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GROWTH RESPONSE OF JUVENILE GRASS CARP (*CTENOPHARYNGODON IDELLA*) FED ISOCALORIC DIETS WITH VARIABLE PROTEIN LEVELS

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ABSTRACT

Sixty days feeding experiment was conducted on grass carp fry fed isocaloric diets with variable protein levels in order to evaluate its growth response. Different diet ingredients were analyzed for % age protein, % age fat, % age ash, % fiber, % age moisture contents and energy. Five isoenergetic (17.91 ± 0.02 Kg MJ^{-1}) and isolipidic (8.56 ± 0.09 g) diets with varying protein levels PL_1 (20%), PL_2 (25%), PL_3 (30%), PL_4 (35%) and PL_5 (40%) were formulated, analyzed and fed to triplicate groups of fish fry each group comprised of 10 fish (average weight 0.57 ± 0.001g $fish^{-1}$). Experimental design was a completely randomized design. Highest mean weight gain, average daily growth, final weight gain, specific growth rate but lowest feed conversion ratio were observed ($P<0.05$) in fish fed diet PL_5 (40% protein level diet). Whereas significantly lowest mean weight gain, average daily growth, percent weight gain, specific growth rate but highest feed conversion ratio ($P<0.05$) were observed for PL_1 (20% protein level diet). Average temperature (29.66–30.0 ºC), pH (8.32–8.55), dissolved oxygen (6.31-6.60 mg/L) were within the optimum range throughout the experiment and did not significantly differ among different treatments ($P<0.05$). Present study revealed that forty percent protein level is best and economical for rearing grass carp fry.

Key words: *Ctenopharyngodon idella* fry; Nutrition; Protein; Dietary requirement; Growth.

INTRODUCTION

The optimization of fish production requires research into feeding techniques, which promotes growth and at the same time reduces the quantity of waste products released in the water as reported by Singh *et al*. (2005). According to Erondu *et al*. (2006); Sheunn *et al*. (2003) fish feed consist of 60% production cost and the protein component is to be the most expensive in terms of over all feed cost. Increasing protein levels in feeds can lead to improved fish production, but excessive dietary protein is not economical for fish culture. Kalla and Garg (2004) concluded that the dietary protein level is one of the major factors influencing growth of fish, feed efficiency and water quality. The protein requirement of fish is recognized as the protein content which gives the maximum growth, maximum economic profit and maximum protein deposition. According to Ajiboye and Yakubu (2009) the determination of protein requirement of fish is a very critical factor in aquaculture production. Bureau *et al*. (2002); Abidi and Mukhtar (2008) emphasize the need to minimize protein levels of the fish, which reduces the proportion of dietary protein that is metabolized, without reducing growth, resulting in undesirable nitrogenous waste production. According to Engin and Carter (2001); Jensen (2003); Kalla *et al*. (2003), determination of the required dietary protein levels is important to get highest growth and reduce the water deterioration problems related with supplementary feed intake of fish. Although toxicity of metabolites in fish varies considerably and depends on many internal and external factors but amount of nitrite, ammonia, and orthophosphate produced by the fish seems to be directly related to the source and levels of protein intake.

Du *et al*. (2006); Mohanta *et al*. (2008a) reported the Grass carp as a very popular economic fish species. Although Du *et al*. (2006) carried out some studies about nutrient requirement of grass carp but according to Gao *et al*. (2009b) the feed ingredients used were neither cost effective nor procurable. In present studies local feed ingredients of plant origin were incorporated with fish meal for the formulation of grass carp fry diet in order to evaluate the best and cost effective diet regarding growth and digestibility.

MATERIALS AND METHODS

Experimental design: The experiment design was a completely randomized design. Experimental set-up comprised of 5 treatments; PL_1 (20% protein level), PL_2 (25% protein level), PL_3 (30% protein level), PL_4 (35% protein level) and PL_5 (40% protein level), each treatment in triplicate to determine the optimum protein requirement of the fish as did by Mohenta *et al*. 2008; Hossain *et al*, 2002. Glass aquaria of 90-litre capacity were used during experimentation and a constant water volume of 70 litters was maintained. Stocking rate was 10 fry per aquarium. Feeding was done @ 2% body weight per day and the feeding frequency was 4 times a day. Experimental duration was 60 days. 12 h light/12 h dark

(12 L: 12 D) photoperiod was maintained throughout the experiment using artificial light.

