Research Article

Effect of Different Dietary Protein Levels on Growth Performance of *Catla catla* (Hamilton) Reared under Polyculture System

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Abstract | A feeding trial to determine the effects of dietary protein levels on growth and to elucidate the optimal dietary crude protein requirement for *Catla catla* (Thaila) in a polyculture system was conducted. Four experimental diets containing graded levels of protein (15, 20, 25 and 30%) were tested. Triplicate groups of *C. catla* stocked in outdoors earthen ponds at 2000 fish/acre were fed at 4% of body weight, 2 times a day, for 180 days. The investigation was spread over three different sites, each providing four earthen ponds. The results revealed that the survival rate, mean individual harvesting weight, feed conversion ratio (FCR) and production of *C. catla* were significantly ($p< 0.05$) affected by different dietary protein levels. However, absolute gain of length and weight, specific growth rate and protein efficiency ratio were not significantly ($p> 0.05$) influenced by dietary protein levels. Absolute weight gain (g) amounted to 736.82±51.95, 749.76±71.20, 863.94±61.24 and 854.73±61.02 g, whereas FCR values were found to be 5.3±0.24, 4.69±0.50, 3.04±0.21 and 3.08±0.24, when the fish were fed upon the formulated diet containing 15, 20, 25 and 30% crude protein (CP), respectively. The maximum production was obtained in the fish group fed on a feed containing 25% CP. Production and FCR values of *C. catla* when fed upon 25% and 30% crude protein did not differ significantly ($p>0.05$) from each other. Thus, the results indicated that inclusion of 25% crude protein in fish feed was sufficient for the efficient production of *C. catla* in a polyculture system rather than using any higher protein level, such as 30%CP used in the present study. This finding should be beneficial in the formulation of economical feed for carps and disseminate high stocking fish culture in the region.

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Introduction

The continuous grooming of the fisheries industry demands attention to boost fish production for improved survival of the industry. Feeding management of fish plays an important role in the efficient and successful culture of fish. Appropriate diet that fish can efficiently digest and provides essential nutrients for the optimum growth of fish leads to success in commercial aquaculture (Mokolensang et al., 2003). The present inclination in fish culture is towards increased intensification which has led to dependence on artificial feeds (Lovell, 1989; Luo et al., 2004); therefore, nutritionally well-balanced and cost-effective feed should be considered for optimizing the fish culture conditions (Abdel-Tawwab, 2012; Kadhar et al., 2012). The quality and quantity of feed consumed by fish have a noticeable influence on their feed conversion ratio (FCR), growth rate and proximate body composition (Siddiqui and Khan,

2009; Abdel-Tawwab, 2012; Zahrani et al., 2013).

Protein is the energy-yielding nutrient; however, it is the most expensive component in fish feeds and is the most important factor for optimizing physiological performance,locomotion,optimumgrowthanddisease resistance (Kiriratnikom and Kiriratnikom, 2012). Recently, numerous studies have been undertaken which show variability in protein requirements of different fish species following development of cost-effective and nutritionally balanced feed (Siddiqui and Khan, 2009; Nasir and Al-Sraji, 2013; Sankian et al., 2017; Khalid and Naeem, 2018). Although increasing the protein level in the fish feeds can improve their production, excessive dietary protein will be metabolized as an energy source and will produce more toxic nitrogenous compounds (Xue et al., 2012) and this process may be detrimental to fish growth. On the other hand, increase in fish density and/or decrease in their dietary protein level would retard and may even decrease fish growth (Abdel-Tawwab, 2012), as in case of higher fish population densities. If same fish species are stocked in the same pond, food competition, competition for space and oxygen requirement is established (Hossain et al., 2014). Moreover, protein level in fish feed also alters the assimilation efficiency and proximate body composition of the fish and it is directly dependent on the quality of nutritional composition, ingredients, and formulation process of the diet (Tacon and Forester, 2000; Siddiqui and Khan, 2009). Therefore, knowledge of the protein requirement of the fish is essential and should be determined for formulating well-balanced and low-cost fish diet(s).

