performance for different carp species at different protein levels in diets, where they showed best performance for *C. cirrhosus* and lower for *G. catla*. In the present study, among the two filter feeder surface feeding carps *G. catla* and *H. molitrix*, comparatively higher final weight was obtained from *G. catla*. Ishtiaq and Naeem (2019) also reported higher growth performance of *G. catla* fed the feed containing 25% protein. Apart from that, among the *L. rohita*, *C. cirrhosus* and *C. carpio*, higher final weight was recorded for *L. rohita*. However, in all

Table 2. Water quality under different treatments of dietary protein levels.

Parameters	Treatments			F-value	P-value
	T_1	T_2	T_3		
Temperature (°C)	28.49±0.14^a	28.50±0.31^a	28.51±0.15^a	0.02	0.99
Transparency (cm)	25.44±0.29^a	25.83±0.25^a	26.28±0.15^a	3.02	0.12
DO (mg L^{-1})	6.61±0.05^a	6.69±0.05^a	6.60±0.11^a	0.40	0.67
pH	7.94±0.13^a	8.13±0.08^a	7.95±0.13^a	0.86	0.47
Alkalinity (mg L^{-1})	149.39±2.64^a	151.50±3.09^a	149.22±0.62^a	0.28	0.76
NH_3-N (mg L^{-1})	0.02±0.01^a	0.03±0.01^a	0.04±0.02^a	3.90	0.08
TDS (mg L^{-1})	671.55±17.07^a	666.78±12.92^a	655.22±8.63^a	0.39	0.69
CO_2 (mg L^{-1})	1.84±0.19^a	1.66±0.04^a	1.70±0.05^a	0.62	0.57
Plankton (×10^4 mg L^{-1})	39.45±2.07^a	39.82±1.64^a	40.25±2.51^a	0.04	0.96

Figures bearing common letter (s) in a row as superscript do not differ significantly ($P < 0.05$).

Table 3. GAP aspects (in terms of heavy metal and pathogenic bacteria) under different treatments of dietary protein levels.

GAP aspects	Treatments			F-value	P-value
	T_1	T_2	T_3		
Cadmium (mg L^{-1})	0.003±0.001^a	0.003±0.002^a	0.002±0.001^a	44.623	0.754
Lead (mg L^{-1})	0.002±0.001^a	0.001±0.001^a	0.003±0.001^a	36.774	0.666
THB (×10^4cfu m L^{-1})	2.23±0.21^a	2.77±0.10^a	2.74±0.15^a	112.10	0.451
Pseudomonas spp. (cfu m L^{-1})	0.00	0.00	0.00	-	-
Aeromonas spp. (cfu m L^{-1})	0.00	0.00	0.00	-	-
Salmonella spp. (cfu m L^{-1})	0.00	0.00	0.00	-	-
Shigella spp. (cfu m L^{-1})	0.00	0.00	0.00	-	-
Escherichia coli (cfu m L^{-1})	0.00	0.00	0.00	-	-

Figures bearing common letter (s) in a row as superscript do not differ significantly ($P<0.05$.

