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Evaluation of formulated diets with different levels of marine protein mixtures in aquariculture of koi carp *Cyprinus carpio* var. *koi* (Linnaeus, 1758)

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ABSTRACT

A marine protein mixture of fish, squid, shrimp and clam meal in equal proportion was incorporated at different levels in the diets formulated and designated as P16, P19, P26, P33, P40, P47, P53 and P59 for koi carp. Triplicate groups of 10 fish each were fed with the experimental diets for 12 weeks and assessed for growth and body composition. On physical evaluation of the extruded feeds, above 80% dry matter retention for 4 h was evident in feeds P26 to P47. Feeds with more starch (wheat) P19 and P16 had stability of more than 90% and feeds with less starch, P53 and P59 had a stability of less than 70%. The body tissue of the experimental animals fed with P59 and P53 had significantly ($p<0.05$) high levels of polyunsaturated fatty acids (PUFA). Total ash in the body decreased with proportionate decrease in nutrient density. Biomass gain was similar with feeds P33, P40, P47 and P53. Growth as a function of incremental levels of marine protein mixtures in diet was linear. Diets containing 250, 300 and 350 g kg^{-1} protein is advocated for normal growth and 400 to 450 g kg^{-1} protein mixture are recommended to promote faster growth.

Keywords: Diet, Koi carp, Marine protein, Ornamental fish nutrition

Introduction

Ornamental aquariculture across the world is a growing sector valued at US$ 8 billion with an average annual growth of 9%, while the entire industry including plants, accessories, aquarium, feed and medications is estimated to be worth more than US$ 18-20 billion (Silas *et al.,* 2011). In India, the sector has a new impetus due to the flourishing export market with an estimated value of US$ 1.17 million in 2009-10 (Silas *et al.,* 2011). Unlike the feed formulations meant for food fish cultivation, ornamental fish feed formulations should be able to maintain health, color and longevity. Koi carps (*Cyprinus carpio* var. *koi*), form a major group of ornamental fish in trade and hence, the present study was conducted with different levels of protein using koi as a model.

Ornamental fish diets sold in India and abroad in general are not evaluated scientifically. The product labels are not uniform and no standard diets exist. Our work on marine ornamentals (Vijayagopal *et al.,* 2008) led to the association with Kerala Aqua Ventures International Limited (KAVIL), a joint venture of the Government of Kerala, India, to promote export and trade of ornamental fish. This partnership prompted an investigation on diets designed specifically for this freshwater ornamental fish. Similarities of koi nutrition with carp nutrition are advantageous owing to the availability of adequate information based on which diets can be formulated and evaluated. However, it is to be remembered that commercially available diets are not always formulated and evaluated using such information, leading to the availability of diets, worldwide without any specified standards. Ornamental fish nutrition is object oriented and during maintenance in the aquarium, the fish should appear healthy and should not lose color. Ornamental fish breeders look to enhance fecundity which would lead to increased production of fry and potential juveniles. Therefore, diets must be appropriately formulated and evaluated to fulfill the desired objectives which may result in extreme alterations of the dietary nutrients and physical composition. Nutritional requirements of koi are not available from published scientific literature.

An exhaustive review of nutrient requirements in carp is available in Takeuchi *et al.* (2002). Essential amino acid requirements of all 10 amino acids for common carp were reported by Nose (1979). However, fatty acid requirements for common carp are available only for the essential fatty acids, namely, linoleic and linolenic acids which are reported to form only one percent of the fatty acids (NRC, 1993). Koi carps mass cultured in manured ponds were reported to attain a marketable size of 4 g in 11 weeks (Jha *et al.,* 2007). They also reported maximum number of deformities and mortality in fish fed with a commercial pellet diet containing 32% protein, 5% fat, 10% crude fibre, 31% carbohydrate and 9% ash. Thus, with the status of knowledge on koi diets being poor, we attempted to evaluate the optimum macronutrient levels required in formulated diets with marine proteins supplying animal protein and soy flour as the vegetable protein source.

Material and methods

Diet preparation

Locally available feed materials were used for formulating the diets. Feedstuffs were evaluated for proximate composition. Diets were formulated in an Excel spreadsheet (Vijayagopal, 2004). All the diets were extruded in a laboratory model twin-screw extruder (BTPL, Kolkata, India) and assessed for their bulk density and hydrostability as per Vijayagopal *et al.* (2008). The extruded diets were crumbled and sieved to obtain 0.75 mm pieces which were found to be the suitable particle size for consumption by the fish during the experiment.

Fish, feeding and water quality

Koi carp fry (approximately 150 mg in size) were obtained from a local aquarium fish dealer (Mareena Aquarium Products and Fishes, Kochi, India). The fish were acclimatised in 250 l fibre reinforced plastic (FRP) tanks with *in situ* biological filters and continuous aeration. Groups of 10 fry averaging 158 mg initial weight were stocked in 24 glass aquaria which were part of a recirculation aquaculture system. The system was filled with decholrinated freshwater. Ninety percent of the water was renewed fortnightly with freshwater.

Water quality parameters *viz.,* temperature, pH, dissolved oxygen (DO) and total ammonia nitrogen (TAN) were analysed fortnightly for the samples drawn from the inlet and outlet points (APHA, 1985). For 84 days, the fish were fed to apparent satiation at 10.00 hrs, 13.00 hrs and 16.30 hrs. In order to arrive at the consumption rates, initial quantity of diet was subtracted from final quantity at the end of the experiment. Feed residue and fecal residue were collected and quantified. The feed residue was observed to be bare minimum and collection of residues was not needed most of the days. Mortalities, if any, were recorded daily (Hopkins, 1992). At the end of the experiment, individual body weights of all the fish as well as the biomass per tank were recorded. Growth performance and feed utilisation pattern were assessed by recording final body weight, determining net body weight gain, specific growth rate (SGR), feed intake, feed conversion ratio (FCR) and protein efficiency ratio (PER).

Biochemical analyses

On termination of the experiment, dried samples of fish were used for whole body proximate analysis. Frozen samples were used for estimating whole body amino acid and fatty acid composition. Triplicate samples of diets and carcass were analysed for proximate composition using standard methods (AOAC, 1990). Dry matter (DM) was determined by drying at 105^{o}C for 24 h in a hot air laboratory oven having forced air circulating system with fan. Crude protein (CP) was determined by Kjeldhal method in a Kjeltec 2300, ether extract (EE) was determined using a Soxtec 2043 and crude fibre (CF) using a Fibertec 2043 (Foss, Denmark). The nitrogen free extract (NFE) of diets considered as total carbohydrates, was derived by subtracting CP, EE, CF and ash from DM.

Amino acid was analysed according to Heinrikson and Meredith (1984) by digesting powdered diet samples (0.1 g) with 10 ml 6 N HCl at 110^{o}C in sealed tubes for 24 h. Samples were used in triplicates and the output was analysed using Breeze GPC software (Waters™, Milford, MA, USA). For fatty acid analysis, the lipid in the diet samples was extracted by the cold extraction method using a chloroform-methanol mixture in a 2:1 ratio (Folch *et al.,* 1957). After isolating the lipid phase, the solvent was evaporated. Two millilitre sample was refluxed with 5 ml of 0.5% alcoholic potassium hydroxide solution for 30 min and then refluxed with 6 ml of BF_3-MeOH (Sigma-Aldrich, Bangalore, India) for an additional 5 min. Refluxing was carried out under nitrogen. The dry fatty acid methyl esters in the flask were extracted with petroleum ether (10 ml x 3) quantitatively. The extract was further washed with 25 ml double distilled water three times and filtered over anhydrous sodium sulphate (10 g) to remove any moisture. The solvent was then evaporated under a stream of nitrogen gas. Two microlitre each of the prepared fatty acid methyl ester samples was injected into an Auto-System XL Gas chromatograph (Perkin Elmer, Waltham, MA, USA) according to Morrison and Smith (1964).

