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Full Length Research Paper

Effect of similar feeding regime on growth and body composition of Indian major carps (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*) under mono and polyculture

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Growth performance and body composition of yearling Indian major carps (*Catla catla, Cirrhinus mrigala* and *Labeo rohita*) was evaluated in semi-intensive (mono and polyculture) systems for 90 days. Prior to stocking, all ponds were fertilized with organic and inorganic manures. This application was repeated every two weeks throughout the study period. Supplementary feed containing 35% protein was applied daily at 3% of wet body weight. In trial 1, all the three species gained significantly higher weights with experimental feed (F1) versus control group (F0). There was non-significant difference observed among species. In trial 2, non-significant difference was observed for net weight gain among species and between feeds. The feed conversion ratio (FCR), protein efficiency ratio (PER), protein utilization (PU), gross nitrogen retention efficiency (GNRE%) and gross energy retention efficiency (GERE%) were found non-significantly different among species in both trials, except GNRE% in polyculture, where *L. rohita* showed significantly higher values than its counterparts. No significant difference was observed in body composition and mineral contents among species and between feeds in both trials. In conclusion, all the three fish species performed well under monoculture system with 35% protein diet and showed significantly higher growth than the control, compared to polyculture, without any significant effect on body composition.

Key words: Artificial feed, Indian major carps, growth, proximate composition, nutrient retention efficiency, cost effectiveness.

INTRODUCTION

Food from aquatic resources is an important dietary component in many countries, the demand for which will continue in the forthcoming years [Food and Agriculture Organization (FAO), 2000; Brugere and Ridler, 2004; FAO, 2006; Failler, 2006; De Silva and Davy, 2010]. Aquaculture is considered to be an important food, providing 50% of the global food fish consumption and over the last few decades it has gained considerable scale in Asia (Tacon and Dominy, 1999; De Silva and Davy, 2010), utilizing variety of available resources to develop fish industry through simple aquaculture practices (Gerking, 1966).

Worldwide, approximately 80% of carps and 65% tilapia are cultured without modern compound feeds (Naylor et al., 2000). Aquaculture in Pakistan is a very new activity and there is huge potential for development due to its rich aquatic resources. The total area under fish

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ponds is about 60,470 hectares (Akhtar, 2001). Semi-intensive fish culture system, with minimum inputs, are predominant and are a primary approach for fish production, providing the majority of fish for domestic and commercial consumption (FAO, 1996). This system helps to effectively utilize all the ecological niches in the aquatic environment, with the stocking of fish species having different feeding habits (Lutz, 2003). Recently, China has initiated intensive culture of carps and other omnivorous species which are more feed intensive to enhance per unit fish production (Cremer et al., 1999; Crook et al., 1999).

In fish culture operations, feed is the major input and represents up to 60% of the total expenditure (De Silva, 1988; Li and Wang, 2004). Supplementary feed is offered in the presence of natural food to augment fish growth (Devaraj and Krishna, 1981). A number of studies have been conducted on fingerlings of Indian major carps and other warm water species in the laboratory and earthen ponds (Hossain et al., 1997; Mazid et al., 1997; Nandeesha et al., 2001; Sarker et al., 2000; Azim et al., 2002; Islam, 2002; Jabeen et al., 2004; El-Ebiary, 2005; Saeed et al., 2005; Biswas et al., 2006; Rahman et al., 2006; Singh et al., 2006; Mondal et al., 2007; Rowland et al., 2007; Sahu et al., 2007; Siddiqui and Khan, 2009; Khan and Abidi, 2010). Work in field condition on grow-out fish is rare. However, these studies can provide important analysis of economic viability of the supplementary feed, which comprises single or more than one ingredient in monoculture, as well as in polyculture system. Provision of more nutrient rich and balanced feed can further enhance the existing level of fish production to cater for the continuously emerging demands of the masses.

The present study is therefore designed with a view to formulate a supplementary feed from locally available ingredients for yearling fish of Indian major carps, and its effect on comparative growth performance, body composition and economic viability, under prevailing fish culture practices.

MATERIALS AND METHODS

Location

The study was conducted at Research and Training Facilities, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki- Pakistan.

Experimental design

Trial 1 (monoculture) was conducted in 12, while trial 2 (polyculture) in 4 earthen ponds with an area of 0.03 ha each, for 90 days. The experiments were designed, following completely randomized design. All the treatment groups had three replicates and one control in both the trials.

