INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2023, Vol. 2, NO. 1, 33-42.
doi: https://doi.org/10.56612/ijaeb.v1i1.29
https://eminentscientists.com/

Impact of feed with varying crude protein (CP) levels on growth performance of different fish species cultured under similar regimes

Kashif Manzoor[1], Fayyaz Rasool[*2], Shakeela Parveen[3], Muhammad Hafeez-ur-Rehman[1], Matiullah[1], Shahid Mahmood[1], Amina Ayub[1]

[1]Department of Fisheries & Aquaculture, Faculty of Fisheries & Wildlife, University of Veterinary & Animal Sciences, Lahore-Pakistan
[2]Department of Zoology University of Education Faisalabad Campus, Faisalabad, Pakistan
[3]Department of Zoology, Wildlife & Fisheries, University of Agriculture, Faisalabad

Abstract
Fish polyculture has resulted in high fish production per unit area, mainly due to the best utilization of all kinds of nutrients. In this nine month's experimental study, five different fish species were stocked in five earthen ponds designated as control (one pond), treatment-T_1 (two ponds) and treatment-T_2 (two ponds). Fish stocked in T_1 and T_2 ponds were fed with a commercial feed containing 20% and 25% crude protein, respectively, whereas fish stocked in the control pond was fed with a conventional mixture of feed ingredients (fish meal, gluten, sunflower, and rice polish) having 20% CP. The fish was fed @ 3% of the fish wet body weight twice a day. The fish was sampled every fortnight. Physicochemical parameters of the ponds were measured daily. The results showed that the maximum weight gain (255 g) was recorded in *Pangasius pangasius* in treatment T_2 and minimum (52.5 g) in *Cirrhinus mrigala* in the control pond. Overall, maximum fish production was recorded after harvest in treatment T_2 followed by that in treatment T_1 and control ponds, respectively. Overall, the commercial fish feed enriched with crude protein was very effective in promoting growth and development of pond fish.

HANDLING EDITOR
Muhammad Ashraf

ARTICLE HISTORY
Received: 14 Nov 2021
Accepted: 27 Mar 2022
Published: 15 Nov 2022

KEYWORDS
Polyculture;
Crude protein;
Growth;
Feed;
Conventional

Introduction

Fish production of the whole world is estimated to be 179 million tons, out of which 156 million tons are consumed by humans at the rate of 20.5 kg per capita. Aquaculture consumption is about 46%, while 56% of fish is consumed by the humans of the whole world. For example, in Pakistan 158.8 thousand tons of fish were produced during 2018, which is about 1% of overall world fish production (FAO, 2018). Fisheries and aquaculture sector provides a source of livelihood to 59.51 million people (20.53 million people in aquaculture and 38.98 in fisheries), of which 14% are women. Asia is the biggest fish producer with the highest percentage of 88.69% (77,812.2 thousand tons), America 4.63% (3,799.2 tons), Europe 3.75% (3,082.6 tons) and Africa 2.67% (2,195.9 tons) (FAO, 2020). Fish has become one of the basic sources of animal protein, and provides about 26.2% animal protein. This is the main reason that fish is considered as the most nutritious food. Fish culture has become the fastest growing sector in Asia and some other countries of the other continents (Delgado, 2002; Louka, 2004; Hussain et al., 2021). Food is the fundamental requirement of all living organisms as well as fish for maintenance of tissues, and fish growth and reproduction. Supplementary diet is the main source of natural feed supply of

***CONTACT** Fayyaz Rasool, fayyazrasool@uvas.edu.pk; drfayyaz1980@gmail.com, +92-0345-1059932: Department of Zoology University of Education Faisalabad Campus, Faisalabad, Pakistan

TO CITE THIS ARTICLE: Manzoor, K., Rasool, F., Parveen, S., Hafeez-ur-Rehman, M., Matiullah, Mahmood, S., Ayub, A. (2023). Impact of feed with varying crude protein (CP) levels on growth performance of different fish species cultured under similar regimes. *International Journal of Applied and Experimental Biology* 2(1): 33-42.

natural ingredients required by fish for proper fish growth in fish culture system (Karapan, 2002; Ayub et al., 2021). Increase in amount and percentage of nutrients in fish diet also increases fish body composition and fish growth performance (Solomon et al., 2007). Physicians consider it a source of valuable protein recommended for a healthy diet, because it constitutes unsaturated fatty acids in the form of Omega-3 and low carbohydrate content (Razvi, 2006). Subjecting fish to any diet containing starchy roots, sugar and cereals is necessary for better human growth (Razvi, 2006; Salim, 2006; Yildrim et al., 2008; Perveen et al., 2020).

Balanced fish diet has a great effect on fish growth and feed utilization. Full understanding is required for fish requirements of lipid and protein in diet formulated for better growth performance of commercially available fish species (Mahmud et al., 2012; Ayub et al., 2021). Fish diet may be supplementary or complete. A complete diet comprises all the nutrients necessary for optimum fish growth and healthy fish. While supplemental diets only provide help to utilize food available naturally in fishponds (Stickney, 1979; Nandakumar et al., 2017). Maximum production may be possible by using easily digestible, good quality and cost-effective feed. Every fish farmer intends to get maximum fish production within a proper time limit and by using low-cost feed, which can give maximum production (Hura et al., 2018). Sufficient dietary protein as the costly nutrient for fish diet, is required for fish optimum growth (Pillay, 1990; Perveen et al., 2020). An optimum amount of protein is essential for fish better growth performance (Ahmad, 2000).