At present, research reports on fish growth in earthen ponds under polyculture system are relatively scarce in the available relevant national and international literature. Thus, the aim of the present study was to observe growth efficiency and FCR of the major carp, *C. catla* (Thaila) raised on artificially formulated feed containing various dietary protein levels (15, 20, 25 and 30% crude protein) under polyculture system.

Materials and Methods

To formulate the four different artificial fish diets, *viz*. Diet-1 containing 15% crude protein (15% CP), Diet-2 containing 20% crude protein (20% CP), Diet-3 containing 25% crude protein (25% CP) and Diet-4 containing 30% crude protein (30% CP), the weighted locally available fish feed ingredients (Table 1) were ground and mixed thoroughly. Thereafter, pellets of the mixed ingredients were prepared by using locally made pellet machine, and the dried pellets were stored in air tight plastic containers.

Table 1: *Feed composition (expressed as percent of used ingredients) of four dietary levels of crude protein (i.e., 15, 20 and 25 and 30%) formulated by locally available fish feed ingredients.*

	Diet-1	Diet-2	Diet-3	Diet-4
Ingredients/Constituents	**15%CP**	**20%CP**	**25%CP**	**30%CP**
Canola Meal	3	5	5	5
Carboxymethyl Cellulose (*CMC*)	2	-	-	-
Corn Gluten Meal 30%	4	6	10	9
Corn Gluten Meal 60%	-	-	10	15
Dicalcium Phosphate ($CaHPO_4$)	2	1	2	1
Fishmeal	4	10	10	10
Rice Polish	40	30	25	15
Sarson (Mustard Seed) Meal	-	2	5	2
Soybean Meal	4	8	10	15
Soybean Oil	2	2	2	2
Sunflower Meal	4	5	5	5
Vitamin Premixes	2	1	1	1
Wheat Bran	33	30	15	20
Total	100	100	100	100

Feeding trials were conducted in earthen ponds maintained at the fish farms at Government Fish Seed Hatchery, Mianchannu, District Khanewal, Pakistan [Site-1/ Replicate-1] (*Latitude*: 30°25'24.83" N; *Longitude*: 72°18'00.09 E); Fish Seed Nursery and Aquaculture, Peerowaal, Pakistan [Site-2/ Replicate-2] (*Latitude*: 30°21'28.25" N; *Longitude*: 72°01'49.78 E); and Bahauddin Zakariya University, Multan, Pakistan [Site-3/ Replicate-3] (*Latitude*: 30°16'02.19" N; *Longitude*: 71°30'05.76 E). The experiment was conducted at three different locations (each providing four earthen ponds) and these locations were considered as replicates by them for the purpose of obtaining the mean values.

Prior to starting the experiment, each earthen pond was disinfected and the water pH was stabilized by liming with CaO dusting method (Hora and Pillay, 1962). All the fish ponds were fertilized with dry cow-dung at the rate of 10,000 kg ha^{-1} (Jhingran, 1995)

Table 2: *Effects of various dietary crude protein (CP) levels on growth parameters of Catla catla, reared under polyculture system.*