Proximate composition, amino acid profiles and fatty acid profiles of all the diets, initial and final whole body proximate composition, amino acid composition and fatty

acid composition of the fish treatment-wise were used to compare the diet induced changes in the body composition of fish.

Statistical analysis

One way analysis of variance (ANOVA) was used to test differences between dietary treatments. Means were evaluated for significance by Student-Newman-Keuls (SNK) test ($p<0.05$). All statistical tests were performed using SPSS software, Ver.13.0.

Results and discussion

Feed analysis and physical properties

Proximate composition of the feed ingredients used and test diets are shown in Tables 1 and 2, respectively. Micronutrient content and cost of the feed ingredients are also indicated in Table 1. The dietary protein content increased from 158-588 g kg^{-1} based on which the diet treatments were numbered as P16, P19, P26, P33, P40, P47, P53 and P59. Gross energy content of the diets increased from 18-19 MJ kg^{-1}. The formulation costs ranged from ₹ 36 – 81 (US$ <1- 1.5) per kg.

Dry matter loss of the test diets increased as dietary protein increased (Table 3). Hydrostability was found to be directly proportional to the wheat flour content in the formulations. Variations in bulk densities (g l^{-1}) are also presented in Table 3. Leaching rates varied significantly ($p<0.01$) from the initial dry matter during the first two hours. Variations in dry matter loss were not significant thereafter during the subsequent two hours.

Average water quality parameters recorded during the experiment were: temperature 24.25°C, pH 7.04, dissolved oxygen 7.03 mg l^{-1} and TAN 0.17 mg l^{-1} Table 4. All the parameters assessed were within the acceptable limits for normal aquatic life (Stickney, 2009).

Dietary amino acid profiles and NRC requirements of amino acids of common carp are presented in Table 5. Table 6 presents the initial and final whole body amino acid profiles. Significant variations ($p<0.05$) were evident between all the diets using SNK test.All diets contained sufficient amount of all essential amino acids required for common carps (NRC, 1993). Final body amino acid profiles are also shown in Table 6. Significant variations ($p<0.05$) in the body amino acid content was noticed when compared with the initial profile, with only few diet treatments having similar treatment means.

Fatty acid profiles of the diets are shown in Table 7 while initial and final body fatty acid profiles are depicted in Table 8. There was a significant decline in the saturated fatty acid (SFA) concentration, and a significant increase in both monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) concentration in diet P16 ($p<0.05$) and not much variation was observed in rest of the experimental diets. Significant decrease was observed in initial and final body saturated fatty acid concentration in fish fed diets P19 and P16 ($p<0.05$). Concentration of MUFA remained similar from treatments P59 to P26 while it increased significantly in treatments P19 and P16 ($p<0.05$). Body PUFA contents of all the treatment fish were significantly lower ($p<0.05$) than the initial body PUFA content. The body tissue levels of SFA in fish fed diet P33 were similar to the control. Significant increase in SFAs was noticed in P59 and P53 ($p<0.05$) and then a declining trend was observed in rest of the diet fed groups. Significantly higher values of MUFA were recorded in fish from all treatments except P59 and P53 ($p<0.05$). Body PUFA levels were similar in P59 and P53 comparable to the initial, and then a decline was observed up to P26 followed by and elevation in P19 and P16. It is noteworthy that all PUFA values were significantly lower ($p<0.05$) than the initial level.

Effects of feeding formulated diet with ascending levels of protein for a period of 84 days are shown in Table 9. Significant differences were observed in growth ($p<0.05$) with ascending levels of protein. The trend observed was poor growth with diets P16 and P19, similar and higher growth with diets P26, P33, P40 and P47 and a still higher growth with diets P53 and P59 indicating that diets containing 26 to 47% protein registers similar biomass gain and percent weight gain. However, there was no significant ($p>0.05$) difference observed in specific growth rate (SGR) among the different experimental groups. Dietary intake was significantly higher with P59 ($p<0.05$). Food conversion ratios (FCR) were above 2 and close to 2 with diets P16-33 and with diets P40-59, FCRs were observed to be below 2. PERs were significantly lower ($p<0.05$) with diets P33 and P40. Feed cost involved in production of one kg koi fry varied from ₹ 67.15 to 103.88 (US $ 1 to 2). The resultant cost of feed for producing a fry of 5 g varies from ₹ 0.36 to 0.52. The relationship between percent protein in the diet and percent growth over initial weight being linear, an optimum level could not be discerned.

Whole body composition of the experimental animals (Table 10) indicated an increase in body protein as protein content in the diets increased ($p<0.05$). Fat deposition showed an increase with the ascending level of carbohydrate in the diet with a complementary decrease in protein content in the formulations. Total ash content was similar to the initial body ash ($p<0.05$) in treatments P59 and P53. Significant decline thereafter, stabilising at less than 2% ash was seen in the remaining diet treatments.

Evaluation of fish feeds is a prerequisite for scaling up of production further leading to commercialisation.

Table 1. Proximate composition of feed ingredients (% dry matter basis) along with details on source and cost

Ingeredient	CP[1]	EE[2]	CF[3]	NFE[4]	Ash	AIA[5]	Cost [6] (₹)	Cost (US$ kg^{-1})	Source of the ingredient
Fish meal	58.36	7.70	0.28	5.96	27.7	0.45	57	1.27	Predominantly made from oil sardines (S*ardinella longiceps*) by Raj Fishmeal and Oil Co., Malpe Karnataka, India
Shrimp meal	68.98	3.42	17.59	3.08	6.93	1.67	60	1.33	Dry *Acetes* spp. procured from Hajee K. A. Abdul Khader Sahib, dry fish merchant, Chennai, India
Squid	84.75	5.62	4.53	0.31	4.79	0.05	100	2.22	Meal prepared form the Indian squid *Uroteuthis (Photololigo) duvauceli* procured through Molluscan Fisheries Division, CMFRI, Kochi, India
Black clam	67.60	7.52	9.12	0.3	15.46	2.95	100	2.22	Meal prepared from fresh black clam (*Villorita cyprinoides*) procured from local sea food market in Kochi, India
Soy flour	52.09	0.51	7.85	32.6	6.95	0.02	65	1.44	Food grade defatted soy flour from Sakthi Soyas Coimbatore, India
Wheat flour	11.15	1.29	0.59	85.13	1.84	0.08	20	0.44	Food grade wheat flour from the local market, Kochi India
Oil							20	0.44	Crude sardine oil from Kiriyanthan Trading Company Narakkal, Kochi, India
Vitamin C							950	21.11	Stay-C, ascorbyl polyphosphate, from DSM Nutritional Technologies, India
Vitamin mixture							120	2.67	Supplevite - M from Sarabhai Zydus Animal Health Pvt. Ltd., Vadodra, India. Composition (per kg): Vitamin A - 2000000IU, Vitamin D3 - 400000IU, Vitamin B2 - 0.08 g, Vitamin E - 300 units, Vitamin K - 0.4 g, Ca. pantothenate - 1g, Nicotinaminde - 4 g, Vitamin B12 - 2.4 mg, Choline chloride – 60 g, Calcium – 300 g, Manganese -11 g, Iodine - 0.04 g, Iron - 3 g, Zinc - 6 g, Copper - 0.8 g and Cobalt-0.18 g
Mineral mixture							83	1.84	Agrimin powder from Virbac Healthcare India Pvt. Ltd. Mumbai, India. Composition (g kg^{-1}): Cobalt -150 mg Copper - 1200 mg, Iodine - 325 mg, Iron - 5000 mg Magnesium - 6000 mg, Manganese - 1500 mg Potassium - 100 mg, Sodium - 5.9 mg, Sulphur - 0.922%, Zinc - 9600 mg, DL -Methionine - 1920 mg, L-lysine Mono hydrochloride - 4400 mg, Calcium - 24% Phosphorus - 12%
Spirulina							800	22.22	Certified organic spirulina (*Arthrospira platensis*) from Parry Nutraceuticals, Chennai, India
Mixed carotenoids							7500	177.78	Mixed carotenoids 7.5% powder form (*Dunaliella salina*), Parry Nutraceuticals, Chennai, India
BHT[7]							1360	6.40	Butylated hydroxyl toluene, from Sisco Research Laboratories Pvt. Ltd., Mumbai, India
Sodium metabisulphate							658	198.40	Nice Chemicals, Cochin, India