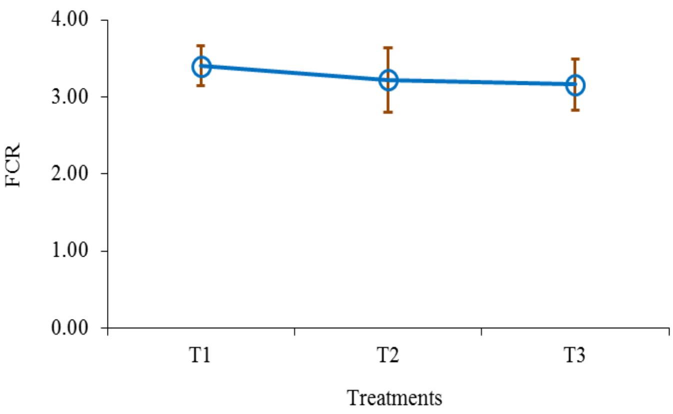

Figure 2. *Feed conversion ratio (FCR) under different treatments.*

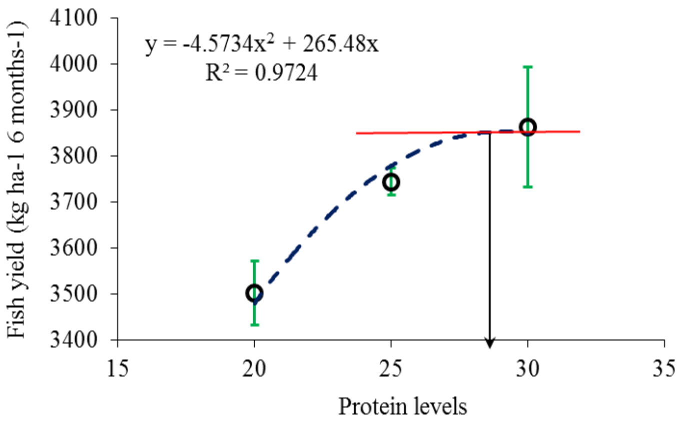

Figure 3. *Second degree polynomial regression analysis between fish yield and protein levels in diets.*

cases, final weight was the highest at treatment T_3 and the lowest at treatment T_1 indicated increasing protein level in diet was beneficial for fish growth. Feed conversion ratio (FCR) did not vary significantly, while numerically better (3.16) performance was detected at treatment T_3 and that of lowest at treatment T_1 (Figure 2). Feed conversion ratio (FCR) of the present experiment was significantly similar among the treatments. The lack of differences in FCR among the different protein level diets was due to optimum consumption of the diets and efficient utilization of the nutrients (Limbu, 2015; Adewolu *et al.*, 2010). Diets of different protein levels were effectively consumed by cultured carp species and they efficiently assimilated and metabolized the nutrients contained in the diets for growth and other body functions.

Fish yield

Individual production of fish species was not found to be varied significantly ($P>0.05$) among the treatments (Table 5). However, numerical value of production was higher for the fishes fed with 30% protein containing diet. Combined production of all the species was found to be varied significantly ($P<0.05$), whereas the highest value was recorded at treatment T_3 and that of lowest at treatment T_1. However, second degree polynomial regression analysis indicated around 28.50% proteins in diet would be optimal for best yield performance of carp species in fattening pond (Figure 3). The present production level was higher than Asadujjaman and Hossain (2016) (3675.33 kg ha^{-1} 6

Table 4. Fish growth performance under different treatments of dietary protein levels.

Species	Treatment	Initial weight (g)	Final weight (g)	Weight gain (gd^{-1})	SGR (%, bwd^{-1})	Survival rate (%)
G. catla	T_1	417.00±4.58^a	2723.00±63.17^b	2306.00±58.61^b	1.04±0.01^b	91.40±0.93^a
	T_2	418.66±2.33^a	2871.00±35.16^{ab}	2452.33±36.99^{ab}	1.07±0.01^{ab}	91.18±1.09^a
	T_3	420.33±4.66^a	2930.67±43.76^a	2510.33±42.39^a	1.08±0.01^a	91.22±1.44^a
	F value	0.17	4.80	5.03	5.17	0.01
	P value	0.84	0.04	0.03	0.04	0.99
H. molitrix	T_1	359.00±3.78^a	2755.67±30.94^b	2396.67±31.97^a	1.13±0.01^a	91.22±0.94^a
	T_2	360.00±7.93^a	2826.66±36.17^a	2466.66±30.77^a	1.14±0.01^a	91.90±1.99^a
	T_3	361.66±4.25^a	2858.67±35.55^a	2497.00±35.55^a	1.15±0.01^a	91.36±1.32^a
	F value	0.06	2.56	2.46	0.69	0.06
	P value	0.94	0.16	0.17	0.54	0.95
L. rohita	T_1	325.00±10.40^a	1503.33±10.40^b	1178.33±19.70^b	0.85±0.01^a	89.33±1.15^a
	T_2	320.00±6.08^a	1602.00±50.48^{ab}	1282.00±45.92^{ab}	0.89±0.01^a	89.69±0.69^a
	T_3	318.33±6.06^a	1648.00±26.69^a	1329.67±32.40^a	0.91±0.01^a	89.78±0.96^a
	F value	0.19	4.87	5.06	3.24	0.05
	P value	0.82	0.04	0.04	0.11	0.95
C. cirrhosus	T_1	328.00±6.42^a	1431.33±23.02^b	1103.33±22.45^a	0.81±0.02^a	87.31±1.90^a
	T_2	330.33±6.38^a	1502.00±48.18^{ab}	1171.67±54.44^a	0.84±0.03^a	88.25±0.53^a
	T_3	324.66±3.17^a	1565.00±80.16^a	1240.33±81.48^a	0.87±0.03^a	88.19±0.68^a
	F value	0.26	1.45	1.39	1.13	0.19
	P value	0.77	0.31	0.32	0.38	0.83
C. carpio	T_1	331.33±6.74^a	1454.66±54.74^a	1123.33±60.26^a	0.82±0.03^a	85.29±1.78^a
	T_2	329.00±5.85^a	1522.00±15.88^a	1193.00±20.03^a	0.85±0.01^a	85.96±2.47^a
	T_3	333.66±4.48^a	1579.67±56.48^a	1246.00±57.23^a	0.86±0.02^a	86.23±1.63^a
	F value	0.16	1.82	1.55	0.86	0.06
	P value	0.85	0.24	0.29	0.47	0.94