Various physicochemical parameters and other growth parameters affect fish protein requirement in the fish diet (Cowey, 1976; Hassan et al., 2021). Protein is the major source of non-essential and essential amino acids required by fish for optimal performance, protein synthesis and energy maintenance (Kim et al., 2002: Ayub et al., 2021). Increase in crude protein level in the fish diet also increases fish body composition, feed utilization, and ultimately fish growth performance (Koprucu, 2012). But increase in crude protein (CP) level in fish diet above a certain level increases cost of production, and disturbs health of the pond (Aslam et al., 2017; Akter et al., 2013; Kim et al., 2020). Each fish species demands specific level of crude protein (CP) in a fish diet formulated for that fish. For example, African catfish requires maximum CP level in comparison with other fish species (Nwanna et al., 2014). Similarly, Nile tilapia gains maximum growth at 25% crude protein level (Mohsen, 2012). Thus, dietary protein level has a great influence on fish growth performance, feed utilization and feed conversion ratio (FCR) (Ahmed and Ahmad, 2020). Determination of minimum crude protein level in fish feed for better growth performance is essential for successful aquaculture practices (NRC, 2011). Mostly, research works have proved that dietary protein requirement for most fish species is 30-50% (Hussain et al., 2021). However, it depends upon environmental factors, dietary protein source, fish species and fish size (Hepher 1988; NRC, 1993; Hussain et al., 2021).

Fish culture in Pakistan depends on culturing of those fishes, which show better production in comparison with other fishes with a same kind of fish feed. Some important fishes being cultured are carps like *Labeo rohita*, *Cyprinus carpio*, *Cirrhinus mrigala*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Catla catla,* and *Pangasius pangasius*. Today polyculture is a popular and traditional practice in Pakistan, and it is found more productive from the economy point of view. Carps are suitable fishes for the polyculture system where optimum manure, feed and aquatic space are utilized, as different fishes have different living habitats and feeding habits (Miah et al., 1997). Fish species, pond fertilization, ecological conditions, stocking densities as well as artificial feed affect fish production in polyculture system. The size of carp seed and quality of seed are also considered critical inputs in better production and success of fish polyculture system (Ali et al., 2017).

Chinese carps and Indian major carps such as *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* are the main fish species which are being cultured in Asia, especially in South Asia. Fish culture of these described fish species is in practice since longtime (Chaudhuri, 1974). Composite and polyculture of these fish species result in higher fish production per unit area. Polyculture of these fish species is possible due to their compassionate behavior while living in the same pond without competition for food and habitat (Ali, 1999). Different fish products are a source of protein due to their protein digestibility ranging from 85% to 90% and a balanced amino acid profile (Rudolf, 1971).

Polyculture of freshwater Indian major carps *Cirrhinus mrigala* (Mori), *Catla catla* (Thaila) and *Labeo rohita* (Rohu) along with Chinese carps *Ctenopharyngodon idella* (Grass carp), *Hypophthalmichthys molitrix* (Silver carp) and *Cyprinus carpio* (Common carp) is a common practice in Pakistan. This is the best combination for polyculture being cultured for maximum fish production and better utilization of all pond resources (Chakrabarti, 1998). It is naïve to expect a lot of variation in all these fish species in terms of their basic requirement for feed and other factors essentially required for their optimum growth. Thus, the present study was conducted to examine that up to what extent growth of different fish species being cultured in Pakistan is affected by varying levels of crude protein present in fish feed.

Materials and Methods

Study area and duration

The experiment was conducted for a period of nine months from 1st February 2020 to 31 October 2020 in two replicates of earthen experimental ponds designated as Control, Treatment-T_1 and Treatment-T_2. The ponds were situated at the Fisheries Research Farms, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan.

Description of ponds

The area of each pond was 0.4 ha and all ponds were similar in shape, size, depth, basin conformation and bottom type. The ponds were free of aquatic vegetation, completely independent and well exposed to sunlight. Each pond had an inlet to maintain water level throughout the experimental period. Each pond was watered up to a level of 1.5 m by a drain water system. This water level was maintained throughout the experimental period.

Pond preparation

The ponds were dried completely, and every type of vegetation was removed manually. All the earthen ponds were disinfected, and the pH was stabilized by liming with CaO using the dusting method (Hora and Pillay 1962). All the fishponds were fertilized with cow-dung @ 3333.33 kg ha^{-1} two weeks prior to stocking of fish as a starter dose for production of phytoplankton and zooplankton.

Experimental design

Five earthen ponds were alienated into two replicates of experimental ponds designated as Control, Treatment-T_1 and Treatment-T_2. Each pond was a polyculture pond where *Cirrhinus mrigala*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Pangasius pangasius* and *Labeo rohita* were stocked to get maximum production.

Fish stocking

Each pond was stocked @ 1050-fish with following proportions: 100 *C. mrigala* (Mori/Mrigal), 250 *L. rohita* (Rohu), 300 *C. idella* (Grass carp), 150 *H. molitrix* (Silver carp), 200 *C. catla* (Thaila), 50 *P. pangasius* (Catfish/Pangasius) with an initial average weight as follows: Rohu and Mori 150 g, Thaila 10 g, Silver carp and Grass carp each 100 g, while Catfish 550 g. The fish was fed @ 3% of the fish wet body weight twice a day at 8:30 and 14:30 throughout the study period.

Feeding and fertilization

This experimental study was conducted by fertilizing each pond only with inorganic fertilizers such as urea for planktonic production. The experimental fish diets were purchased from the market for treatments T_1 (20%) and T_2 (25%). The fish stocked in the control pond was fed with a conventional mixture of feed ingredients (fish meal, gluten, sunflower, and rice polish) having 20% CP. The fish stocked in two ponds (T_1) was fed with a feed (obtained from the Oryza Organics) containing 20% CP, while the remaining two ponds were fed with 25% CP (obtained from AMG and Supreme Feeds). A standard fish diet for the control group was formulated having 20% crude protein using fish meal, sunflower meal, rice polish, canola meal and vitamin premix added @ 1% of the feed weight. The composition of the two treatments as well as control is given in **Table 1**.