[1]Crude protein, [2]Ether extract or crude fat, [3]Crude fibre, [4]Nitrogen free extracts, [5]Acid insoluble ash, [6]One Indian Rupee (₹) was 0.55 US$ at the time of sourcing the feed ingredients, [7]Butylated hydroxytoluene

Table 2. Formulation of the experimental diets (g kg^{-1}), proximate composition (g kg dry $matter^{-1}$) and cost

Feed no.	P59	P53	P47	P40	P33	P26	P19	P16
Ingredients								
Fishmeal	175.00	150.00	125.00	100.00	75.00	50.00	25.00	15.00
Shrimp meal	175.00	150.00	125.00	100.00	75.00	50.00	25.00	15.00
Squid meal	175.00	150.00	125.00	100.00	75.00	50.00	25.00	15.00
Clam meal	175.00	150.00	125.00	100.00	75.00	50.00	25.00	15.00
Soya flour	175.00	150.00	125.00	100.00	75.00	50.00	25.00	15.00
Wheat flour	17.80	142.80	267.80	392.80	517.80	642.80	767.80	817.80
Oil	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Vitamin C	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin mixture	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Mineral mixture	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Spirulina	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mixed carotenoids	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
BHT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sodium metabisulphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proximate composition								
Crude protein	588	534	468	401	326	258	190	158
Crude fat	98	84	72	60	65	53	52	55
Total carbohydrates	161	234	332	423	504	604	686	713
Ash	150	141	122	107	93	75	66	63
Acid insoluble ash	1.0	2.0	0.9	0.8	1.1	0.6	0.5	0.4
*Energy MJ kg^{-1}	19.12	18.72	18.52	18.18	18.18	17.95	17.90	17.79
Cost kg^{-1}								
₹	81	74	67	60	53	46	39	36
US$	1.47	1.64	1.48	1.33	1.17	1.01	0.86	0.80

*Calculation based on Cuzon and Guillaume (1997): 21.3, 17.2 and 39.5 MJ kg^{-1} of protein, carbohydrate and lipid, respectively

Table 3. Hydrostability (% dry matter remaining after elapse of 0 - 4 h) and bulk density (g l^{-1}) and crumble buoyancy[1]

Time (h)	0	1	2	3	4	Bulk density (g l^{-1})	Pellet buoyancy
P59	100^{a}	77.54 ± 0.31^{b}	77.10 ± 0.15^{c}	76.44 ± 0.12^{d}	75.64 ± 0.21^{d}	612.31 ± 21.31^{a}	Sinking
P53	100^{a}	76.25 ± 0.74^{b}	76.07 ± 0.33^{c}	74.76 ± 1.23^{d}	73.98 ± 0.21^{d}	583.24 ± 18.88^{b}	Slow sinking
P47	100^{a}	84.43 ± 0.18^{b}	83.67 ± 0.25^{c}	83.44 ± 0.17^{d}	82.85 ± 0.18^{d}	580.70 ± 20.33^{b}	Slow sinking
P40	100^{a}	85.66 ± 0.09^{b}	85.05 ± 0.21^{c}	84.83 ± 0.12^{d}	83.30 ± 0.12^{d}	516.57 ± 18.19^{b}	Neutral
P33	100^{a}	90.26 ± 0.13^{b}	90.00 ± 0.28^{c}	89.40 ± 0.25^{d}	88.37 ± 0.11^{d}	483.83 ± 27.32^{c}	Neutral
P26	100^{a}	92.52 ± 0.21^{b}	91.36 ± 0.28^{c}	90.87 ± 0.09^{d}	88.83 ± 0.12^{d}	486.77 ± 22.57^{c}	Neutral
P19	100^{a}	94.02 ± 0.09^{b}	93.10 ± 0.26^{c}	92.70 ± 0.28^{d}	91.55 ± 0.46^{d}	467.67 ± 31.28^{c}	Floating
P16	100^{a}	95.59 ± 0.17^{b}	94.97 ± 0.20^{c}	93.16 ± 0.28^{d}	91.04 ± 0.30^{d}	413.26 ± 15.11^{d}	Floating

[1]Mean±SE values bearing the same superscript in rows for hydrostability are not significantly different ($p<0.05$)
[2]Mean±SE values bearing the same superscript in column 7 for bulk density are not significantly different ($p<0.05$)

Table 4. Water quality parameters during the feeding experiment, sampled at inlet (In) and outlet (Out of the recirculation system (Average of six fortnightly observations).

Parameter	In	Out
Temperature (°C)	24.25±0.54	24.25±1.33
pH	6.98±0.013	7.11±0.38
Dissolved oxygen (mg l^{-1})	6.97±0.19	7.10±0.38
Total ammonia nitrogen (mg l^{-1})	0.15±0.07	0.20±0.15

This approach was attempted, so that, a scientifically evaluated product is available off the shelf for aquarium hobbyists and traders. Since the ornamental fish diets are retailed at a high cost mainly because of low sales volumes, high quality ingredients were used. All ingredients used in the formulation are of a grade fit for human consumption. Costly additives such as carotenoids and an antifungal (sodium metabisulphate) are included at less than 1%. The formulation costs vary from ₹ 46-81 kg^{-1} (US$ 0.65-1.47 kg^{-1}). In the manufacturer - distributor - retailer value chain, there is ample scope for value

Table 5. Amino acid profiles of the diets tested (g 100 g^{-1} protein)