Figures bearing common letter (s) in a column as superscript do not differ significantly (*P<0.05*).

Table 5. Fish yield (kg ha^{-1} 6 $months^{-1}$) under different treatments fed with different levels of protein diets.

Species	Treatments			F- value	P- value
	T_1	T_2	T_3		
G. catla	1535.09±37.41^a	1629.35±24.69^a	1670.33±56.28^a	2.79	0.14
H. molitrix	532.15±3.25^a	552.70±16.07^a	555.84±14.21^a	1.05	0.41
L. rohita	754.55±26.32^a	828.17±37.82^a	860.88±32.47^a	2.80	0.14
C. cirrhosus	455.73±23.29^a	491.79±26.07^a	521.07±32.11^a	1.42	0.31
C. carpio var. specularis	224.22±8.11^a	241.99±11.00^a	254.50±18.50^a	1.31	0.34
All species	3501.76±69.23^b	3744.01±28.72^a	3862.64±130.76^a	4.47	0.04

Figures bearing common letter (s) in a column as superscript do not differ significantly (*P<0.05*).

$months^{-1}$) and within the level reported by Khan *et al.* (2018) (3743.30±59.18 to 4011.20±90.98 kg ha^{-1} 6 $months^{-1}$) and beyond the range reported by Hosen *et al.* (2014) (2680.75 to 5660.85 kg ha^{-1} 6 $months^{-1}$) in carp polyculture pond. However, analysis of second degree polynomial regression analysis directed the optimal level of protein as around 28.50% to be incorporated in diets for carp species. Zeb and Javed (2018) showed that net fish yield increased as the level of dietary protein increased in diets and further increase of protein level might hinder fish growth. But in our present finding, such decreasing trend was not so light to visualize.

Economic performance

Economic performance of different treatments is shown in Table 6. Major variable costs of the experiment were seed and feed cost, whereas seed cost did not differ significantly among the treatments. However, incorporation of different protein levels in different treatments caused significant difference (*P<0.05*) in total feed cost among the treatments, with the highest feed cost recorded at treatment T_3 (BDT 453320.00±2469.33 ha^{-1} 6 $months^{-1}$) (5431.95±29.59 USD) and that of lowest at treatment T_1 (BDT 297477.00±554.28 ha^{-1} 6 $months^{-1}$) (3564.55±6.64 USD). Fixed cost items were kept similar in all treatments to reduce biasness during the experiment. Therefore, total cost was found to vary significantly among the treatments and the highest total cost was recorded at treatment T_3 (BDT 777754.39±1750.65 ha^{-1} 6 $months^{-1}$) (9319.51±20.98 USD) and the lowest at treatment T_1 (BDT 622439.54±665.76 ha^{-1} 6 $months^{-1}$) (7458.44±7.98 USD). Feed costs were 47.79, 50.71 and 58.29% of total cost at treatment

Table 6. Economics of carp fattening pond (BDT ha^{-1} 6 $months^{-1}$) under different treatments of dietary protein levels. Figures in the parenthesis indicated values in USD on 30 June 2020.