Sampling procedure

After every fortnight, cultured fish species were captured and sampled randomly by using a nylon drag net at 9:00 a.m. from each experimental treatment and these fishes were released back into their respective ponds after recording the data. At least 20 fish samples of each fish species for recording morphometric data were captured for the determination of their body weight (g).

Table 1: Composition of standard and experimental diets

Ingredients (g/100 g)	Standard diet 20% Control	Experimental diet composition T_1 (CP 20%)	T_2 (CP 25%)
Fish meal	24	18	12
Sunflower meal	24	24	24
Rice polish	30	28	22
Canola meal	22	24	22
Maize gluten	0	06	20
Soybean meal	0	06	20
Vitamin premix	1%	1%	1%

Total fish production under different treatments

At the end of the experiment, total harvested fish species were weighed to calculate the total fish production under different treatments.

Physico-chemical parameters of water

Since physico-chemical parameters play an important role in the productivity of a pond, so some important physico-chemical parameters of pond water such as water temperature (^{o}C), dissolved oxygen-DO (mg/L), pH, and total dissolved solids (TDS) were recorded daily throughout the experimental period.

Water temperature

The temperature of the water was noted on the spot at 9:00 to 10:00 a.m. which was indicated with the help of a dissolved oxygen meter (HI-9146) along with DO measurements by fixing the temperature factor at 0 ^{o}C.

pH

To measure the pH on the spot at 9:00 to 10:00 a.m., the microprocessor pH meter (HANNA-HI-8520) was used after setting its range at “pH” point.

Dissolved oxygen (DO)

The dissolved oxygen in each pond was measured on the spot at 9:00 to 10:00 a.m. by a dissolved oxygen meter (HI-9146) after setting its range at “ppm”.

Total dissolved solids (TDS)

Total dissolved solids in pond water were measured by a TDS meter (HANNA-HI-98302).

Statistical analysis

The data obtained for all variables were subjected to an appropriate statistical analysis (Steel et al., 1996). The variation for different parameters, the significance, and their interaction among different treatments for the growth data and various parameters were tested by using analysis of variance (ANOVA).

Results

Limnological parameters

Water temperature

Temperature is one of the limnological parameters vital for growth of fish and biological organisms (biological productivity). The overall range indicated in **Table 2** of water temperature was 10.42-37.67 ^{o}C in control, 10.51-37.59 ^{o}C in treatment T_1 and 10.56–37.45 ^{o}C in treatment T_2. Maximum value of temperature (37.69 ^{o}C) was recorded during the month of July-2020 in treatment T_1, while minimum value of temperature 10.42 ^{o}C recorded during the month of February-2020 in the control pond.

The data obtained for temperature was subjected to a statistical analysis for obtaining ANOVA (**Table 3**). The results showed that average monthly values of the temperature of three treatments were found highly significant and different from each other, but there was a non-significant difference among the average monthly temperature values of each treatment.

Dissolved oxygen

Dissolved oxygen is another most vital limnological parameter which is required in its optimum value by all biological organisms of a pond. Phytoplankton are considered as the most important source for dissolved oxygen in any fishpond. The overall range of dissolved oxygen was 5.5-8.4 mg/L in control, 5.3-8.3 mg/L in treatment T_1 and 5.2-8.1 mg/L in treatment T_2. The maximum value (8.4 mg/L) was recorded during the month of February, while the minimum value (5.2 mg/L) of dissolved oxygen was recorded during the month of September. The statistical analysis showed that the average monthly dissolved oxygen values differed significantly from each other of all treatments, but the values of DO of each treatment did not differ significantly.

Table 2: Monthly observations on water temperature (°C), DO, pH and TDS under control, treatment T_1 and treatment T_2

Month	Temp. (°C)			DO (mg/L)			pH			TDS (mg/L)		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
February	10.42	10.51	10.56	8.4	8.3	8.1	8.0	8.1	8.2	1426	1433	1431
March	16.32	16.42	16.89	7.4	6.7	7.0	7.8	7.9	7.8	1418	1428	1423
April	23.49	23.34	23.66	7.1	6.3	6.8	8.2	8.0	7.9	1453	1450	1443
May	30.05	30.25	30.30	6.8	6.2	6.4	8.3	8.1	8.0	1356	1351	1342
June	35.17	35.63	35.80	6.5	6.0	6.1	7.9	7.8	8.3	1371	1376	1381
July	37.67	37.59	37.45	6.1	5.9	6.0	8.1	8.0	8.1	1317	1334	1335
August	35.2	35.45	35.67	5.8	5.6	5.5	8.0	8.1	7.8	1324	1341	1348
September	30.66	30.56	30.71	5.5	5.3	5.2	8.2	7.8	7.9	1351	1358	1366
October	24.68	24.61	24.52	6.3	6.4	6.0	7.9	8.0	8.1	1367	1368	1371
Mean	**27.07**	**27.15**	**27.28**	**6.7**	**6.3**	**6.44**	**8.0**	**8.0**	**8.0**	**1375**	**1381**	**1382**

Table 3: ANOVA (mean squares) of each parameter

Source	DF	Temp.	DO	pH	TDS
Treatments	2	0.103ns	0.350ns	0.001ns	142.43ns
Months	8	84.86**	0.748**	0.028**	1698.24**
Corrected Total	10				

**, significant at 0.01 probability level; ns, non-significant.

pH

The pH values ranged from 7.8-8.3 in control, 7.8-8.3 in treatment T_1 and 7.8-8.3 in treatment T_2. The maximum value of pH (8.3) was recorded during the month of August, while the minimum (7.8) in the month of November 2020.