Feeds	P59	P53	P47	P40	P33	P26	P19	P16	NRC Requirements of *Cyprinus carpio* (1993)
Asp	6.58±0.07^{b}	7.05±0.03^{a}	5.31±0.02^{e}	5.89±0.09^{d}	6.10±0.03^{c}	6.04±0.01^{c}	5.24±0.03^{e}	4.57±0.02^{f}	-
Glu	10.55±0.06^{a}	11.11±0.05^{b}	13.08±0.02^{c}	11.20±0.06^{b}	13.00±0.01^{c}	10.74±0.07^{a}	16.30±0.04^{d}	17.75±0.22^{e}	-
Ser	4.78±0.09^{c}	4.91±0.02^{c}	4.05±0.00^{a}	4.45±0.06^{b}	4.83±0.05^{c}	4.56±0.03^{b}	4.66±0.11^{b}	4.76±0.06^{c}	-
Gly	11.16±0.02^{b}	10.57±0.06^{b}	11.25±0.01^{b}	11.94±0.03^{c}	10.42±0.02^{b}	12.46±0.12^{c}	9.44±0.11^{a}	9.44±0.71^{a}	-
His	1.55±0.03^{a}	1.93±0.02^{b}	1.80±0.02^{b}	2.05±0.01^{b}	2.00±0.04^{b}	2.03±0.04^{b}	1.86±0.03^{b}	1.86±0.14^{b}	0.8
Arg	5.28±0.05^{c}	5.95±0.03^{e}	4.34±0.04^{a}	5.67±0.07^{d}	5.58±0.08^{d}	5.84±0.07^{d}	4.63±0.14^{b}	4.27±0.02^{a}	1.6
Thr	4.76±0.02^{c}	4.84±0.00^{d}	3.55±0.05^{a}	4.19±0.02^{b}	4.45±0.07^{b}	4.47±0.08^{b}	3.83±0.11^{a}	3.67±0.17^{a}	1.5
Ala	7.47±0.06^{e}	7.14±0.01^{d}	7.51±0.03^{e}	7.25±0.12^{d}	6.74±0.02^{c}	7.11±0.01^{d}	6.01±0.07^{b}	5.67±0.12^{a}	
Pro	6.47±0.07^{b}	6.03±0.01^{a}	10.46±0.05^{e}	8.11±0.06^{d}	7.80±0.01^{c}	7.89±0.02^{c}	10.39±0.12^{e}	12.01±0.07^{d}	
Tyr	3.17±0.06^{d}	2.34±0.03^{a}	2.91±0.01^{b}	2.82±0.05^{b}	2.53±0.02^{a}	2.89±0.06^{b}	2.53±0.06^{a}	2.52±0.24^{a}	
Val	5.34±0.02^{b}	5.25±0.03^{b}	5.33±0.01^{b}	5.31±0.02^{b}	5.33±0.01^{b}	5.21±0.03^{a}	5.34±0.04^{b}	5.31±0.06^{b}	1.4
Met	2.18±0.02^{e}	2.14±0.03^{d}	1.76±0.01^{ab}	1.95±0.02^{c}	1.93±0.02^{c}	2.01±0.03^{c}	1.78±0.04^{b}	1.67±0.06^{a}	1.2
Cys	0.44±0.02^{b}	0.41±0.01^{b}	0.21±0.00^{a}	0.38±0.06^{b}	0.38±0.01^{b}	0.45±0.04^{b}	0.27±0.03^{a}	0.43±0.03^{b}	
Ile	4.95±0.07^{b}	4.69±0.01^{c}	4.77±0.06^{c}	4.75±0.06^{c}	4.56±0.06^{c}	4.72±0.09^{c}	4.28±0.08^{a}	4.19±0.04^{a}	0.9
Leu	8.66±0.06^{a}	8.44±0.00^{b}	8.38±0.04^{b}	8.35±0.06^{b}	8.29±0.03^{b}	8.38±0.09^{b}	8.35±0.07^{b}	8.19±0.05^{b}	1.3
Phe	4.41±0.02^{a}	4.07±0.02^{a}	4.31±0.04^{a}	4.37±0.06^{a}	4.16±0.06^{a}	4.44±0.21^{a}	4.18±0.05^{a}	4.34±0.12^{a}	2.5
Lys	11.05±0.01^{a}	11.59±0.02^{b}	9.47±0.02^{c}	10.12±0.06^{d}	10.51±0.13^{e}	9.69±0.06^{c}	9.10±0.05^{f}	7.51±0.14^{g}	2.2

Mean±SE values with the same superscripts in rows do not differ significantly ($p<0.05$)

Table 6. Initial and final body amino acid profiles (g 100 g^{-1} protein)

Amino acid	Initial	P59	P53	P47	P40	P33	P26	P19	P16
Asp	6.00±0.04^{b}	7.56±0.09^{e}	7.74±0.02^{f}	5.75±0.05^{a}	5.44±0.05^{a}	5.98±0.05^{b}	6.77±0.05^{d}	7.34±0.05^{e}	6.26±0.03^{c}
Glu	8.37±0.07^{b}	9.68±0.13^{d}	9.83±0.08^{d}	7.95±0.02^{a}	7.88±0.05^{a}	8.11±0.05^{b}	9.09±0.00^{c}	9.19±0.03^{c}	8.39±0.06^{b}
Ser	4.25±0.04^{bc}	4.59±0.01^{d}	4.59±0.04^{d}	3.43±0.04^{a}	4.09±0.04^{b}	4.36±0.05^{cd}	4.43±0.05^{cd}	4.86±0.04^{e}	4.39±0.07^{cd}
Gly	14.47±0.17^{f}	11.68±0.04^{b}	11.21±0.05^{a}	15.84±0.01^{h}	15.17±0.05^{g}	14.14±0.03^{e}	12.22±0.05^{c}	12.65±0.04^{d}	15.23±0.03^{g}
His	1.75±0.04^{a}	2.49±0.02^{d}	2.48±0.05^{d}	1.95±0.04^{b}	2.09±0.00^{c}	2.40±0.05^{d}	2.14±0.04^{c}	2.19±0.01^{c}	1.91±0.01^{b}
Arg	5.63±0.05^{a}	5.57±0.00^{a}	5.62±0.05^{a}	4.92±0.05^{b}	5.71±0.05^{a}	5.76±0.06^{a}	5.53±0.05^{a}	5.75±0.04^{a}	5.67±0.03^{a}
Thr	3.94±0.01^{b}	4.25±0.02^{c}	4.32±0.05^{c}	3.35±0.05^{a}	3.98±0.02^{b}	4.17±0.05^{c}	4.19±0.02^{c}	4.54±0.05^{d}	4.00±0.01^{b}
Ala	8.07±0.02^{a}	8.24±0.04^{ab}	8.21±0.04^{ab}	10.32±0.05^{e}	8.82±0.08^{d}	8.43±0.05^{c}	8.12±0.05^{a}	8.38±0.05^{c}	8.55±0.05^{c}
Pro	6.64±0.08^{a}	5.28±0.03^{b}	5.12±0.03^{b}	9.33±0.04^{e}	7.12±0.05^{d}	6.74±0.05^{a}	5.85±0.05^{c}	5.64±0.06^{c}	6.73±0.12^{a}
Tyr	2.61±0.02^{a}	2.16±0.02^{b}	2.16±0.02^{b}	2.52±0.02^{a}	2.51±0.05^{a}	2.56±0.05^{a}	2.47±0.04^{a}	2.04±0.01^{b}	2.09±0.04^{b}
Val	4.98±0.04^{ab}	5.29±0.04^{c}	5.25±0.05^{bc}	5.04±0.02^{b}	5.25±0.05^{bc}	5.13±0.05^{b}	5.08±0.05^{b}	5.49±0.09^{d}	4.80±0.06^{a}
Met	2.23±0.10^{c}	2.14±0.03^{b}	2.23±0.05^{c}	1.91±0.00^{b}	2.04±0.02^{b}	2.17±0.05^{b}	2.15±0.05^{b}	2.02±0.02^{b}	1.73±0.04^{a}
Cys	0.24±0.01^{a}	0.13±0.01^{a}	0.13±0.05^{a}	0.06±0.01^{b}	0.14±0.02^{a}	0.23±0.05^{a}	0.17±0.04^{a}	0.06±0.01^{b}	0.11±0.01^{a}
Ile	4.36±0.06^{b}	4.29±0.07^{ab}	4.31±0.01^{ab}	4.70±0.05^{c}	4.53±0.05^{bc}	4.45±0.05^{b}	4.46±0.05^{b}	4.37±0.01^{b}	4.11±0.05^{a}
Leu	7.31±0.04^{bc}	7.58±0.04^{e}	7.57±0.05^{e}	7.49±0.04^{d}	7.46±0.05^{bd}	7.36±0.04^{c}	7.42±0.05^{d}	7.18±0.03^{ab}	7.09±0.03^{a}
Phe	3.98±0.05^{d}	3.54±0.05^{a}	3.55±0.06^{a}	3.81±0.01^{c}	3.99±0.01^{d}	4.00±0.01^{d}	3.76±0.05^{bc}	3.49±0.05^{a}	3.62±0.05^{ab}
Lys	13.73±0.02^{d}	14.46±0.01^{f}	14.63±0.04^{f}	10.67±0.04^{a}	12.75±0.05^{b}	13.01±0.00^{c}	15.05±0.01^{g}	13.79±0.08^{d}	14.24±0.05^{e}