Pond preparation

The ponds were cleaned and exposed to sunlight. All the ponds were filled with tube well water up to 1.5 m, and same level was maintained throughout the study period by compensating the daily losses. Prior to stocking of fish, all ponds were fertilized with cattle dung at 90 kg/pond (3 ton/ha) (Jena and Das, 2006; Sahu et al., 2007) and poultry manure at 45 kg (50% of cattle dung), 2.5 kg single super phosphate and 1.25 kg urea/pond to accelerate the production of planktonic life. These doses were repeated after every two weeks during the study period to maintain a constant level of plankton.

Experimental fish and stocking density

Yearling of Indian major carps (*C. catla, L. rohita* and *C. mrigala*) were used as experimental animals. Fish were collected from the local fish hatchery and farm, and were randomly stocked at 70/pond (2333/ha) in monoculture. In polyculture ponds, ratio of 30% *C. catla*, 20% *C. mrigala* and 50% *L. rohita* was maintained, with a total 100 fish/pond. All the fish were weighed and measured individually before initiation of each feeding trial. Later on, random sample of 20 fish of each species was taken every two weeks from all ponds, weighed and measured, and then released into respective pond.

Procurement of ingredients, feed formulation and preparation

Feed formulation and composition percentage of ingredients used is given in Table 1. All the ingredients were fine ground individually, mixed, steam cooked at 140°C and passed through 5 mm extruder die, dried and crumbled to prepare a mash feed at National Feed Mill, Sheikhupura, Pakistan, and were transported to experimental site and carefully stored.

Feeding protocol

The fish were fed twice a day at 3% body weight by dusting method at 8:30 to 9:00 and 16:30 to 17:00 h (Javed and Sial, 1991). F1 represents experimental feed and F0 control. Feeding rate was adjusted after every 2 weeks with fish growth increments.

Other growth parameters

Condition factor (K), net weight gain (NWG), percent gain in weight, specific growth rate (SGR%), feed conversion ratio (FCR), protein efficiency ratio (PER), protein utilization (PU), gross nitrogen retention efficiency (GNRE%) and gross energy retention efficiency (GERE%) were calculated, according to the following formulae:

Condition factor (K) = $W \times 10^5/L^3$

The value 10^5 is the factor bringing the ponderal index or condition factor (K) near the unity (Carlander, 1970).

Net weight gain (NWG) = Average final weight (g) - Average initial weight (g)

Percent weight gain = Final weight (g) - Initial weight (g) / Initial weight (g) × 100

Specific growth rate was estimated by the formula given by Hopkins (1992).

SGR% = ln (Final wet body weight) - ln (Initial wet body weight) / Number of days × 100

Table 1. Formulation and percentage composition of experimental feed (F1).

Ingredient	Composition (%)
Fish meal	25
Soy bean meal	32
Canola meal	15
Wheat bran	5
Rice polish	5
Maize grains	8
Molasses	8
Mineral mixtures	1
Vitamins	1
Total	100

Feed Conversion Ratio (FCR) = Feed intake (g) / Wet weight gain (g)

Protein Efficiency Ratio (PER) = Wet weight gain of fish (g)/protein intake (g)

Protein Utilization (PU) = protein content (g) fish at the end of experiment - protein content (g) fish at the start of experiment / dry protein fed (g)

Gross Nitrogen Retention Efficiency (GNRE%) = {[(FBW × N content$_{final}$) - (IBW × N content$_{initial}$)] / GNI} × 100

Where: FBW = final body weight; IBW = initial body weight; GNI = gross nitrogen intake.

Gross Energy Retention Efficiency (GNRE%) = {[(FBW × E Content$_{final}$) - (IBW × E content$_{initial}$)] / GEI} × 100

Where: FBW = final body weight; IBW = initial body weight; GEI = gross energy intake.

Proximate analysis

Feed and feed ingredients were analyzed for dry matter, moisture, crude proteins, crude lipids, ash, amino acids, minerals, gross energy and crude fiber. Nitrogen free extract (NFE) was calculated by difference. Fish reared on different feeds were also analyzed for same parameters to assess its nutritional value following (Association of Official Analytical Chemists (AOAC), 2003). All samples were dried in a vacuum oven (Model: 524 Precision Scientific, USA) at 105°C for 18 h to determine dry matter. Crude protein was determined by Kjeltec Auto analyzer Tecator 1030 (FOSS, Hoganas, Sweden). Samples were digested in sulphuric acid (15 ml) at high temperature (415°C) in the presence of potassium sulphate and copper sulphate (catalyst). Crude fat was determined by Soxtec System (Model: HT 1043 Extraction Unit Tecator, Hoganas, Sweden), using diethyl ether as a solvent. Ash contents were determined by incinerating 1 g of the samples in a muffle furnace (Thermolyne, Dubuque, Iowa, USA) at 550°C overnight. Crude fiber content of each feed and feed ingredients was determined by digesting dry sample in 1.25% H_2SO_4, followed by 1.25% NaOH solutions in ankom fiber analyzer (Ankom 200/220, Model: A200, Macedon, NY, USA). Gross energy was determined bomb calorimeter (Parr 6300 Calorimeter, Moline, IL, USA), using benzoic acid as a standard.