Feed ingredients and feed preparation: All the diet ingredients (fish meal, corn flour, wheat flour, gluten, rice polish) were analysed in triplicate following the methods described in AOAC (2006). Crude protein (CP) was analyzed by Kjeldahl method (inkjel M Behr Labor-Technik D-40599 Dusseldorf) after acid hydrolysis (N ×6.25); crude lipid (CL) after extraction with petroleum ether by Soxhlet method (KB 8 Gerhardt Bonn); total ash by igniting the sample at 550 °C for 12 h in muffle furnace (Carbolite CWF 1200); moisture after oven drying (memmert GmbH+Co.KG D-91126 Schwabach FRG) at 105 °C till constant weight; fiber contents by acid digestion (1.25%) followed by alkali digestion (1.25%) using crude fiber apparatus (OSK 1352OA, Ogawa Seiki Co). Organic matter (OM) was calculated by subtracting total ash from DM (dry matter); total carbohydrates (TCHO) were calculated by subtracting crude protein (CP) and CL (crude lipid) from OM; nitrogen free extract (NFE) was calculated as 100 % (moisture + protein + lipid + ash + crude fiber) and digestible energy (DE) was determined using bomb calorimeter (K-C2000 basic IKA^R WERKE). Proximate composition of feed ingredients used in formulation of different diets is given in Table I.

All these feed ingredients were mixed in different proportions to formulate five isoenergetic (17.91 ± 0.02 Kg MJ^{-1}), isolipidic (8.4-8.6%) diets with variable protein levels of 20% (PL_1), 25% (PL_2), 30% (PL_3), 35% (PL_4) and 40% (PL_5). The lipid level was maintained using equal proportions of cod liver oil and variable proportions of soyabean oil (Habib Pure). α-cellulose was used as filler and carboxymethyl cellulose was used as feed stabilizer (binder). All the diets were fortified with prepared vitamins and minerals as reported by Hasan (2001). All major dry ingredients were mixed for 15 min in a food mixer. The cod liver oil and soya bean oil were blended in a mixer, then added to the mash and mixed for an additional 15 min. Hot water (approximately 60 °C) was mixed into the mash to provide a consistency appropriate for pelleting and this is mixed for another 15 min following the procedure adopted by Hernández *et al*. (2008a,b). The resulting mash was passed through a meat grinder equipped with a 1.0 mm diameter die to produce pellets. The pellets were air dried, crumbled and stored frozen at -20 °C until utilization as reported by Meyer and Fracalossi (2004) in air-tight plastic jars. All the prepared diets were analyzed following the same procedures and equipments as for the feed ingredients. Ingredient composition of experimental feeds fed to fingerlings of *Ctenopharyngodon idella* is given in Table II and chemical composition, digestible energy value, protein energy ratio of experimental feeds is given in Table III. Fresh feeds were prepared every 15 days.

Experimental setup: Fry of *Ctenopherangodon idella* (grass carp) were procured from Himalaya Fish Hatchery, Muriedke and transported to the SDSC laboratory in oxygen filled polyethylene bags. All the aquaria were washed with potassium permanganate solution for disinfection and fish was also treated with potassium permanganate solution (1.0 mg L^{-1}) to remove any external parasites as reported by Giri *et al*. (2009). Fry of *Ctenopharyngodon idella* were stocked in these aquaria for a period of one month. During acclimatization they were fed with 35% protein diet as Hossain *et al*. (2002) did. Prior to feeding of experimental diets, the fish were starved overnight to empty their gut and increase their appetite and reception for the new diets.

After 1 month of acclimatization, fry of *Ctenopharyngodon idella,* (Ave. wt. 0.57±0.001g $fish^{-1}$) were weighed using Mettler Toledo PB602 top-loading balance before introduction into the experimental aquaria. All fish were fed 4 times a day i. e., 8:00 AM, 11:00 AM, 2:00 PM, 5:00. Fish were batch weighed after every fortnight to determine the growth and health status of the fish. The quantity of diet given was readjusted every fortnight after weighing the fish. Unconsumed feed was collected and dried in a hot air oven at 80 °C. Food consumption was estimated by subtracting the weight of the unconsumed dry feed from the weight of the offered feed following the procedure of Chakrabarty *et al*. (2008). The faecal samples were siphoned daily.

Monitoring of water quality: Temperature (°C), pH (pH meter) and dissolved oxygen (DO meter) were monitored daily three times a day. Total ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO^{2-}-N), nitrate-nitrogen (NO_3^--N), phosphate-phosphorus were determined daily. Total alkalinity, total hardness and chlorides were measured fortnightly following the standard procedures of APHA (2005).

Nutritional indices: Fish were weighed fortnightly to determine weight gain for different treatments and adjust the feed inputs. Final weight gain (WG), average daily growth (ADG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were calculated in order to determine the growth response of juvenile grass carp (*Ctenopharyngodon idella*) fed on experimental diets.