Mean±SE values bearing same superscript in rows do not differ significantly ($p<0.05$)

addition of these diets with attractive packaging. Contrary to the report by Bashir *et al.* (2010), inclusion of costly animal and vegetable protein ingredients in formulations, varied from a maximum to a minimum level (Table 2). While examining the water stability of these diets, dry matter retention above 80% for 4 h is evident in diets P26 to P47. Obviously diets with more starch (wheat), P19 and P16 had stability of more than 90% and diets with less starch, P53 and P59 had stability of less than 70%. As no other synthetic binders were used, starch at different degrees of gelatinisation seemed to impart such variations in the hydrostability. The crumbles of the diets followed buoyancy patterns indicated in Table 3 as reported by Riaz (2009) and Rokey and Plattner (2006). Even though, diets with three types of buoyancies were produced, a definite preference could not be noticed while feeding. All the diets were consumed round the clock from the floating crumbles to the crumbles settled at the bottom. Carp being an agastric fish, feeding is continuous unless otherwise restricted. Even though, statistically significant variations

Table 7. Fatty acid profiles of the diets tested (g 100 g^{-1} lipid)

Fatty acids	P59	P53	P47	P40	P33	P26	P19	P16	Requirement (% dry diet)[1]
14:0	8.71±0.04^{a}	8.95±0.05^{b}	9.04±0.09^{b}	9.07±0.07^{b}	9.80±0.02^{cd}	9.94±0.05^{cd}	10.02±0.00^{d}	9.72±0.05^{c}	-
15:0	0.27±0.02^{a}	0.97±0.04^{d}	0.83±0.01^{c}	0.86±0.02^{cd}	0.86±0.02^{cd}	0.88±0.00^{cd}	0.93±0.02^{cd}	0.62±0.02^{b}	-
16:0	24.92±0.04^{c}	24.73±0.05^{c}	26.32±0.17^{e}	24.29±0.04^{d}	24.75±0.05^{c}	23.87±0.03^{b}	30.29±0.05^{e}	13.97±0.04^{a}	-
17:0	1.42±0.02^{e}	1.29±0.05^{de}	1.21±0.05^{d}	0.93±0.01^{c}	0.83±0.05^{bc}	0.85±0.04^{bc}	0.84±0.05^{b}	0.27±0.00^{a}	-
18:0	6.72±0.05^{g}	6.39±0.05^{f}	5.86±0.05^{e}	5.50±0.03^{c}	5.15±0.05^{b}	5.04±0.01^{b}	5.70±0.02^{d}	2.31±0.04^{a}	-
20:0	0.98±0.05^{a}	0.94±0.02^{a}	0.93±0.05^{a}	0.90±0.03^{a}	0.97±0.01^{a}	0.95±0.01^{a}	1.17±0.01^{b}	0.49±0.04^{c}	-
22:0	0.52±0.00^{c}	0.21±0.05^{a}	0.20±0.05^{a}	0.38±0.01^{b}	0.35±0.05^{b}	0.28±0.03^{b}	0.41±0.05^{bc}	0.20±0.00^{a}	-
24:0	0.08±0.00^{ab}	0.07±0.01^{a}	0.05±0.00^{a}	0.13±0.01^{b}	0.08±0.02^{ab}	0.05±0.02^{ab}	0.07±0.01^{ab}	0.09±0.00^{ab}	-
ΣSFA	43.62±0.15^{d}	43.55±0.08 ^{d}	44.44±0.18^{e}	42.07±0.15^{c}	42.80±0.11^{c}	41.85±0.11^{b}	49.43±0.10^{f}	27.65±0.02^{a}	-
16:1ω7	9.64±0.10^{c}	9.60±0.05^{c}	9.70±0.04^{c}	9.15±0.03^{b}	9.70±0.05^{c}	9.90±0.14^{c}	7.90±0.05^{a}	13.11±0.03^{d}	-
18:1ω9	11.33±0.05^{c}	11.12±0.05^{c}	9.32±0.05^{a}	12.33±0.04^{d}	10.33±0.08^{b}	12.60±0.11^{e}	10.35±0.04^{b}	18.31±0.04^{f}	-
17:01	0.28±0.02^{a}	0.73±0.05^{c}	0.75±0.05^{c}	0.72±0.03^{c}	0.76±0.05^{c}	0.79±0.03^{c}	0.57±0.05^{c}	0.22±0.04^{b}	-
20:1n11	0.91±0.05^{c}	0.87±0.04^{bc}	0.77±0.04^{b}	1.24±0.01^{d}	0.06±0.01^{a}	0.09±0.00^{a}	0.10±0.04^{a}	0.00±0.00^{a}	-
22:1n9	1.96±0.01^{a}	2.13±0.04^{b}	1.67±0.04^{c}	1.53±0.01^{d}	1.32±0.05^{e}	1.14±0.02^{f}	0.77±0.05^{g}	1.08±0.03^{f}	-
ΣMUFA	24.12±0.01^{c}	24.45±0.04^{c}	22.21±0.04^{b}	24.98±0.08^{d}	22.17±0.12^{b}	24.52±0.27^{cd}	19.68±0.13^{a}	32.72±0.29^{e}	-
18:2n6 cis	3.80±0.05^{a}	5.39±0.05^{b}	7.57±0.04^{c}	9.50±0.09^{d}	10.59±0.04^{e}	13.96±0.00^{f}	13.06±0.03^{g}	18.40±0.07^{h}	-
18:3n6	1.18±0.05^{c}	1.06±0.02^{b}	0.87±0.04^{a}	1.49±0.03^{d}	1.20±0.05^{c}	1.29±0.00^{c}	1.03±0.00^{b}	0.80±0.01^{a}	-
18:3ω3	1.00±0.05^{d}	1.08±0.05^{ac}	1.15±0.05^{ac}	0.11±0.01^{bc}	0.14±0.03^{bc}	0.13±0.03^{b}	0.11±0.03^{b}	1.48±0.05^{bc}	-
20:2n6	1.83±0.04^{d}	1.77±0.05^{c}	1.75±0.10^{c}	1.65±0.01^{bc}	1.55±0.05^{bc}	1.45±0.00^{b}	1.02±0.00^{a}	1.52±0.05^{bc}	-
20:3n6 NS[2]	0.07±0.00	0.15±0.05	0.08±0.01	0.19±0.01	0.17±0.01	0.13±0.01	0.16±0.05	0.17±0.01	-
20:4ω6 NS[2]	0.38±0.05	0.37±0.04	0.38±0.05	0.36±0.02	0.36±0.02	0.29±0.01	0.28±0.02	0.41±0.01	-
20:5ω3	7.24±0.02^{a}	6.87±0.03^{b}	6.75±0.04^{c}	6.34±0.01^{d}	6.18±0.05^{e}	5.96±0.02^{f}	4.44±0.05^{g}	6.21±0.03^{e}	-
22:5ω3	0.30±0.00^{ab}	0.48±0.05^{c}	0.46±0.05^{c}	0.40±0.02^{bc}	0.44±0.02^{c}	0.19±0.00^{a}	0.29±0.01^{ab}	0.27±0.03^{ab}	-
22:6ω3	7.60±0.05^{a}	7.21±0.05^{b}	6.76±0.03^{c}	5.84±0.00^{d}	5.23±0.02^{e}	3.86±0.05^{f}	2.98±0.05^{g}	3.12±0.01^{h}	-
ΣPUFA	23.41±0.07^{a}	24.39±0.10^{b}	25.77±0.29^{c}	25.88±0.10^{c}	25.88±0.23^{c}	27.26±0.05^{d}	23.36±0.07^{a}	32.39±0.09^{e}	-
% fat in dry diet	9.80±0.05^{a}	8.40±0.05^{b}	7.20±0.05^{c}	6.00±0.00^{d}	6.50±0.02^{e}	5.30±0.04^{g}	5.20±0.05^{g}	5.50±0.02^{f}	-
ω 3/ ω6	2.22±0.05^{a}	1.79±0.04^{b}	1.42±0.05^{c}	0.96±0.02^{d}	0.86±0.02^{d}	0.59±0.03^{e}	0.50±0.05^{e}	0.52±0.02^{e}	-
ω3 % of dry diet	1.58±0.07^{a}	1.31±0.05^{b}	1.09±0.04^{c}	0.76±0.01^{d}	0.78±0.01^{d}	0.54±0.01^{d}	0.41±0.04^{e}	0.61±0.03^{d}	0.05
ω 6 % of dry diet	0.71±0.01^{a}	0.73±0.05^{ab}	0.77±0.05^{ab}	0.79±0.03^{ab}	0.90±0.00^{b}	0.91±0.01^{b}	0.81±0.05^{ab}	1.17±0.03^{b}	1.00
DHA/EPA	1.05±0.02^{a}	1.05±0.04^{a}	1.00±0.02^{a}	0.92±0.01^{ab}	0.85±0.04^{b}	0.65±0.00^{c}	0.67±0.05^{c}	0.50±0.02^{d}	-