Parameters	T1	T2	T3	T4
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Survival rate of fish (%)	72.00±2.08^{c}	83.69 ±0.96^{b}	91.89±1.13^{a}	91.03±1.24^{a}
Mean Individual Stocking Length (cm) (TL_s)	10.8±3.11^{a}	11.2±3.87^{a}	11.24±4.07^{a}	10.97±3.67^{a}
Mean Individual Harvesting Length (cm) (TL_h)	38.57±0.95^{b}	38.82±1.33^{b}	40.48±0.98^{a}	40.28±1.01^{a}
Absolute Length gain (cm) (ALG)	27.77±2.96^{a}	27.62±3.39^{a}	29.24±3.65^{a}	29.31±3.26^{a}
Relative Length gain (RLG %)	72.13±7.82^{a}	71.44±9.32^{a}	72.43±9.58^{a}	72.37±9.26^{a}
Mean Individual Stocking Weight (g) (W_s)	38.78±28.95^{a}	45.01±39.36^{a}	47.98±42.53^{a}	46.75±41.06^{a}
Mean Individual Harvesting Weight (g) (W_h)	775.60±52.13^{b}	794.77±78.57^{b}	911.92±70.92^{a}	901.47±122.84^{a}
Absolute Weight Gain (g/fish) (AWG)	736.82±51.95^{a}	749.76±71.20^{a}	863.94±61.24^{a}	854.73±61.02^{a}
Daily Growth Rate (g/day) (DGR)	4.09±0.29^{a}	4.17±0.40^{a}	4.80±0.34^{a}	4.75±0.34^{a}
Relative Weight Gain, (RWG %)	95.18±3.58^{a}	94.71±4.56^{a}	95.09±4.31^{a}	95.15±4.21^{a}
Feed Conversion Ratio (FCR)	5.30±0.24^{a}	4.69±0.50^{a}	3.04±0.21^{b}	3.08±0.24^{b}
Specific growth rate (in percent per day) (SGR%)	0.97±0.21^{a}	0.95±0.24^{a}	0.99±0.26^{a}	0.98±0.25^{a}
Protein efficiency ratio (PER)	1.26±0.06^{a}	1.09±0.10^{a}	1.33±0.09^{a}	1.09±0.08^{a}
Net Production (kg ha^{-1} $year^{-1}$)	4205.12±492.88^{b}	4977.10±719.19^{a,b}	6309.45±786.44^{a}	6192.91±882.35^{a}

Mean values of three replicates; Mean values sharing the same superscripts in a row are not significantly different ($p > 0.05$); T = Treatments; T-1 = 15%CP; T-2=20%CP; T3 = 25%CP; T4 = 30%CP.

two weeks prior to stocking of fish and there after inorganic fertilizer (urea) was applied at monthly interval at the rate of 200 kg ha^{-1} $year^{-1}$ (Garg and Bhatnagar, 2000), as cited in Afzal et al. (2008).

Young *C. catla* were stocked randomly in each earthen pond at the rate of 2000 fish/acre (3942 fish/ hectare) comprising 30%, with *Labeo rohita* (60%) and *Cirrhinus mrigala* (10%), in earthen ponds under polyculture system in July, 2014. At the time of stocking, body weight and total length (TL) of some fish samples were taken to record initial fish size. Four different artificial diets containing 15%, 20%, 25% and 30% of crude protein were offered at the rate of 4% of body weight of fish stock in four different treatments, T1, T2, T3 and T4, respectively. The fish were fed experimental diets twice daily at 0900 and 1700 hours, 7 days a week. To adjust the feeding rate, health condition and growth of fish, sampling was carried out at an interval of 30 days. The feeding trials lasted for 180 days. Various growth parameters were calculated by using standard formulae following Zaikov et al. (2008), Siddiqui and Khan (2009) and Sawhney and Gandotra (2010).

The data obtained was statistically analyzed by using one-way analysis of variance (ANOVA) to determine the effects of feed on the growth of *C. catla*. Duncan's Multiple Range Test was conducted to separate treatment means only in case of significant difference (ANOVA, $p< 0.05$). Statistical analyses were performed using MS-Excel for Windows 7 and SPSS (version 23.0).

Results and Discussion

Results related to various growth parameters, including FCR, SGR%, PER and production of *C. catla* for different treatments are presented in Table 2, whereas descriptive statistics for the overall data of all studied treatments for *C. catla* fed upon varying dietary protein levels under polyculture system is presented in Table 3. Monthly mean (±SE) values of length and weight gain (g) of *C. catla* in different treatments are shown in Figures 1 and 2, respectively. Maximum length gain (cm) of *C. catla* was recorded in first month (July), while it was lowest in fifth month (November) of the study in all treatments (Figure 1). Maximum length gain (cm) of *C. catla* was recorded in the fourth month (October) for T1 and T2, while in the sixths month of the study for T3 and T4 (Figure 2). ANOVA on TLs, ALG, RLG%, Ws, AWG, DGR and RWG % of *C. catla* showed no significant difference ($p>0.05$) between different treatments (Table 2).