Calcium, magnesium and potassium, were determined by atomic absorption spectrophotometry (Model: Z-8100 polarized zeeman atomic absorption spectrophotometry, Hitachi, Limited, Tokyo Japan) (Association of Official Analytical Chemists, 2002), while phosphorus was determined by colorimetric spectrophotometer at 400 nm wavelength (Spectronic 1201 and 1001-plus, Milton Roy, Ivyland Road, USA). Amino acids profile was determined by Water Acquity UPLC System (Milford, Massachusetts, USA) according to Bidlingmeyer et al. (1987) and Cohen and Strydom (1988). The system comprises a binary solvent manager module, a sample manager module, a TUV detector module and a Water Acquity UPLC BEH C18 column (2.1 × 100 mm). Column temperature was adjusted at 48°C. Amino acids were detected on Water Acquity TUV Detector at 254 nm. Data was collected, stored and processed, using waters empower 2 chromatography software. Drying was done using a TOMY CC-181 centrifugal concentrator (Tokyo, Japan), with a Sargent-Welch Model 8821 vacuum pump (Skokie, Illinois, USA).

Water quality parameters

Water temperature, dissolved oxygen (DO), pH, salinity, total dissolved solids (TDS), electrical conductivity, and secchi disc visibility were observed on daily basis. Nitrates were determined on fortnightly basis. Water samples were collected between 9.00 and 10.00 a.m (American Public Health Association APHA, 1998).

Statistical analysis

Data were analyzed using general linear model (GLM) procedure and the mixed procedure of Science Analysis System (SAS) (2009) software version 9.2 (TSIM0), SAS Institute, Cary, NC, USA. Pair wise comparisons were made, using Tukey adjustment. Normality and homogeneity of variance were verified, and transformation was used if necessary. The results were accepted at the probability value of 0.05 or less for significant difference. The results in the tables are presented as mean, with pooled mean standard error (PSEM) throughout the text.

RESULTS

Proximate analysis, mineral composition of experimental feed (F1), and feed ingredients are given in Table 2, whereas amino acid profile of the experimental feed is presented in Table 3.

In monoculture (trial 1), a significant difference ($P \leq 0.05$) was observed in the final body weight, net increase in weight, final body length and gain in length among different species *C. catla*, *C. mrigala* and *L. rohita* and between treatment and control group. Condition factor (K) was also found significantly different ($P \leq 0.05$) among species. SGR%, FCR, PER, PU, GNRE% and GERE% did not differ among all the three fish species (Table 4).

In polyculture (trial 2), significant difference ($P \leq 0.05$) was observed in the final weight gain and final body length among the fish species fed supplemented feed (F1) and in the control (F0). Condition factor (K) was found significantly different ($P \leq 0.05$) among species

Table 2. Proximate composition of experimental feed (F1) ingredients for grow-out of Indian major carps.

Proximate composition	F1	Fish meal	Soybean meal	Canola meal	Wheat bran	Rice polish	Maize grain
Dry matter (%)	89.6	90.4	89.4	91.1	83.9	90.0	88.4
Crude protein (%)	35.2	42.9	45.9	39.5	15.5	13.9	9.0
Crude lipid (%)	4.0	12.4	0.7	1.03	2.3	10.9	4.3
Ash (%)	16.9	31.9	8.9	7.3	5.4	15.7	1.3
Crude fiber (%)	7.4	2.6	10.9	13.2	13.1	39.2	2.81
Nitrogen free extract (%)	26.1	0.7	22.9	30.1	47.6	10.3	71.0
Gross energy (MJ g^{-1})	17.9	17.3	18.9	19.1	18.3	18.6	18.5
Mineral composition							
Ca	2.8	4.3	0.3	0.8	0.2	0.3	1.6
Mg	0.4	0.06	0.2	0.5	0.4	0.5	0.2
K	1.5	0.1	1.1	1.2	1.3	1.1	0.3
P	0.9	1.6	0.5	1.0	1.0	1.2	0.1

(Table 5). Net weight gain, SGR% FCR, PER, PU, and GERE% were also non significant ($P > 0.05$) among species, as well as between treated and control groups. Gross nitrogen retention efficiency (GNRE%) was comparatively higher in *L. rohita* ($P \leq 0.05$) than its counter parts (*C. catla* and *C. mrigala*) (Table 5). Nutrient level was comparatively higher in the treated than in the control but differences were statistically not distinguishable ($P > 0.05$) (Tables 6 and 7).