[1]Tocher (2010), [2]NS Not significant ($p>0.05$), Mean ± SE values bearing the same superscripts in rows do not differ significantly ($p<0.05$)

($p<0.05$) were observed in the diet intake between diets P59, P53, P40, P19 and P16, no variation in feed intake was noticed among the diets P47, P33 and P26. Overall consumption ranged between 3-5 g. Considering the above in juxtaposition with growth, the physical property of diets has not affected linearity in growth of these fish. Though pellet buoyancy increased with increase in the starch content (from wheat flour) following a definite pattern, consumption and growth was not affected by this variation. It appears that, koi are amenable to homemade diets equally well.

Optimum growth in carp spawn, fry and fingerlings was reported at 45% and 26% protein (casein) and carbohydrate (dextrin) respectively (Sen *et al.,*1978). Subsequently, Singh (1991) reported a protein requirement of 31-38% for cultivated carp *Cyprinus carpio*. NRC (2011) recommended a dietary protein level of 45% for fish having <20 g size. Due to the diversity in culture conditions and feed material used, it is difficult to adopt any of these findings in an aquariculture situation using formulated feed with natural ingredients. Therefore, the range of 16-59% protein was covered in this investigation. Total carbohydrates varied from 16 to 71% and total fat varied from 5.5-10% depending upon the formulations. The gross macronutrient levels in the formulations being so, amino acid content in the diets were profiled and

Table 8. Initial and final body fatty acid profiles (g 100 g^{-1} lipid)