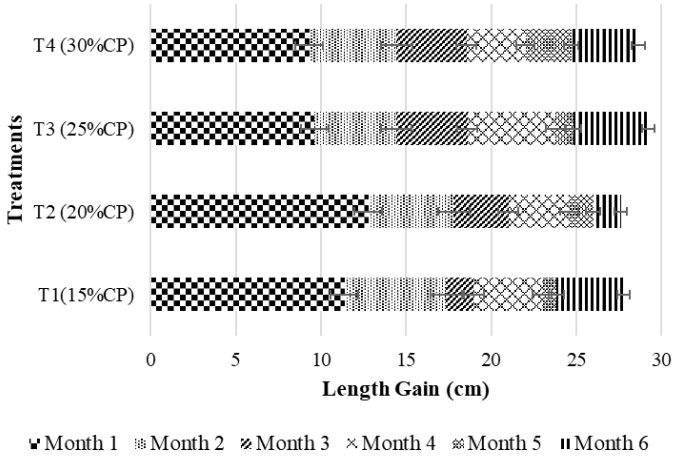

Figure 1: *Monthly length gain (cm) of C. catla in different treatments.*

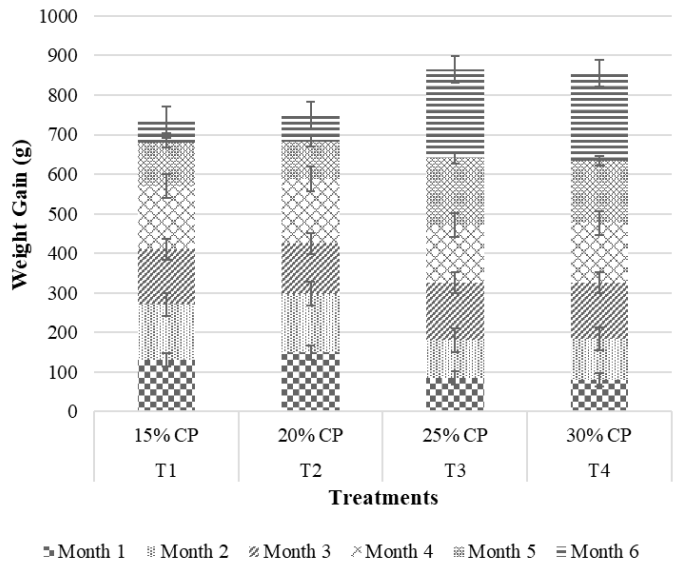

Figure 2: *Monthly weight gain (g) of C. catla in different treatments.*

The highest absolute length gain (ALG) value was found to be 29.24±3.65 cm in T3, followed by 28.64±3.23 cm in T4, and the lowest 27.62±3.39 cm in T2. Whereas, highest absolute weight gain (AWG) value was found to be 863.91±61.26 g in T3, followed by 854.73±61.02 g in T4, and the lowest being 736.82±51.95 g in T2.

Percent survival of *C. catla* ranged from 68.67% to 93.33% among different treatment in the present study. Survival rate (%) of *C. catla* reared under polyculture system in different treatments showed significant differences (p<0.05) among the fish groups fed upon the fish feed containing 15%, 20%, 25% and 30% crude protein in four different treatments. Percent survival in T3 and T4 was found to be similar (p> 0.05) when compared with 25% and 30% crude protein; however, it differed (p< 0.05) from T1 (15% crude protein) and T2 (20% crude protein).