Total dissolved solids

Total dissolved solids (TDS) are an important and useful limnological parameter for estimating the chemical nature, quality, and solubility of water. The TDS values ranged from 1317 mg/L to 1453 mg/L in control, 1334 mg/L to 1450 mg/L in treatment T_1 and 1335 mg/L to1443 mg/L in treatment T_2. The maximum value of TDS, 1453 mg/L, was recorded during the month of April, while the minimum TDS 1317 mg/L during the month of July.

Fish growth studies

The data regarding growth performance of five fish species, *Labeo rohita*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, *Pangasius pangasius* and *Ctenopharyngodon idella* fed with 20% CP (Treatment T_1) and 25% CP (Treatment T_2) are presented in **Table 4**. The data was subjected to statistical analysis for working out ANOVA for comparison of growth performance of fishes subjected to three treatments.

Growth performance in different treatments

The initial and final average weights of *Ctenopharyngodon idella* were 150.05 g and 1325.05 g, *Hypophthalmichthys molitrix* 150.2 g and 878.2 g, *Pangasius pangasius* 250.65 g and 1690.65 g, *Labeo rohita* 101.05 g and 553.05 g, while *Cirrhinus mrigala* was 99.5 g and 444.5 g, respectively, in the control treatment. The maximum weight gain of *Ctenopharyngodon idella* was 135 g, of *Hypophthalmichthys molitrix* 80 g, *Pangasius pangasius* 135 g, *Labeo rohita* 50 g and of *Cirrhinus mrigala* 33 g after 11th fortnight. The minimum weight gain of *Ctenopharyngodon idella* was 10 g, *Hypophthalmichthys molitrix* 22 g, *Pangasius pangasius* 40 g, *Labeo rohita* 5 g and of *Cirrhinus mrigala* 15 g after 18th fortnight in the control treatment (**Table 4**).

The data obtained for monthly weight gain in the control treatment was subjected to statistical analysis for working out ANOVA. The results showed that with respect to fortnightly increase in body weight, there was a non-significant difference among *Ctenopharyngodon idella, Hypophthalmichthys molitrix*, *Pangasius pangasius*, *Labeo rohita* and *Cirrhinus mrigala* (**Table 5**).

The initial and final average weights of *Ctenopharyngodon idella* were 146.55 g and 2265.55 g of *Hypophthalmichthys molitrix* 146.7 g and 976.2 g, *Pangasius pangasius* 248.05 g and 2266.55 g, *Labeo rohita* 99.55 g and 712.05 g, while of *Cirrhinus mrigala* was 94.4 g and 564.4 g, respectively, in treatment T_1. The maximum weight gain of *Ctenopharyngodon idella* was 225 g, *Hypophthalmichthys molitrix* 100 g, *Pangasius pangasius* 210 g, *Labeo rohita* 57.5 g and of *Cirrhinus mrigala* 48 g after 11th fortnight. The minimum weight gain of *Ctenopharyngodon idella* was 57 g, *Hypophthalmichthys molitrix* 18 g, *Pangasius*

pangasius 55 g, *Labeo rohita* 22.5 g and of *Cirrhinus mrigala* 12.5 g after 18th fortnight of growth in treatment T_1.

The initial and final average weights of *Ctenopharyngodon idella* were 150.05 g and 2755.55 g, of *Hypophthalmichthys molitrix* 145.25 g and 1027.25 g, *Pangasius pangasius* 250.05 g and 2821.05 g, *Labeo rohita* 98.05 g and 872.55 g, while of *Cirrhinus mrigala* 94.9 g and 956.4 g, respectively, in treatment T_2. The maximum weight gain in *Ctenopharyngodon idella* was 255 g, in *Hypophthalmichthys molitrix* 102.52 g, *Pangasius pangasius* 265 g, *Labeo rohita* 67.5 g and in *Cirrhinus mrigala* 52.5 g after 11th fortnight of growth. The minimum weight gain in *Ctenopharyngodon idella* was 62.5 g, in *Hypophthalmichthys molitrix* 20 g, *Pangasius pangasius* 110 g, *Labeo rohita* 27.5 g, and in *Cirrhinus mrigala* 19 g after 18th fortnight of growth in treatment T_2.

Total fish production under Control, Treatment T_1 and Treatment T_2

A total of 20 samples of each fish species were stocked in each of control, T_1 and T_2 treatments and fish survival rate was recorded as 100% due to intensive fish culture system. Initial average weights of all fish species in control, T_1 and T_2 were found to be 751.45 g, 640.85 g and 738.3 g, respectively. The final average weights of all fish species under control, T_1 and T_2 were recorded as 824.00 g, 712.05 g and 872.55 g, respectively. The average weight gains in all fish species under control, T_1 and T_2 were recorded as 452 g, 612.5 g and 774.5 g, respectively.