In monoculture, the mean values of key physico-chemical parameters such as temperature, ranged from 25.5 to 25.9°C, dissolved oxygen 4.8 to 5.6 mg L^{-1}, pH 7.6 to 7.5, light penetration 19.3 to 28.3 cm, salinity 0.9 to 1.4 g L^{-1}, total dissolved solids 1725 to 1879 mg L^{-1}, electrical conductivity 2.0 to 2.3 µS cm^{-1} and nitrates 0.2 to 0.4 mg L^{-1}, respectively in both the trials. All the parameters showed non-significant differences ($P > 0.05$) among species and between feeds except dissolved oxygen, which was found significantly ($P \leq 0.05$) higher in *L. rohita* and *C. mrigala* ponds than *C. catla*.

In polyculture system, the water quality parameters were found non-significantly different ($P > 0.05$). The mean values of temperature ranged from 28.3 to 28.4°C, dissolved oxygen 4.9 to 5.4 mg L^{-1}, pH 7.5 to 7.6, total dissolved solids 1643 to 1654 mg L^{-1}, nitrates 0.2 to 0.3 mg L^{-1}, light penetration was 24.2 cm, salinity was 0.9 g L^{-1} and electrical conductivity was 2.1 µS cm^{-1}.

Total fish production, using 35% protein in monoculture, was recorded as 9019.3 kg and in polyculture 4571.66 kg/ha/year. Total cost of feed and fertilizers in monoculture was Rs. 910854/ha/year and Rs. 848188/ha/year in polyculture system, respectively. Total income in both experiments, under different culture systems were Rs.1803867 and Rs. 914333/ha/year, respectively. The results of net profit for polyculture system projection was indicated with a positive trend of Rs. 66145/ha/year, while for monoculture system, a negative cash flow of Rs. -106987/ha/year was found.

DISCUSSION

The analysis of experimental feed revealed that feed was deficient in some essential amino acids (lysine 1.34% and tryptophan 0.03%) as compared to optimum requirement (lysine 2.3% and tryptophan 0.38 to 1.13% (Murthy and Varghese, 1997; Ahmed and Khan, 2004, 2005) for *L. rohita* and *C. mrigala*. The deficiency of essential amino acids resulted in reduced growth and higher feed conversion (Wilson, 1985). Higher ash contents (31.97%) in fish meal used in the experimental feed indicate that fish meal might not be of a high quality, which failed to provide required amino acids and resulted in poor growth of fish. The higher ash content (31.97%) in fish meal is another indicator of its poor quality. According to Giri et al. (2010) fish meal in India and also in neighboring countries is not of high quality and is mostly processed from traditionally dried by-catch fishes and mainly comprised of bony fishes with low nutritive value and high level of inorganic ash.

In monoculture system, experimental feed application (F1) resulted in significantly ($P \leq 0.05$) higher growth of all the fish species, as compared to control (F0), but these differences were mitigated in polyculture system. Length increments followed the same pattern as in growth, since all the three fish species performed well in experimental diet (F1) as compared to control (F0) in monoculture system. In polyculture system, growth trend of three species was comparatively higher in experimental diet (F1) versus control (F0), but not statistically significant ($P > 0.05$). There was no significant difference among three fish species in both experiments. This suggests that experimental diet was equally acceptable to all the three fish species.

The overall growth performance of grow-out fish in both experimental trials, under different culture systems, with the application of 35% protein supplementary feed remained poor. A possible reason of poor fish growth

Table 3. Analyzed amino acid composition (% dry matter basis) of the experimental feed (F1) and requirement of Indian major carps.