Table 3: *Descriptive Statistics for the overall data of all studied treatments for Catla catla fed upon varying dietary protein levels under polyculture system.*

Parameters	N	Min.	Max.	Mean	
	Statistic	Statistic	Statistic	Statistic	Std. Error
Survival	12	68.67	93.33	84.6533	2.49066
TL_s	12	6.90	19.37	11.1192	1.64827
TL_h	12	36.17	41.47	39.4342	.51727
ALG	12	21.19	33.97	28.3150	1.47353
RLG	12	52.83	82.09	72.0417	3.95545
Ws	12	3.48	133.00	44.6275	16.76789
Wh	12	638.44	983.55	845.9317	35.32748
AWG	12	634.22	976.10	801.3042	32.29044
DGR	12	3.52	5.42	4.4517	.17936
RWG	12	85.60	99.55	95.0325	1.83589
FCR	12	2.82	5.86	4.0267	.32970
SGR	12	.47	1.30	.9692	.10440
PER	12	.88	1.42	1.1950	.04798
Production	12	3681.80	7189.92	5421.142	1107.667

Statistical analysis of FCR values revealed significant differences (p<0.05) between different treatment groups. Values of FCR were similar in T1 and T2 but were significantly lower (p<0.05) in T3 and T4. Lowest FCR value (3.04) was noted in T3 where fish were fed upon the artificial diet containing 25% crude protein whereas the highest value of FCR, 5.03, was noted, in T1 which was fed upon 15% crude protein.

Mean±SE values of percent specific growth rate per day (SGR%) of *C. catla* were found to be 0.97±0.21, 0.95±0.24, 0.99±0.26 and 0.98±0.25 % in T1, T2, T3 and T4. However, SGR (%) of *C. catla* reared under polyculture system in different treatments indicated no significant difference (p>0.05) among different treatment groups. Protein efficiency ratio (PER) also showed no significant (p>0.05) difference among treatments. However, higher PER value was obtained in T3, when the fish stock was provided with 25% dietary protein.

Net production (kg $ha^{-1}year^{-1}$) of *C. catla* cultivated by feeding varying levels of crude protein levels (15%, 20%, 25% and 30%) in the studied treatments differed significantly (p<0.05; Sig 0.011). The maximum production was obtained in the fish group fed upon the feed containing 25% crude protein and reared in T3.

This study evaluated the influence of different dietary protein levels on growth of a major carp, *C. catla*,

when reared in high stocking (2000 fish/acre) under polyculture system along with other cultureable carps and fed with laboratory prepared artificial fish feed containing 15%, 20%, 25% and 30% crude protein.

Generally, local fish farmers practice a stocking density of 800-1000 fish/acre in earthen ponds. However, it can be enhanced by proper management and provision of good quality feed to obtain higher production. Chakraborty and Banerjee (2010) found the highest weight, length, daily weight gain, growth rate and protein content in the fingerlings of Monosex Nile Tilapia in 20000 fish/ha density class. More recently, Hassan and Naeem (2017) evaluated the production of carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) when stocked at various densities and documented maximum production in the treatment in which fish were stocked at 2000 fish/acre and reared. These authors had reported weight gain of 733.09g, FCR 5.24, PER 0.6 and survival 91.5%in *C. catla* reared in poly-culture system with 2000 fish/acre stocking density. Findings of the present study for treatment 1 (15% crude protein) correspond with those of Hassan and Naeem (2017). However, in the present study, the same fish species has shown better growth performance when fed upon the diet containing 25% crude protein with same stoking density (2000 fish/acre).