Table 4: Increase in average body weight (g) with time of different fish species of control and two treatments

Fortnights	*C. idella*			*H. molitrix*			*P. pangasius*			*L. rohita*			*C. mrigala*		
/Date	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
28-02-2020	20.0	37.5	46.5	15.0	18.4	23.0	30.0	36.0	42.5	7.0	10.0	17.0	3.0	7.0	11.5
15-03-2020	30.0	60.0	75.0	25.0	25.1	27.5	50.0	52.5	55.0	10.0	17.0	28.5	4.5	10.0	18.0
31-03-2020	50.0	74.0	100.0	30.0	35.0	34.9	70.0	82.5	77.5	15.0	23.0	34.0	8.5	15.0	23.5
15-04-2020	70.0	92.0	27.5	35.0	38.0	39.0	90.0	95.0	100.0	23.0	26.5	37.5	14.0	21.5	28.5
30-04-2020	80.0	110.0	145.0	40.0	47.0	46.0	100.0	110.0	120.0	28.0	30.0	42.0	18.0	26.5	33.5
15-05-2020	90.0	132.5	167.5	45.0	50.0	50.0	110.0	132.5	140.0	35.0	36.5	47.0	23.0	32.5	37.5
31-05-2020	100.0	155.0	182.5	50.0	55.0	55.0	115.0	150.0	165.0	40.0	40.0	53.0	25.0	40.0	42.5
15-06-2020	110.0	182.5	200.0	60.0	61.0	63.0	120.0	177.5	190.0	42.0	45.0	57.0	28.0	42.0	47.5
30-06-2020	120.0	200.0	237.5	70.0	85.0	85.0	130.0	195.1	220.0	45.0	51.5	62.5	30.0	44.0	53.5
15-07-2020	135.0	225.0	255.0	80.0	100.0	102.5	135.0	210.0	265.0	50.0	57.5	67.5	33.0	48.0	52.5
31-07-2020	90.0	200.0	227.5	70.0	85.0	92.5	100.0	180.0	235.0	45.0	51.5	64.0	30.0	43.0	47.5
15-08-2020	85.0	172.5	200.0	50.0	65.0	75.0	90.0	150.0	220.0	38.0	50.0	58.5	28.0	38.0	38.5
31-08-2020	65.0	135.0	187.5	45.0	50.0	57.5	80.0	132.5	192.5	25.0	48.0	54.0	25.1	33.0	32.5
15-09-2020	50.0	112.5	160.0	35.0	44.9	45.0	70.0	105.0	162.5	18.0	41.5	46.5	21.8	25.0	29.0
30-09-2020	30.0	95.0	131.5	30.0	30.0	35.0	60.0	87.0	150.0	15.0	35.0	42.5	20.0	17.5	25.0
15-10-2020	20.0	78.0	100.0	26.0	22.0	31.0	50.0	67.5	126.0	11.0	27.0	35.0	18.0	14.5	21.5
31-10-2020	10.0	57.0	62.5	22.0	18.0	20.0	40.0	55.0	110.0	5.0	22.5	27.5	15.0	12.5	19.0

Table 5: ANOVA (mean squares) of control and each treatment

Source	DF	Control	T_1	T_2
Fortnights	16	2382.12***	5079.78*	6073.06**
Species samples	68	919.17*	2633243.0**	4274.44***
Corrected total	84			

*, significant at 0.05 probability level; **, significant at 0.01 probability level; ***, significant at 0.001 probability level

Table 6: Fish production in control (One acre), treatment T1 (One acre) and treatment T2 (One acre)

Treatments	Standard diet	Diet with 20% CP	Diet with 25% CP
No. of stocked fishes	20	20	20
No. of fishes survived	20	20	20
Survival rate	100%	100%	100%
Initial average weight (g)	751.45	640.85	738.3
Final average weight (g)	824	712.05	872.55
Gain average weight (g)	452	612.5	774.5
Gross fish production/acre/y (kg)	4.89	6.78	8.13
Gross fish production/ha/y (kg)	12.08	16.76	20.09
Net fish production/acre/y (kg)	3.37	5.41	6.66
Net fish production/ha/y (kg)	8.32	13.36	16.44
Total gross fish production/pond/y (kg)	10.18	63.43	75.53
Total gross fish production/acre/y (kg)	10.18	63.43	75.53
Total gross fish production/ha/y (kg)	25.14	156.67	186.55

The total fish production of all five fishes, *Labeo rohita*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, *Pangasius pangasius* and *Ctenopharyngodon idella* as well as overall fish production are presented in **Table 4**. The gross fish production of all five fishes per pond per year in the control, T_1 and T_2 were recorded as 4.89 kg, 6.78 kg and 8.13 kg, respectively. The gross fish production of all five fishes per acre per year in control, treatment T_1 and treatment T_2 was recorded as 4.89 kg, 6.78 kg and 8.13 kg, respectively (**Table 6**).

The net fish production of all five fishes per pond per year in control, T_1 and T_2, were calculated as 3.37 kg, 5.41 kg and 6.66 kg, respectively. The net fish productions of all five fishes per acre per year in control, treatment T_1 and treatment T_2 were calculated as 8.32 kg, 13.36 kg and 16.44 kg, respectively.

Discussion

Growth, survival, reproduction and other biological activities of fish are influenced by water temperature (Hussain et al., 2021). During the whole experimental period, water temperature varied form 10.42 ^{o}C -37.67 ^{o}C in control, 10.51 ^{o}C -37.59 ^{o}C in treatment T_1, and 10.56 ^{o}C -37.45 ^{o}C in treatment T_2. Phytoplankton are considered as the most important source of dissolved oxygen in any fishpond. During the whole experimental period dissolved oxygen (DO) varied from 5.5 mg/L-8.4 mg/L in control, 5.3 mg/L-8.3 mg/L in treatment T_1, and 5.2 mg/L-8.1 mg/L in treatment T_2. Amon et al. (2014) also reported similar findings of water quality parameters. Buentello et al. (2000) reported that DO plays a critical role as a limiting factor for fish growth. Iwama et al. (2000) observed that water quality parameters fluctuate daily. Similarly, Keppeler et al. (2012) also concluded that chemical and biological factors influence water quality parameters of a fishpond.