Amino acid	Experimental feed	Requirement (%) of dry feed	Reference
Essential amino acids	**F1**		
Arginine	1.9	1.8	Ahmed and Khan (2004)
Histidine	0.6	0.9	Ahmed and Khan (2005)
Isoleucine	1.2	1.3	Benakappa and Varghese (2003)
Leucine	2.0	1.7	-do-
Valine	1.3	1.6	-do-
Lysine	1.3	2.3	Ahmed and Khan (2004)
Methionine	0.4	1.2	Ahmed et al. (2003)
Cystine	0.4	-	-
Phenylalanine	1.3	1.8	Benakappa and Varghese (2004)
Tyrosine	0.8	1.0	Khan and Abidi (2007)
Thrionine	0.9	1.3	Ahmed (2007)
Tryptophan	0.03	1.1	Murthy and Varghese (1997)
Non-essential amino acids			
Alanine	1.4	-	-
Aspartic acid	2.2	-	-
Glutamic acid	4.4	-	-
Glycine	1.6	-	-
Proline	1.5	-	-
Serine	1.0	-	-
Total	23.8		

might be due to low appetite and low feed utilization (Islam, 2002). This low growth might be due to the inclusion of higher amount of plant based protein soybean meal and canola meal in the experimental feed (F1) that contains a variety of anti-nutritional substances (Francis et al., 2001; Islam, 2002) which could not manage to digest, and ultimately, they affected the protein utilization and digestion of useful nutrients (Makkar, 1993). High amount of ash contents in fish meal used in experimental feed might hinder the digestibility, as well as nutrient utilization by fish that cause poor growth in fish (Giri et al., 2010). The comparatively lower temperature values were recorded in both study trials conducted in autumn (September to November), when temperature was on declining trend which may also affect the growth. Fish are poikilothermic by nature and temperature plays a significant role in controlling most aspects of their metabolism, feed utilization and growth (De Silva and Anderson, 1995). SGRs values of C. catla were higher in experimental feed (F1) than control (F0) in experiment 1, followed by L. rohita and C. mrigala. In polyculture system, L. rohita showed higher SGR values, followed by C. catla and C. mrigala. Differences between two treatments however, were statistically not significant ($P > 0.05$). Similar minor variation of SGR values in carps were also reported by Azim et al. (2001) and Sahu et al. (2007). The growth observed in present studies, on supplementary feed with high crude protein, agrees well with previous studies (Shankar, 1988; Joshi et al., 1989; Sunder et al., 1998; Islam, 2002), while SGR values are comparable with those of Dhawan and Kaur (2002) whose SGR values ranged from 0.9 to 1.23 for *C. catla* and *L. rohita*, in pig dung ponds.

Natural feed contributed to fish growth in both fed and non-fed ponds. Sohail (2010) observed in his studies that the amount of chlorophyll ‘a’ pigment and plankton abundance in fish, fed on supplemented diet (F1) and control (F0) pond of *C. catla, C. mrigala* and *L. rohita* was high in pond containing *C. mrigala*, ranging from 2.056 to 4.421 µg L^{-1} than pond containing *C. catla*, where it was low (ranging from 0.313 to 0.768 µg L^{-1}). It was also observed that treated ponds showed significantly higher ($P \leq 0.05$) chlorophyll ‘a’, phytoplankton and zooplanktons than control.

The importance of fertilizers and supplementary feed in increasing fish production, in commercial carp polyculture system, has also been well documented by Chaudhuri et al. (1975), Rappaport et al. (1977), Geiger (1983), Moll (1986), Nandeesha (1993) and Garg and Bhatnagar (1996). The results of significantly higher growth in treated (F1) in trial 1 are in line with the findings of Yadava and Garg (1992) and Mahboob et al. (1995), who observed higher production of major carps, with the combination of supplementary feed, organic and

Table 4. Growth performance of three species of Indian major carps grow out fed 35% protein diet (F1) with control (F0) for 90 days in monoculture system.

Parameter	Species/feed								ANOVA P value		
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*						
	F0	F1	F0	F1	F0	F1	SEM F0	SEM F1	Species	Feed	Sp* feed
Initial weight (g)	365.4	339.3	334.1	323.6	312.6	355.8	27.3	15.8	0.82	0.95	0.66
Final weight (g)	562.5^{a}	711.1^{b}	496.2^{a}	594.4^{b}	539.2^{a}	666^{b}	35.4	20.5	0.26	0.02	0.88
Net weight gain (g)	197.1^{a}	371.9^{b}	162.2^{a}	270.8^{b}	226.6^{a}	310.2^{b}	27.5	15.9	0.26	0.00	0.52
Initial length (mm)	292.2	292.4	313.4	311.1	289.9	292.4	5.8	3.4	0.07	0.99	0.96
Final length (mm)	341.7^{a}	364.6^{b}	349.9^{c}	368.1^{d}	343.7^{ac}	364.7^{bd}	0.01	0.004	0.05	0.00	0.55
Gain in length (mm)	49.5^{a}	72.2^{b}	36.5^{a}	57.0^{b}	53.8^{a}	72.3^{b}	5.7	3.3	0.17	0.02	0.96
Percent gain in weight	53.9	109.8	48.5	84.3	72.5	90.9	10.3	7.0	0.44	0.14	0.06
SGR%	0.4	0.8	0.4	0.7	0.5	0.7	0.08	0.05	0.69	0.13	0.14
K	1.4^{a}	1.4^{a}	1.1^{c}	1.1^{c}	1.3^{b}	1.4^{b}	0.02	0.01	0.00	0.19	0.61
FCR	0	3.7	0	4.6	0	4.6	0	0.3	0.07		
PER	0	0.8	0	0.6	0	0.6	0	0.05	0.05		
PU	0	0.4	0	0.3	0	0.4	0	0.05	0.21		
GNRE%	0	9.5	0	5.8	0	8	0	1.02	0.12		
GERE%	0	29.7	0	24	0	20.9	0	1.9	0.07		