Fatty acids	Initial	Final P59	P53	P47	P40	P33	P26	P19	P16
Saturated fatty acids									
14:00	1.04±0.02^{a}	6.07±0.01^{g}	5.81±0.01^{f}	3.89±0.04^{e}	3.59±0.05^{d}	3.91±0.04^{e}	3.34±0.05^{c}	3.11±0.01^{b}	3.5±0.05^{d}
15:00	0.2±0.00^{a}	0.3±0.04^{a}	0.93±0.04^{c}	0.93±0.05^{c}	0.22±0.05^{a}	0.59±0.05^{b}	0.5±0.05^{b}	0.24±0.05^{a}	0.28±0.05^{a}
16:00	19.53±0.05^{a}	20.77±0.05^{c}	21.48±0.05^{d}	20.09±0.05^{b}	20.16±0.05^{b}	20.59±0.04^{c}	20.03±0.03^{b}	6.88±0.05^{a}	7.8±0.05^{a}
17:00	0.36±0.01^{b}	0.93±0.03^{f}	0.61±0.03^{e}	0.54±0.04^{be}	0.48±0.05^{be}	0.53±0.05^{be}	0.48±0.05^{be}	0.16±0.05^{a}	0.16±0.05^{a}
18:00	6.26±0.05^{a}	3.43±0.02^{b}	3.6±0.02^{b}	2.6±0.12^{c}	2.83±0.04^{e}	3.49±0.04^{b}	3.52±0.09^{b}	1.41±0.04^{d}	1.52±0.05^{d}
20:00	1.14±0.05^{a}	0.32±0.05^{b}	0.3±0.05^{b}	0.21±0.05^{b}	0.19±0.05^{b}	0.19±0.04^{b}	0.17±0.05^{b}	0.08±0.03^{b}	0.26±0.05^{b}
22:00	0.67±0.05^{d}	0.12±0.00^{a}	0.08±0.00^{a}	0.09±0.01^{a}	0.11±0.05^{a}	0.2±0.05^{ab}	0.23±0.04^{ab}	0.32±0.05^{bc}	0.41±0.05^{c}
24:00	0.15±0.03^{a}	0.07±0.00^{b}	0.08±0.00^{b}	0.04±0.00^{b}	0.04±0.00^{b}	0.06±0.01^{b}	0.05±0.02^{b}	0.06±0.02^{b}	0.06±0.02^{b}
ΣSFA	29.35±0.05^{d}	32.01±0.17^{b}	32.89±0.17^{c}	28.39±0.37^{c}	27.62±0.06^{c}	29.56±0.05^{d}	28.32±0.14^{c}	12.26±0.08^{a}	13.99±0.26^{b}
Monounsaturated fatty acids									
16:1n7	3.71±0.05^{a}	11.58±0.07^{cd}	11.25±0.07^{b}	12.24±0.04^{e}	11.32±0.10^{bc}	11.62±0.05^{d}	11.43±0.07^{bcd}	12.76±0.04^{f}	12.56±0.05^{f}
17:01	0.58±0.05^{b}	0.5±0.10^{a}	0.52±0.09^{a}	1.06±0.02^{b}	0.3±0.01^{c}	0.33±0.04^{b}	0.28±0.05^{c}	0.31±0.05^{d}	0.21±0.05^{d}
18:1n9	36.86±0.01^{b}	24.00±0.94^{ab}	23.75±0.94^{ab}	36.23±0.05^{c}	40.21±0.08^{ab}	36.21±0.05^{ab}	39.53±0.04^{ab}	49.85±0.05^{ab}	48.81±0.04^{a}
20:01n11	0.08±0.00^{a}	1.34±0.05^{a}	0.79±0.05^{c}	0.58±0.05^{ac}	0.48±0.05^{a}	0.59±0.04^{ac}	0.52±0.05^{b}	0.61±0.04^{bc}	0.61±0.04^{bc}
22:01n9	0.13±0.01^{a}	1.89±0.05^{d}	1.79±0.05	0.93±0.03^{bc}	0.79±0.05^{bc}	0.84±0.07^{bc}	0.72±0.05^{bc}	0.97±0.05^{c}	0.99±0.05^{d}
ΣMUFA	41.36±0.09^{b}	39.31±1.08^{a}	38.10±1.08^{a}	51.04±0.18^{d}	53.10±0.17^{e}	49.59±0.16^{c}	52.48±0.16^{de}	64.50±0.14^{g}	63.18±0.23^{f}
Polyunsaturated fatty acids									
18:2n6	15.75±0.04^{g}	4.08±0.03^{a}	5.15±0.03^{b}	4.36±0.04^{c}	4.76±0.05^{d}	6.16±0.05^{e}	6.29±0.04^{e}	8.44±0.05^{f}	8.36±0.05^{f}
18:3n6	2.09±0.04^{b}	1.41±0.05^{a}	2.45±0.05^{c}	2.12±0.04^{b}	2.05±0.01^{b}	2.13±0.04^{b}	2.05±0.03^{b}	2.44±0.05^{d}	2.33±0.05^{d}
18:3n3	0.52±0.05^{c}	0.8±0.05^{a}	0.04±0.05^{b}	0.06±0.01^{b}	0.04±0.01^{b}	0.35±0.05^{c}	0.37±0.04^{c}	0.48±0.05^{c}	0.49±0.05^{c}
20:2n6	0.14±0.02^{a}	1.31±0.05^{b}	1.21±0.05^{b}	0.82±0.04^{b}	0.67±0.05^{b}	0.74±0.07^{b}	0.59±0.04^{b}	0.69±0.04^{b}	0.64±0.05^{b}
20:3n6	0.14±0.01^{abc}	0.2±0.00^{abc}	0.15±0.00^{bcd}	0.08±0.03^{abc}	0.23±0.02^{bc}	0.27±0.04^{cd}	0.29±0.05^{c}	0.05±0.02^{ad}	0.41±0.05^{d}
20:4n6	1.4±0.016^{a}	0.51±0.05^{b}	0.5±0.05^{b}	0.28±0.02^{c}	0.22±0.04^{c}	0.24±0.02^{c}	0.2±0.05^{c}	0.21±0.02^{c}	0.22±0.04^{c}
20:5n3	0.43±0.02^{a}	4.05±0.09^{f}	3.64±0.09^{e}	2.27±0.05^{d}	1.81±0.05^{c}	1.93±0.02^{c}	1.48±0.05^{b}	1.5±0.05^{b}	1.49±0.05^{b}
22:5n3	0.27±0.05^{ab}	0.48±0.05^{ab}	0.37±0.05^{ab}	0.34±0.05^{ab}	0.21±0.06^{b}	0.2±0.05^{a}	0.5±0.05^{a}	0.25±0.04^{ab}	0.25±0.05^{ab}
22:6n3	1.48±0.05^{ab}	7.78±0.08^{ab}	7.36±0.08^{ab}	3.94±0.02^{ab}	3.28±0.02^{a}	2.71±0.05^{a}	2.16±0.04^{b}	2.75±0.05^{ab}	2.74±0.05^{ab}
ΣPUFA	22.28±0.24^{a}	20.62±0.20^{b}	20.87±0.20^{b}	14.27±0.05^{e}	13.27±0.06^{f}	14.73±0.05^{d}	13.93±0.13^{e}	16.81±0.09^{c}	16.93±0.12^{c}
n3/n6	0.14±0.01^{a}	1.75±0.07^{e}	1.21±0.07^{d}	0.86±0.05^{c}	0.67±0.05^{bc}	0.54±0.04^{b}	0.48±0.05^{b}	0.42±0.09^{b}	0.42±0.05^{b}
DHA/EPA	3.44±0.05^{a}	1.92±0.05^{bc}	2.02±0.05^{b}	1.74±0.04^{c}	1.81±0.05^{bc}	1.4±0.02^{d}	1.46±0.05^{d}	1.83±0.05^{bc}	1.84±0.05^{bc}

Mean±SE values bearing the same superscript in rows do not differ significantly ($p<0.05$)

Table 9. Growth and feed utilisation of koi fry fed formulated diets with varying nutrient densities[1]

Feed no.	Initial weight (g)	Final weight (g)	Biomass gain[2] (g)	Percent body weight gain[3]	Specific growth rate (% per day)[4]	Diet intake (g)	FCR[5]	PER[6]	Diet cost per kg fish produced[7] (₹)	Diet cost for 5 g fry (₹)
P59	0.149±0.00	4.000±0.60^{b}	3.851±0.60^{b}	2585±409^{b}	2.64±0.49^{a}	4.313±0.01^{b}	1.12±0.65^{d}	0.52±0.93^{b}	90.72	0.45
P53	0.162±0.00	3.544±0.35^{b}	3.382±0.35^{b}	2088±235^{ab}	3.11±0.12^{a}	3.855±0.01^{a}	1.14±0.55^{cd}	0.46±1.17^{b}	84.35	0.42
P47	0.161±0.01	3.366±0.44^{ab}	3.205±0.44^{ab}	2007±305^{ab}	3.05±0.17^{a}	3.461±0.06^{c}	1.08±0.63^{abc}	0.43±1.20^{b}	72.35	0.36
P40	0.167±0.00	2.833±0.30^{ab}	2.666±0.30^{ab}	1991±179^{ab}	2.85±0.12^{a}	3.492±0.05^{f}	1.31±0.76^{bcd}	0.30±1.13^{a}	78.59	0.39
P33	0.157±0.00	2.702±0.00^{ab}	2.545±0.00^{ab}	1621±49^{ab}	2.88±0.06^{a}	4.988±0.00^{e}	1.96±1.13^{abc}	0.17±0.92^{ab}	103.88	0.52
P26	0.146±0.01	2.511±0.20^{ab}	2.364±0.20^{ab}	1619±120^{ab}	2.89±0.09^{a}	3.451±0.03^{c}	1.46±0.84^{a}	0.18±1.56^{ab}	67.15	0.34
P19	0.163±0.00	1.654±0.16^{a}	1.491±0.16^{a}	915±102^{a}	2.34±0.13^{a}	3.071±0.00^{e}	2.06±1.19^{a}	0.09±1.53^{ab}	80.33	0.40
P16	0.159±0.01	1.542±0.18^{a}	1.383±0.18^{a}	870±94^{a}	2.29±0.11^{a}	3.291±0.02^{d}	2.38±1.38^{ab}	0.07±1.61^{a}	85.67	0.43