pH of pond water is also considered the most important limiting factor for fish growth (Uzoka et al., 2012). pH indicates the acidity and alkalinity conditions of a fish pond. It is also called the productivity index of a fishpond (Carlos et al., 2011). Neutral or slightly alkaline pH is the most suitable for fish culture (Ivoke et al., 2007). An acidic pH of water reduces the growth rate, metabolic and other physiological activities of fishes (Uzoka et al., 2015). During the whole experimental period, pH varied from 7.8-8.3 in control, 7.8-8.3 in treatment T_1 and 7.8-8.3 in treatment T_2. Total dissolved solids (TDS) also play an important role in fish production. All natural waters of the planet Earth contain different amounts of dissolved solids. Total dissolved solids (TDS) are an important and useful limnological parameter to estimate chemical nature, quality, and solubility of water (Greenberg et al., 1992). During the whole experimental period, total dissolved solids (TDS) varied from 1317 mg/L-1453 mg/L in control, 1334 mg/L-1450 mg/L in treatment T_1, and 1335 mg/L-1443 mg/L in treatment T_2.

The present findings regarding the physicochemical parameters measured in this study such as temperature, dissolved oxygen (DO), pH and total dissolved solids (TDS) agree with the findings of Okomoda et al. (2016), Dixit et al. (2015) and Rehman et al. (2006) who also recorded all these physicochemical attributes of pond water containing different types of fish species. However, dissimilar findings have been reported by Nakkina et al. (2016) and Hura et al. (2018), which could have been due to selection of different water quality and limnological parameters.

Average weight gain of all cultured five fish species was recorded highest in treatment T_2 followed by that in treatment T_1 and control. The maximum weight gain in T_2 could be easily related to high amount of crude protein (25%) present in the diet of this treatment. The present findings strongly relate to those of Tibihika et al. (2020), Hura et al. (2018), Iram et al. (2013) and Solomon et al. (2007) who reported that increase in dietary protein level in fish diet is favorable for proper fish growth. However, contrasting findings have been reported by Aslam et al. (2017), Mansour et al. (2021) and Islam et al. (2017) who reported that lipid and other ingredients included in diets may greatly affect the fish growth. Overall production of ponds of treatment T_2 was more than that of the T_1 and control ponds. This clearly shows that high amount of crude protein in the fish diet played a significant role in enhancing fish growth and weight gain in this treatment.

Conclusion

In conclusion, 25% crude protein proved to be more efficient in increasing growth performance of different fish species cultured under similar environmental conditions. Therefore, it is recommended that high protein feed in floating or extruded form is much better than the conventional mixture of ingredients.

References

Ahmad, M.H. (2000). Improve productive performance in fish. *Ph.D. Dissertation*, Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt.

Ahmad, M.H., Al-Tawwab, M., Khattab, Y.A. (2010). Effect of dietary protein levels on growth performance and protein utilization in Nile tilapia (*Oreochromis niloticus* L.) with different initial body weights. *In* "The Sixth International Symposium on Tilapia in Aquaculture, Manila, Philippines", pp. 249-263.

Ahmed, I., Ahmad I. (2020). Effect of dietary protein levels on growth performance, hematological profile and biochemical composition of fingerlings rainbow trout, *Oncorhynchus mykiss* reared in Indian Himalayan region. *Aquaculture Reports* 16:100268.

Akter, R., Sumi, K., Ali, M.M., Sarker, M. (2013). Nutritive value of three commercial fish feeds and their effects on the growth of tilapia (*Oreochromis niloticus*) FRY. *Journal of Patuakhali Science and Technology University* 4:29-35.

Ali, M.S., Islam, M.S., Begum, N., Suravi, I.N., Mia, M., Kashem, M.A. (2017). Effect of monoculture and polyculture systems on growth and production of fishes in seasonal water bodies of Haor villages, Sunamganj District. *Journal of Scientific Research* 9(3):307–316.

Ali, S.S. (1999). Freshwater fishery biology. *In* "Pond Fish Culture", pp. 236-251. Naseem Book Depot Ltd. Shahra-e-Quaid-e Azam, Hyderabad, Pakistan.

Aslam, S., Zuberi, A. (2017). Effect of duckweed by replacing soybean in fish feed on growth performance of grass carp *Ctenopharyngodon idella* and sliver carp *Hypophthalmichthys molitrix*. *International Journal of Fisheries and Aquatic Studies* 5(5):278-282.

Ayub, A., Rasool, F., Khan, N., Qaisrani, S.N., Parveen, S., Anjum, K.M., Fatima, M., Matiullah, Mahmood, S., Zulfiqar, T. (2021). Limiting amino acids supplementation in low crude protein diets and their impacts on growth performance and carcass composition in *Labeo rohita* (rohu) adult fish. *Brazilian Journal of Biology* 83:e249422.

Banerjee, S.M. (1967). Water quality and soil conditions of ponds in some states of India in relation to fish production. *Indian Journal of Fisheries* 14:115-144.

Buentello, J.A., Gatlin, D.M., Neill, W.H. (2000). Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish. *Aquaculture* 182:339-352.

Carlos, E.C., Garcia, L.O., Kochhann, D., Cunha, M.A., Becker, A.G., Baldisserotto, B. (2011). Low water hardness and pH affect growth and survival of silver catfish juveniles. *Ciencia Rural, Santa Maria* 41(8):1482-1487.

Chakrabarti, N.M. (1998). "Biology, Culture and Production of Indian Major Carps", pp. 175. Fisheries Scientist in Indian Council of Agricultural Research, India.

Chaudhuri, H., Chakrabarty, R.D., Rao, N.G.S., Janakiram, K., Chatterjee, D.K., Jena, S. (1974). Record fish production with intensive culture of Indian and exotic carps. *Current Science* 43:303-304.

Cowey, C. (1976). Use of synthetic diets and bio-chemical criteria in assessment of nutrients requirements of fish. *Journal of Fisheries Research Board of Canada* 33:1040-1045.