F0 = Control; F1= feed 1; SEM F0 = pooled standard error of diet F0; SEM F1 = pooled standard error of feed F1; SGR% = specific growth rate; K= condition factor; FCR = feed conversion ratio; PER = protein efficiency ratio; PU = protein utilization; GNRE% = gross nitrogen retention efficiency; GERE% = gross energy retention efficiency. Values in the same row with different superscripts are significantly ($P \leq 0.05$) different; means are of three replicates of F1 and one of F0.

Table 5. Growth performance of three species of Indian major carps grow-out fed 35% protein diet (F1) with control (F0) for 90 days in polyculture system.

Parameter	Species/feed								ANOVA P value		
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*						
	F0	F1	F0	F1	F0	F1	SEM F0	SEM F1	Species	Feed	Sp*feed
Initial weight (g)	310.7	300.2	403	370.7	312.5	309	40.9	23.6	0.19	0.77	0.93
Final weight (g)	493^{a}	607.2^{b}	564.7^{a}	674.5^{b}	535.9^{a}	679.5^{b}	16.8	9.7	0.19	0.02	0.86
Net weight gain (g)	182.3	307.1	161.7	303.9	223.4	370.5	50.8	29.3	0.13	0.14	0.91
Initial length (mm)	273.1^{a}	276.1^{a}	338.9^{b}	329.6^{b}	292.4^{ac}	291.1^{ac}	19.8	11.4	0.01	0.94	0.95
Final length (mm)	315.2^{a}	349.9^{b}	366^{c}	383.2^{d}	336.8^{ac}	367.4^{bd}	2.9	1.7	0.02	0.01	0.62
Gain in length (mm)	42.1	73.8	27.1	53.6	44.4	76.3	19.5	11.2	0.09	0.32	0.44
Percent gain in weight	58.7	102.3	40.1	85.2	71.5	126.9	26.2	15.1	0.25	0.25	0.94
SGR%	0.5	0.8	0.4	0.6	0.6	0.9	0.1	0.08	0.17	0.23	0.96
K	1.6^{a}	1.5^{a}	1.1^{b}	1.1^{b}	1.3^{c}	1.3^{c}	0.04	0.02	0.00	0.79	0.66
FCR	0	3.9	0	4.7	0	3.6	0	0.57	0.24		

Table 5. Contd.

PER	0	0.7	0	0.7	0	0.8	0	0.09	0.53
PU	0	0.5	0	0.3	0	0.5	0	0.07	0.37
GNRE%	0	10.3^{b}	0	8.2^{b}	0	12.5^{a}	0	0.8	0.00
GERE%	0	28.9	0	30.3	0	32.5	0	3.3	0.55

F0 = Control; F1= feed 1; SEM F0 = pooled standard error of diet F0; SEM F1 = pooled standard error of feed F1; SGR% = specific growth rate; K= condition factor; FCR = feed conversion ratio; PER = protein efficiency ratio; PU = protein utilization; GNRE% = gross nitrogen retention efficiency; GERE% = gross energy retention efficiency. Values in the same row with different superscripts are significantly (P ≤ 0.05) different; means are of three replicates of F1 and one of F0.

Table 6. Whole body proximate composition of three fish species grow-out post-treatment fed 35% protein diet (F1) and control (F0) in monoculture system.