[1]Mean±SE values are of 3 replicates, [2]Biomass gain = mean final weight – mean initial weight, [3]Percent body weight gain = (Final weight - Initial weight)/ Initial weight x 100, [4]SGR = (ln. mean final weight) − (ln. mean initial weight) x 100/ no. of days, [5]FCR = Dry food fed (mg) / wet weight gain (mg), [6]PER = Weight gain (mg, wet weight basis) / protein intake (mg, dry weight basis), [7]Diet cost per kg of production = (feed price per kg x feed fed/fish produced), Mean values sharing the same superscripts in columns are not significantly different ($p<0.05$)

Table 10. Initial and final body composition of experimental animals (% wet weight basis)

Parameters	Initial	P59	P53	P47	P40	P33	P26	P19	P16
Moisture	80.69±0.05^{g}	79.08±0.03^{f}	80.99±0.38^{g}	77.02±0.02^{e}	74.25±0.05^{d}	73.63±0.07^{c}	69.84±0.06^{a}	70.78±0.05^{b}	73.72±0.05^{c}
Crude protein	10.83±0.05^{c}	12.23±0.05^{hi}	11.55±0.10^{def}	10.55±0.10^{b}	11.71±0.06^{fg}	11.37±0.04^{de}	12.37±0.05^{i}	11.96±0.05^{gh}	9.65±0.12^{a}
Crude lipid	4.40±0.05^{a}	4.81±0.05^{b}	4.49±0.05^{a}	9.78±0.04^{c}	11.31±0.04^{d}	12.32±0.05^{e}	16.32±0.05^{h}	15.77±0.04^{f}	14.18±0.05^{g}
Total cabohydrates	1.67±0.05^{e}	1.54±0.05^{e}	0.81±0.01^{b}	0.94±0.02^{b}	1.41±0.05^{d}	1.36±0.04^{c}	0.05±0.00^{a}	0.11±0.02^{a}	0.84±0.05^{b}
Ash	2.41±0.05^{d}	2.34±0.02^{d}	2.16±0.04^{d}	1.72±0.04^{c}	1.31±0.12^{a}	1.33±0.05^{a}	1.42±0.05^{ab}	1.38±0.05^{ab}	1.62±0.05^{bc}

Mean±SE values bearing the same superscript in rows do not differ significantly ($p<0.05$)

compared with the amino acid requirements published by NRC (1993) for common carp along with the initial and final body amino acid profiles. The results indicate a proportionate reduction in the amino acid content with reduction in inclusion of protein rich ingredients. The level of all the essential amino acids required for common carp was met by all the experimental diets used in this study (NRC, 1993; NRC, 2011). The amino acid composition of final body mass of differential experimental groups was better than the initial body amino acid content except P16. Final body amino acid profiles shown in Table 5 do not reflect the variations in dietary amino acid profiles as reported by Schwarz and Kirchgessner (1988).

Fatty acids in the diet (exogenous) are reflected in the body fatty acid profile (Ackman, 1980; 1989). Gross protein deposition was found to increase proportionately with the protein content in diet and fat deposition increased with fat and carbohydrate content in the diet (Table 10). According to Hasan *et al.* (1997), in carp fry, carcass composition is not influenced by the protein content in the diet. Moisture and lipid are the other two major components which tend to vary (Atack *et al.,* 1979; Hasan *et al.,* 1990; Hasan and Macintosh, 1993). In this study also, the most evident variation was with respect to lipid which tends to accumulate with increasing starch content in the diet indicating the utilisation of cooked starch (Wilson, 1994) for lipid synthesis. However, when compared with a report in koi carp (Bashir *et al.* 2010), in the present study, the body protein on a dry matter basis was found to be lesser, probably due to the difference in size of the fish; 12 g *vs* 4 g in the present study. Moreover in diets used in the present study, the non-protein energy yielding nutrients (fat and carbohydrate) were more than 76% which would have contributed to higher fat deposition when compared with the former report.

Further, when fatty acid profiles of the diets were examined for their sufficiency (Tocher, 2010) in ω3 and ω6 fatty acids, ω6 fatty acids were deficient (Table 7). As, there are no other reports on the fatty acid profiles of *Cyprinus carpio* var. *koi,* high content of ω6 fatty acid docosahexaenoic acid (DHA) initially itself and its diet-induced accumulation is noteworthy. ω3/ω6 ratio reported by Jabeen and Chaudhry (2011) is 0.27 for carps weighing 600 g with 0.37% DHA and 0.34% EPA. In this study, the initial DHA and EPA were 1.48 and 0.43% respectively which increased to 7.78% and 4.08% in treatment P59 indicating the availability of these fatty acids from feed in substantial quantity. These elevated levels of PUFA in the initial body composition itself could be due to breeding and rearing of these fish in controlled conditions with high quality formulated diets or live diets prior to their use as experimental animals. Moreover, compared to marine fish, it is also known that freshwater fish have the ability to convert linolenic acid (ALA) to EPA and DHA (Farkas, 1984; Olsen *et al*., 1990; Tocher, 2003; Zheng *et al*., 2004) which could be the main reason for elevated PUFA levels.

From the growth data (Table 9) it is evident that, biomass gain is similar with the diets P26 – P47 ($p<0.05$). Percent body weight gains were statistically similar for the diets P26 - P53. Similarity in SGRs can be noticed with P33, P40, P47 and P53. Significantly highest growth rate was recorded with the diet P59 containing almost 60% protein. Bashir *et al.* (2010) evaluated different levels of protein in formulated diets for koi carp with levels ranging from 24% to 43%. Since growth was statistically similar with 35% and 43% protein, they opined that 35 – 45% protein would be an economical range. The growth increments observed in the present study, with ~60% protein without any adverse effects on the growth or health of the fish indicates the adaptive mechanisms inherent in the fish to survive on varying nutrient densities which make it a popular candidate in outdoor garden ponds. Uniformity in the PER with all diets except P59 is another indication that a diet with 60% protein can be avoided. Lowest FCR was obtained with diet P47. Diets with protein content between 25 - 50% are considered appropriate for carps (Kaushik, 1995). Diets P16 and P19 can be excluded because of predictable long term effects of protein insufficiency. Above this level of protein, diets with protein content 25, 30 and 35% can be advocated as an economical range with all nutrient fortifications and diets with 40 and 45% protein can be provided as diets for faster growth. Since high level of carbohydrate utilisation

was evident, probably due to microflora in the hindgut (Pannevis and Earle, 1994) different permutations and combinations of protein, carbohydrate and lipid could be examined for an economically optimum level. Cost of fry production using these diets varied from ₹0.34 to 0.52. Procurement cost of these fishes was ₹0.55 with a weight of less than 0.2 g. These fish with a weight gain of over 1619 to 2000% would fetch more than double the cost (₹1 to 1.5) in bulk wholesale and another 100% increase in cost when retailed in pairs (personal communication, Santosh Baby, Ornamental fish producer and trader, Nellayi, Kerala, India). Therefore, koi can be reared on diets with varying nutrient densities ranging from 25 -55% proteins, 5.5 – 10% fat and 20-80% carbohydrate. The pace of growth can be staggered according to the trade requirements. As mentioned earlier, 20-50% protein as suggested by Kaushik (1995) for maintenance and 40-55% protein for faster growth can be recommended..

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