Craig, S. (2002). "Understanding Fish Nutrition, Feeds and Feeding", Virginia Cooperative Extension, Virginia State University.

Delgado, C.L., Rosegrant, M.W., Wada, N., Meijer, S., Ahmed, M. (2002). "Fish as Food: Projections to 2020 under Different Scenarios", Markets and Structural Studies Division, International Food Policy Research Institute, 2033 K Street, N.W. Washington, D.C. 20006 U.S.A.

Dixit, A.K., Pandey, S.K., Mehta, R., Ahmad, N., Gunjan Pandey, J. (2015). Study of physico-chemical parameters of different pond water of Bilaspur district, Chhattishgarh, India. *Environmental Skeptics and Critics* 4(3):89-95.

FAO (1996). "Aquaculture Potential in African Documentation Issued on the Occasion of the World Food Summit in Rome, November 1996. Rome, FAO, pp. 20.

Greenberg, A.E., Clesceri, L.S., Eaton, A.D. (1992). "Standard Methods for the Examination of Water and Wastewater", 18th ed. American Public Health Association Publication (APHA), Washington DC.

Hassan, H.U., Ali, Q.M., Ahmad, N., Masood, Z., Hossain, M.Y., Gabol, K., Khan, W., Hussain, M., Ali, A., Attaullah, M., Kamal, M. (2021). Assessment of growth characteristics, the survival rate and body composition of Asian sea bass *Lates calcarifer* (Bloch, 1790) under different feeding rates in closed aquaculture system. *Saudi Journal of Biological Sciences* 28(2):1324-1330.

Hepher, B. (1988). "Nutrition of Pond Fishes", Cambridge University Press, Sydney.

Hura, M.U.D., Zafar, T., Borana, K., Prasad, J.R., Iqbal, J. (2018). Effect of commercial probiotic *Bacillus megaterium* on water quality in composite culture of major carps. *International Journal of Current Agricultural Sciences* 8(1):268-273.

Hussain, M., Hassan, U.H., Siddique, M.A., Mahmood, K., Abdel-Aziz, M.F., Laghari, M.Y., Abro, N.A., Gabol, K., Rizwan, S. (2021). Effect of varying dietary protein levels on growth performance and survival of milkfish *Chanos chanos* fingerlings reared in brackish water pond ecosystem. *The Egyptian Journal of Aquatic Research* 47(3):329-334.

Iram, A., Ahmed, N., Amir, I., Hussain, T., Saif, F., Naz, S. (2013). Growth performance of indigenous and exotic fish species after yearling stage in polyculture system. *Middle East Journal of Scientific Research*16 (2):256-263.

Ivoke, N., Mgbenka, B.O., Okeke, O. (2007). Effect of pH on the growth performance of *Heterobranchus bidorsalis* (♂) x *Clarias gariepinus* (♀) hybrid juveniles. *Animal Research International* 4(1):639-642.

Iwama, G.K., Vijayan, M.M., Morgan, J.D. (2000). "The Stress Response in Fish. Ichthyology, Recent Research Advances", Oxford and IBH Publishing Co, Pvt. Ltd, New Delhi.

Kader, M.A., Hossain, M.A., Hossain, M.D. (2003). A comparative study on the effect of commercial fish feeds on the growth of Thai Pangas, *Pangasius hypophthalmus*. *Bangladesh Journal of Fisheries Research* 7(1):53-58.

Karapan, A. (2002). Studies to optimize polyunsaturated fatty acid composition of tilapia for human consumption in S.E. Asia. *Aquaculture News* 28:6-7.

Keppeler, E.C., Valenti, P.M.C.M., Pereira, L.V. (2012). Temporal variation in the water quality of ponds and effluent of grow-out ponds of Amazon River prawn *Macrobrachium amazonicum*. *Revista Peruana de Biología* 19:299-306.

Keshavanath, P., Shivanna, Gangadhara, B. (2006). Evaluation of sugarcane by-product press mud as a manure in carp culture. *Bioresource Technology* 97(4):628–634.

Kim, K.W., Kim, K.D., Han, H.S., Won, S., Moniruzzaman, M., Lee, J.H., Choi, Y.H., Bai, S.C. (2017). Evaluation of the dietary protein requirement of a selectively bred (F-5 generation) strain of olive flounder, *Paralichthys olivaceus*. *Turkish Journal of Fisheries and Aquatic Science* 17:1009-1016.

Kim, K.W., Wang, X.J., Bai, S.C. (2002). Optimum dietary protein level for maximum growth of juvenile olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). *Aquatic Research* 33:673-679.

Koprucu, K. (2012). Effects of dietary protein and lipid levels on growth, feed utilization and body composition of juvenile grass carp, *Ctenopharyngodon idella*. *Journal of Fisheries Science* 6(3):243-251.

Louka, N., Juhel, F., Fazilleau, V., Loonis, P. (2004). A novel colorimetry analysis used to compare different drying fish processes. *Food Control* 15:327-334.

Mahmud, N.A., Hasan, M.D.R., Hossain, M.B., Minaz, M.H. (2012). Proximate composition of fish feed ingredients available in Lakshmipur region, Bangladesh. *American-Eurasian Journal of Agricultural and Environmental Sciences* 12(5):556-560.

Mansour, A.T., Allam, B.W., Srour, T.M., Omar, E.A., Nour, A.A.M., Khalil, H.S. (2021). The feasibility of monoculture and polyculture of striped catfish and Nile tilapia in different proportions and their effects on growth performance, productivity, and financial revenue. *Journal of Marine Science and Engineering* 9(6):586.