Generally, fish farmers of the region feed the stock with fish diet containing 30% crude protein. Results of the present study suggested that inclusion of 25% crude protein is sufficient for optimum growth of *C. catla*, as it provided lowest FCR and highest production. Previously, Suharmili et al. (2015) conducted a study to determine the optimal dietary protein requirement for lemon fin barb hybrid fingerlings by feeding 20, 25, 30, 35 and 40% dietary protein and recommended a diet containing 30% protein for the practical culture of lemon fin barb hybrid fingerlings as it gave best protein retention. Renukaradhya and Varghese (1986) used four different types of feeds with 20, 30, 40 and 45% protein content and concluded that *C. catla* require around 30% dietary protein. These authors recommended further experimentation using feeds with only 3-4% variation in protein levels. Hence, the present study may be considered as an extension of their work and reveals that 25% dietary crude protein is the best for rearing of *C. catla*.

In the present work, commonly used and suitable fish feeding rate of 4% of the fish body weight was applied as recommended by other fisheries researcher on carps, such as Salim and Sheri (1999) and Sahzadi et al. (2006), who had conducted feeding trials in glass aquaria.

Results of the present study showed that size (total length and body weight) at the time of harvesting of *C. catla* was significantly higher ($p< 0.05$) in T3. However, Renukaradhya and Varghese (1986) had reported best growth in weight of *Labeo rohita* and *C. catla* when fed on feed containing 30% protein content. Whereas, studies of Bechara et al. (2005) had documented no significant improvements in final weight of *Piaractus mesopotamicus* with high dietary protein diets. The variations in growth of fish may be due to different factors and mostly depend on biotic factors, such as age (Deniel, 1990) and sex (Imsland and Jonassen, 2003) and abiotic factors, such as oxygen level (Brett, 1979), temperature and water chemistry (Imsland et al., 2007), photoperiod (Imsland and Jonassen, 2003); stocking density (Ma et al., 2006) and feed quality and its consumption (Slawski et al., 2011).

Present study on *C. catla* shows that absolute length gain (ALG) and absolute weight gain (AWG) was the highest in T3, in which fish stock was fed with laboratory-formulated fish feed containing 25% crude protein. However, absolute growth rate (ALG and AWG) of *C. catla* represented insignificant difference ($p> 0.05$) for different treatments. These results are a in accordance with studies of Abou-Daoud et al. (2014), who had reported no significant difference in weight gain of *Siganus rivulatus* by feeding 30, 35, 40, 45and 50% crude protein level in feed. Whereas, other workers had reported significant difference in AWG feeding different crude protein levels in fish feed (Siddiqui and Khan, 2009; Suharmili et al., 2015). Moreover, in the present work, no significant difference ($p<0.05$) for different treatments was found for RWG and RLG. Relative growth rate (RGR) statistically depends on the AGR and is considered a reasonable way for growth comparison (Lugert et al., 2014).

In the present work, survival rate (%) of *C. catla* showed statistically significant differences ($p<0.05$) among the fish groups that were fed containing 15%, 20%, 25% and 30% crude protein in four different treatments and cultured in earthen ponds under polyculture system with high stocking density of 2000 fish/acre. Highest mean survival percentage of *C. catla* was found in T3 receiving 25% crude protein in the diet. Results indicated that protein levels in

diet significantly affected the survival rate of fish, as also noted by Sawhney and Gandotra (2010).

The lowest FCR value (3.04) of *C. catla* in the present study was noted in the 25% crude protein treatment. Similar observations had previously been reported by Chatta et al. (2015); these authors had reported FCR value of 3.29 in *C. catla*. Khan et al. (2012) had reported FCR value of 3.9 in *C. catla* when fed upon 35% protein diet for 90 days in a polyculture system. Results of the present study, indicate that the FCR value decreases with an increase in the dietary protein level. Previously, Abdel-Tawwab (2012) had also reported that FCR decreases with increase in the dietary protein level. Contrary to this, Basade and Mohan (2012) and Islam (2002) had discovered an increase in the FCR value with the increase in protein level in the fish diet. The variation in FCR values might be due to poor digestibility, inefficient utilization of feed, higher ration size or wastage of feed. As the present study was conducted in earthen ponds it is assumed that all feed provided to the fish was consumed by them. However, feed consumption by *C. catla* may vary due to different dietary protein levels.