Proximate composition	Species/feed								ANOVA P value		
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*						
	F0	F1	F0	F1	F0	F1	SEM F0	SEM F1	Species	Feed	Sp* feed
Dry matter (%)	22.5^{ab}	24.4^{ab}	24.2^{ab}	24.9^{a}	24.6^{ab}	20.9^{b}	0.6	0.3	0.17	0.60	0.03
Crude protein (%)	12.8	13.9	14.5	13.4	15.2	12.1	0.7	0.4	0.82	0.27	0.22
Crude lipid (ether extract) (%)	3.2	5.4	3.2	7.31	5.2	4.7	1.0	0.6	0.79	0.14	0.31
Ash (%)	4.1	4.1	4.1	3.5	3.7	3.1	0.4	0.2	0.44	0.38	0.83
Gross energy (MJ g^{-1})	4.9	5.6	5.4	6.1	5.7	5.0	0.3	0.2	0.40	0.53	0.16
Mineral composition											
Ca (%)	1.1	0.9	0.9	0.8	0.9	0.8	0.1	0.1	0.55	0.39	0.95
Mg (%)	0.03	0.02	0.03	0.03	0.03	0.02	0.00	0.004	0.86	0.27	0.86
K (%)	0.2	0.1	0.2	0.2	0.2	0.1	0.03	0.02	0.44	0.15	0.78
P (%)	0.6	0.5	0.5	0.4	0.5	0.4	0.04	0.02	0.40	0.22	0.79

F0 = Control; F1 = feed 1; SEM F0 = pooled standard error of diet F0; SEM F1 = pooled standard error of feed F1. Values in the same row with different superscripts are significantly (P ≤ 0.05) different; means are of three replicates of F1 and one of F0 and the values in each represent means of two determinations.

inorganic fertilizers.

Condition factor values in experiments 1 and 2 were observed to be significantly different among species. This difference among species regarding condition factor might be due to their genetic makeup, size and status of pond productivity or nature of their different feeding habit and seasonal effect (Javed et al., 1993; Kartha and Rao, 1990).

Regarding feed conversion and nutrient retention studies, higher FCR and lower PER, PU, GNRE% and GERE% values indicated that lower feed conversion and feed utilization take place in grow-out fish. *L. rohita* (12.5%) in polyculture showed significantly higher nitrogen retention efficiency than *C. catla* (10.3%) and *C. mrigala* (8.2%). These differences among Indian major carps and Chinese carps occur due to variation of voluntary feed intake, weight gain, composition, digestion, absorption, transport and metabolism (Gjoen et al., 1993; Medale et al., 1993; Mahboob et al., 1995).

The proximate composition of grow-out fish under experimental supplementary feed (F1 35% protein), along with control (F0) in experiments 1 and 2 showed no significant differences (P ≤ 0.05) among species and diets. Studies on whole body composition of *Aristichthys nobilis* (Naeem and

Table 7. Whole body proximate composition of three fish species grow-out post-treatment fed 35% protein diet (F1) and control (F0) in polyculture system.

Proximate composition	Species/feed						ANOVA P value				
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*						
	F0	F1	F0	F1	F0	F1	SEM F0	SEM F1	Species	Feed	Sp* feed
Dry matter (%)	27.1	19.5	19.4	27.6	12.9	19.9	1.8	1	0.56	0.34	0.45
Crude protein (%)	13.4	11.9	10.5	15.8	6.6	12.7	0.8	0.5	0.66	0.07	0.61
Crude lipid (ether extract) (%)	5.3	3.0	5.3	6.6	3.7	3.1	0.6	0.4	0.26	0.54	0.47
Ash (%)	4.9	3.6	2.9	4.1	2.1	3.3	0.1	0.1	0.16	0.13	0.21
Gross energy (MJg^{-1})	6.8	4.1	4.6	6.6	3.2	4.4	0.4	0.2	0.48	0.82	0.35
Mineral composition											
Ca (%)	1.4	0.9	0.8	1.0	0.5	0.9	0.04	0.02	0.15	0.68	0.13
Mg (%)	0.06	0.03	0.02	0.02	0.02	0.02	0.005	0.003	0.03	0.21	0.14
K (%)	0.2	0.1	0.1	0.2	0.1	0.1	0.01	0.01	0.74	0.95	0.27
P (%)	0.7	0.5	0.4	0.6	0.3	0.5	0.02	0.01	0.30	0.28	0.21

F0 = ontrol; F1 = feed 1; SEMF0 = pooled standard error of feed F0; SEMF1 = pooled standard error of feed F1. Means are of three replicates of F1 and one of F0 and the values in each represent means of two determinations.