Items	Treatments			F-value	P-value
	T_1	T_2	T_3		
Variable cost					
Seed	108402.53±792.58^a (1298.94±9.50)	108100.78±670.78^a (1295.33±8.04)	107874.36±1183.14^a (1292.61±14.18)	0.08	0.92
Feed	297477.00±554.28^c (3564.54±6.64)	333970.00±2000.89^b (4001.826±23.98)	453320.00±2469.33^a (5431.948±29.59)	1914.95	0.00
				-	-
Fixed cost					
Lease value	110000.00±0.00 (1318.09±0.00)	110000.00±0.00 (1318.09±0.00)	110000.00±0.00 (1318.09±0.00)	-	-
Water (pump)	9000.00±0.00 (107.84±0.00)	9000.00±0.00 (107.84±0.00)	9000.00±0.00 (107.84±0.00)	-	-
Lime	14000.00±0.00 (167.76±0.00)	14000.00±0.00 (167.76±0.00)	14000.00±0.00 (167.76±0.00)	-	-
Fertilizer	16060.00±0.00 (192.44±0.00)	16060.00±0.00 (192.44±0.00)	16060.00±0.00 (192.44±0.00)	-	-
Labour	52500.00±0.00 (629.09±0.00)	52500.00±0.00 (629.09±0.00)	52500.00±0.00 (629.09±0.00)	-	-
Harvest	15000.00±0.00 (179.74±0.00)	15000.00±0.00 (179.74±0.00)	15000.00±0.00 (179.74±0.00)	-	-
Total cost	622439.54±665.76^c (7458.44±7.98)	658630.79±2630.71^b (7892.10±31.52)	777754.39±1750.65^a (9319.51±20.98)	1899.72	0.00
Total return	914416.06±14544.73^b (10957.07±174.28)	995276.59±6270.95^{ab} (11925.99±75.14)	1035641.45±25724.21^a (12409.67±308.24)	5.70	0.04
Net benefit	291976.53±15161.90^b (3498.64±181.68)	336644.71±3643.77^a (4033.88±43.66)	257887.08±24043.46^c (3090.16±288.10)	17.21	0.00
CBR	0.47±0.02^a	0.51±0.00^a	0.33±0.03^b	17.21	0.00

Figures bearing common letter (s) in a column as superscript do not differ significantly (*P<0.05*).

T_1, T_2 and T_3, respectively. Significant difference (*P<0.05*) was also observed in total and net return, whereas the total return was the highest at treatment T_3 (BDT 1035641.45±25724.21 ha^{-1} 6 $months^{-1}$) (12409.67±308.24 USD). On the contrary, the highest net return was obtained from treatment T_2 (BDT 336644.71±3643.77 ha^{-1} 6 $months^{-1}$) (4033.88±43.66 USD). 5% increased of protein level from T_1 to T_2 caused 2.91% higher feed costs, while 13.27% higher net benefit. However, further increase of 5% protein level from T_2 to T_3 caused 7.58% higher feed cost, but 30.54% reduction in net benefit. Incorporation of higher level of protein at treatment T_3 was responsible for higher feed and total cost. As a consequence, significantly lower net return and CBR was recorded at treatment T_3. The present study was able to successfully reduce around 10-20% production cost without compromising the economics of carp fattening in pond compared to Jasmine *et al.* (2011) and Hossain *et al.* (2020), respectively. Therefore, although higher protein level (30%) in diet was responsible for higher growth and production performance of carp species, 25% protein diet would be economically viable and sustainable for carp fattening pond. Moreover, significantly higher cost-benefit ratio (CBR) was perceived from treatment T_2 (0.51±0.00), where the fishes were fed with 25% protein containing diet. However, the difference in CBR between T_1 and T_2 was insignificant.

Conclusion

Findings clearly indicated that comparatively higher ammonia and plankton concentration was obtained with increased utilization of protein level at treatment T_3. Incorporation of higher level of protein at treatment T_3 was also responsible for higher feed and total cost. No significant difference between T_2 and T_3 was found for the combined fish yield and significantly (*P<0.05*) highest cost-benefit ratio was obtained with 25% protein containing diet. Therefore, the present study suggested maintaining 25% protein content in diet for profitable carp fattening in pond. Since the study used a single feeding strategy (twice daily feeding of commercial feed), further research is also required for testing the efficacy of commercial and home-made feed under different feeding strategies (restricted feeding) towards cost minimization and sustainability of carp fattening technique in pond.

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