Mean SGR values of *C. catla* among treatments varied within a narrow range of 0.95-0.99% day^{-1} which values are comparable with those (0.9 to 1.23) reported by Dhawan and Kaur (2002) for *C. catla*. Other authors had reported lower SGR values, such as Khan et al. (2012) reporting this value to be 0.8 when fed upon 35% protein diet, and Chatta et al. (2015) reported 0.74% SGR in polyculture system for *C. catla*. The higher SGR values of *C. catla* in the present study suggests its growth potential, especially in the treatment where fish stock was fed with feed containing 25% crude protein.

Results for PER are in accordance with the study of Abdel-Tawwab (2012) who had also reported higher values of PER with 25% crude protein for *Oreochromis niloticus*, as compared 45% and 35% crude protein. In the present study, the value of PER for T4 (containing 30% crude protein) was the same as noted for T2 (containing 20% crude protein) indicating that 25% crude protein in fish feed is optimum for rearing of *C. catla* in earthen ponds under polyculture system and higher 25% crude protein level perhaps negatively affects the PER of *C. catla*. This may be due to the fact that fish weight gain is associated with the protein deposition. The results of the Abdel-Tawwab et al. (2010) were similar to the results of present study; these authors had reported a decrease in PER value under increasing dietary protein level.

Maximum production (kg $ha^{-1}year^{-1}$) in T3 (fed with 25% crude protein) can be attributed to considerably better growth rate of *C. catla* as well as higher survival rate indicating the fish in better condition, as compared to other rates of treatment used in the present study. Additionally, production of T3 (6309.45 kg) and T4 (6192.91 kg) was not significantly different from each other. This confirms that inclusion of 25% crude protein in fish feed is enough for the production of *C. catla* in polyculture system and indicates that inclusion of 30% or higher crude protein level in fish feed for *C. catla* under polyculture system is not economical and therefore not advisable.

However, a recent study by Khalid and Naeem (2018) on another carp species, *Ctenopharyngodon idella*, showed maximum growth performance, SGR, survival rate and production by feeding the fish on diet containing 20% crude protein. This lower dietary protein requirement of the grass carp might be due to different feed supplied and feeding habits of the species, as feed influences the growth pattern of fish (Iqbal and Naeem, 2018).

Conclusions and Recommendations

The present study suggests that among the different experimental dietary protein levels of 15, 20, 25 and 30% crude protein, growth of *C. catla* under a polyculture system with high stocking density (2000 fish/acre), the diet containing 25% crude protein gave better FCR, survival and production results indicating the best dietary protein level for optimum growth of *C. catla* to be 25%; this level in terms of growth and production terms was not significantly (p>0.05) different from that achieved by the 30% dietary protein level. The present study also revealed feasibility of higher stocking density (up to 2000 fish/acre) especially in a polyculture system. Since locally available fish feed ingredients were used in the present study and yielded satisfactory growth of the fish, these ingredients can be wisely used for formulating nutrient-rich and cost-effective diet for raising carps on large scale. Doing so will not only improve income of fish farmers but will also help in availability of cheaper source of quality protein to the general public of Pakistan and elsewhere. The results of the present study should be helpful in improving fish production as well as promotion of aquaculture industry at large. It will also be helpful in popularization of high

stocking (2000/acre) fish culture and use of artificial diets in public and private sectors. However, further studies on growth performance by feeding various levels of dietary protein levels, by reducing feed rate up to 1-3% of fish body weight in pond culture system to reduce the FCR value, and inclusion of other fish species and hybrid fish are recommended and may help in enhancing fish production.

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Novelty Statement

Findings of the study will be beneficial in the formulation of economical feed for carps, improving fish production as well as to disseminate high stocking fish culture in the region.

Author's Contribution

Abir Ishtiaq performed experiments, analyzed the data, and wrote the first draft of the manuscript. Muhammad Naeem designed the experimentation and supervised the research work.

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