Miah, M.S., Uddin, M.S., Shah, M.S. (1997). Effect of stocking ratios on the growth and production of fishes in mixed polyculture system. *Bangladesh Journal of Fisheries* 20:135-138.

Mohsen, N.A. (2012). Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticus* (L.). *International Aquatic Research* 4:3.

Mohseni, M., Pourkazemi, M., Hosseni, M.R., Hassani, M.H.S., Bai, S.C. (2013). Effects of the dietary protein levels and the protein to energy ratio in sub-yearling Persian sturgeon, *Acipenser persicus* (Borodin). *Aquaculture Research* 44:378-387.

Nakkina, M. (2016). Study of growth rate in Nile tilapia (*Oreochromis niloticus*). *Journal of Aquaculture Research & Development* 7:8.

Nandakumar, S., Ambasankar, K., Ali, S.S.R., Syamadayal, J., Vasagam, K. (2017). Replacement of fish meal with corn gluten meal in feeds for Asian seabass (*Lates calcarifer*). *Aquaculture International* 25:1495-1505.

National Research Council (NRC) (1993). "Nutrient Requirements of Fish", National Academy Press, Washington DC.

National Research Council (NRC). (2011). "Nutrient Requirements of Fish and Shrimp", National Academy Press, Washington DC.

Nwanna, L.C., Omojola, I., Nwanna, E., Abiodun E. (2014). Effects of protein deficient diets on the growth performance and carcass protein ash ratio of African catfish *Clarias gariepinus*. *Journal of Applied Sciences and Environmental Management* 18(3):537-541.

Okomoda, V.T., Tiamiyu, L.O., Lortim, M. (2016). The effect of water renewal on growth of *Clarias gariepinus* fingerlings. *Croatian Journal of Fisheries* 2016:25-29.

Parveen, S., Ahmed, I., Mateen, A., Hameed, M. (2020). Replacement of fish meal by corn gluten meal in fish feed: impacts on growth and body composition of *Labeo rohita* and overall production of fish pond. *Pakistan Journal of Agricultural Sciences* 57(5):1271-1278.

Pillay, T.V.R. (1990). "Aquaculture: Principles and Practices. Fishing News Book", pp. 575. Blackwell Scientific Publications, Ltd., Oxford, UK.

Razvi, M. (2006). "Lahore-Absolutely Fishy: Nutritional Value", pp. 12–13. The Review, Dawn, Lahore, Pakistan.

Rehman, M.H., Ahmed, I., Javaid, A., Akhtar, H.M.N. (2006). Comparative growth and yield of exotic Chinese and indigenous major carps under polyculture system. *Journal of Agriculture and Social Sciences* 2(4):256-259.

Rudolf, K. (1971). "Fish Inspection and Quality. Fishing News Books Ltd.", London, UK.

Sahu, P.K., Jena, J.K., Das, P.C., Mondal, S., Das, R. (2007). Production performance of *Labeo calbasu* (Hamilton) in polyculture with three Indian major carps *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) with provisions of fertilizers, feed and periphytic substrate as varied inputs. *Aquaculture* 262:333-339.

Salim, M. (2006). Role of fish as food to human nutrition. *In* "International Conference on Solving Problems of Freshwater Fish Farming in Pakistan", pp. 20. The University of Veterinary and Animal Sciences, Lahore, Pakistan.

Sandhu, G.S. (2005). "A Textbook of Fish and Fisheries", pp. 39-40. Dominant Publishers and Distributors, New Delhi, India.

Shoko, A.P., Limbu, S.M., Mrosso, H.D.J., Mgaya, Y.D. (2014). A comparison of diurnal dynamics of water quality parameters in Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) monoculture and polyculture with African sharp tooth catfish (*Clarias gariepinus*, Burchell, 1822) in earthen ponds. *International Aquatic Research* 6:56.

Solomon, S.G., Tiamiyu, L.O., Agaba, U.J. (2007). Effect of feeding different grain sources on the growth performance and body composition of tilapia, *Oreochromis niloticus* fingerlings fed in outdoor hapas. *Pakistan Journal of Nutrition* 6(3):271-275.

Steel, R.G.D., Torrie, J.H., Dinkey, D.A. (1996). "Principles and Procedures of Statistics", 2nd ed. McGraw Hill Book Co. Singapore.

Stickney, R.R. (1979). "Principles of Warmwater Aquaculture", pp. 375. John Wiley and Sons, New York.

Tibihika, P.D.M., Barekye, A., Byakora, E. (2015). Critical analysis of the growth performance of three fish species; mirror carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and sharp toothed African catfish (*Clarias gariepinus*), cultured in earthen ponds in Kigezi region South western Uganda. *International Journal of Fisheries and Aquatic Studies* 2(3):63-73.

Uzoka, C.N., Anyanwu, J.C., Uche, C.C.,Ibe, C.C., Uzoma, A. (2015). Effect of pH on the growth performance and survival rate of *Clarias gariepinus* Fry. *International Journal of Research in Biosciences* 4(3):14-20.

Uzoka, C.N., Nwigwe, H.C., Ihejirika, C.E., Ibe, C.C., Onwuagba, J.I. (2012). Growth and survival of hatchlings of *Clarias gariepinus* subjected to various pH treatments. *Journal of Biodiversity and Environmental Sciences* 2(10):35-39.

Yildrim, O. (2008). Aquafeed industry in Turkey: Its aquafeed projections towards the year 2015. *Turkish Journal of Fisheries and Aquatic Science* 8:93-98.

Yones, A.M.M. (2015). Partial and complete replacement of fish meal with soybean meal in diets of Nile tilapia, *Oreochromis niloticus* and mullet *Mugil cephalus* reared in polyculture system. *Egyptian Journal of Experimental Biology* 1:51-55.