Salam, 2010) and *Mystus bleekeri* (Naeem and Ishtiaq, 2011) revealed that nutrient composition of fishes vary with species, body size and condition factor. Zeitler et al. (1984) reported that there is an inverse relationship of dietary protein supply with body fat and energy contents, and direct relationship of body protein and water contents in *Cyprinus carpio*. Satpathy et al. (2003) observed that with the increase in dietary protein level, there is a significant ($P \leq 0.05$) increase in whole body protein and lipid contents. Khan and Abidi (2010) reported that a high protein 40% diet produced fish with higher fat content. In experiments 1 and 2, an increasing trend of protein deposition in *L. rohita* was noticed at post treatment. The non-significant difference in body composition among species and diets, during the present study period, might be due to low intake of artificial feed and their preference to natural food organisms. Another possible reason might be due to higher level of inclusion of plant protein sources that did not affect any significant change in fish body.

Incorporation of plant protein by replacing fish meal did not have a significant effect on the whole body composition of *Oreochromis niloticus* (El-Saidy and Gaber, 2003).

The percentage of macro minerals (Ca, Mg, K and P) determined during the present study exhibited non-significant difference ($P \leq 0.05$) among species and treatments. Minor variation among species, were observed at post treatment where *C. catla* showed higher concentration of Ca and P contents than *C. mrigala* and *L. rohita*, that showed almost equal trend in mineral contents, which might be due to genetic factor. The results of the present study regarding Ca and Mg were found much higher, while K and P were almost comparable with the findings of Kirchgessner and Schwarz (1986), who reported mineral contents of *C. carpio* to be 41% protein and 16.9 to 20.1 MJ/g energy.

Additionally, they stated that increased mineral contents in the carp carcass are directly related with increasing amount of crude protein contents in the feed, while with the increase in energy supply and values of Ca and P decreased. They also reported that differences in live weights of carp (600 to 1000 g) did not influence the mineral contents. This statement is also justified with the present results obtained from same weights of fish of Indian major carps. It was observed during the present study that neither weights nor culture system has any affect on the mineral composition of Indian major carps. Non-significant differences ($P > 0.05$) among treatments (F1 and F0) indicated that treatments had no affect on the composition of mineral contents in fish carcass. This is also reflected from the low values of protein utilization for both experiments. Results of

the present study are contrary to the findings of Pfeffer et al. (1977) who observed increasing trend of mineral contents with the increase in live weights of carp (400 to 1000 g). Zeitler et al. (1984) stated that increase in mineral contents is related with the increase in nutrient contents (crude ash, crude protein, and crude fat) of the carcass. Carps contain higher mineral contents than trout on fresh weight basis (Pfeffer and Potthast, 1977).

All the water quality variables remained within an acceptable range except temperature. Being cold blooded, fish is easily influenced by the surrounding water temperature that play major role and directly influence metabolism, feed intake, FCR and nutritional efficiency (De Silva and Anderson, 1995; Burel et al., 1996; Britz et al., 1997).

The economic analysis of the two experiments with same experimental feed indicate that experimental feed due to its deficient profile for certain indispensible amino acids, resulted in overall poor growth, which led to high feed conversion, low fish production and lower economic return. As far as the culture system is concerned, the polyculture was found more satisfactory than monoculture system. The reason of high feed cost might be due to the inclusion of higher levels of (fish meal and soybean meal) almost 57% of the experimental diet that increased the overall cost of the supplementary feed, compared to net profit. Thus, the major challenge for the carp feed manufacturer is to find alternative feed resources that are sustainable, and have all the necessary nutrients and quality, ensuring good growth and minimizing feed cost. Secondly, feed was prepared in mash type that was not fully utilized by the fish, so pelleted feed should be formulated to check its efficacy on these species in earthen ponds. Thirdly, the trials were conducted during the autumn season for a three months period, when temperature was slightly lower than the optimal growth of carps. Hence, there is a need to test such diet in other season of the year like spring and summer for long term, to check the efficacy and economic suitability. Higher protein (35%) at this grow-out stage might be another possible reason of low feed utilization and its conversion to fish somatic growth. It has been urged that supplementary feeds are not difficult to make in laboratory research condition using low cost practical ingredients. Under field conditions, however, it is also critical to consider many other factors, including physico-chemical parameters, feed palatability, fish growth, fish quality, disease resistance, and so forth. All, these practical requirements need to be considered to develop sustainable supplementary feed for carp aquaculture.

In conclusion, the present study indicate that experimental feed 35% protein showed satisfactory results, in terms of gain in weight, as compared to control, but overall growth performance, feed conversion ratio and protein utilization remained poor. The overall growth percentage of three species remained higher in polyculture than monoculture. Economically, monoculture is very expensive and not suitable; however, polyculture is best for overall production and economic point of view. Supplementary feed did not affect the whole body proximate composition nor mineral contents of three fish species under both culture system.

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