Statistical analysis: The difference among the treatments was tested by one-way analysis of variance (ANOVA). All analysis was conducted using SPSS Version 13.0. A Duncan's multiple range was used to compare the means significant differences between the feeds following Zar (2001). Treatment effects were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Growth in terms of final weight gain (WG), average daily growth (ADG), and specific growth rate (SGR) were significantly (P<0.05) higher in fish fed on PL_5 as compared to PL_1 (P< 0.01), PL_2, PL_3 and PL_4 (P< 0.05). Feed conversion ratio (FCR) was significantly high in PL_1 as compared to PL_5 (P< 0.01), PL_2, PL_3 and PL_4 (P<0.05). No significant difference (P>0.05) was observed regarding growth parameters and FCR among PL_2, PL_3 and PL_4 (Table IV). 100% survival of grass carp fry was observed in all the treatments (PL_1 to PL_5) throughout the experiment.

According to Hossain *et al*. (2002), growth response of fish varies from species to species due to difference in feeding habit, water temperature and fish size. Gao *et al*. (2009a) reported improved growth in grass carp when dietary protein level increased from 25% to 38%. However in another study Gao *et al*. (2009b) reported 39% optimum protein level in grass carp. In this study, the approximate level of dietary protein, which showed maximum growth in grass carp (*Ctenopharyngodon idella*) fry was found to be 40%. Similar results for growth indices were observed by Jana *et al*. (2006) for *Chanos chanos*; Ogunji and Wirth (2000) for *Oreochromis niloticus* and Stapathy *et al*. (2003) for *Labeo rohita.*

Hossain *et al*. (2005) reported that average daily growth (ADG) and weight gain (WG) in *Tor putitora* had maximum value for 40% protein level fed diet and minimum value for 20% protein level fed diet. Their results are in agreement with the results obtained during present studies.

Sa *et al*. (2008) reported that unbalanced diets do not fulfill the basic requirements of animals. In present study lowest growth in fish fed 20% protein diet (PL_1) may be due to the reduced feed intake of the diet with a protein level lower than that required for body repairing and maintenance. It was also observed that as an energy source, protein was used more efficiently than carbohydrates and digestibility of protein was not influenced by different carbohydrate to protein ratios. Similar findings were reported by Sa *et al*. (2006, 2007, 2008).

In this study specific growth rate (SGR) was found to be significantly lower (P<0.05) for the fish fed 20% protein level diet which increased with increasing protein level and maximum SGR was found for fish fed 40% protein level diet. The feed conversion ratio (FCR) value ranged between 1.71 and 3.95 which decreased with increasing protein level and found to be lowest at highest protein level fed diet (PL_5) but highest FCR was found at lowest protein level fed diet (PL_1). Hossain *et al.* (2002) in *Tor Putitora* at 40% protein level but Gao *et al.* (2009a) at 38% protein level in grass carp reported similar findings.

Different water quality parameters ranged as pH 8.32–8.55, temperature 29.66–30.0 (ºC), dissolved oxygen 6.31-6.60, ammonia- nitrogen 0.054-0.082, nitrite-nitrogen 0.037–0.063, nitrate-nitrogen 3.440–5.100, phosphate 0.077–0.110, total alkalinity 262–361 ($CaCO_3$), total hardness 287.36–375.21 ($CaCO_3$) and chloride (Cl^{-1}) 86.98–91.42, mg L^{-1}. Ammonia concentration was significantly low (P< 0.05) in PL_1 and PL_5 as compared to PL_2, PL_3, PL_4; nitrite concentration was significantly high in PL_4 (P<0.05) than PL_1, PL_2, PL_3 and PL_5; nitrate concentration was significantly low in PL_5 than PL_4 (P<0.01) and PL_1, PL_2 and PL_3 (P<0.05); phosphate concentration was significantly high (P< 0.05) in PL_4 as compared to PL_1, PL_2, PL_3 and PL_5 (Table V).

The overall slow growth observed in the last 30 days of experiment may be due to abrupt dial fluctuations in temperature. Song *et al*. (2009) also found the differences in growth of Chinese sucker due to dial fluctuation of temperature.

pH values for the treatments PL_4 and PL_5 were non significantly high (P>0.05) than other treatments (PL_1, PL_2 and PL_3) but suitable for fish culture as reported by Afzal *et al*. (2008). Overall dissolved oxygen did not significantly differ (P>0.05) among different treatments and was within acceptable range as reported by Boyd (2000). Other water quality parameters (mgL^{-1}) i. e., ammonia, nitrite, nitrate, phosphate, total alkalinity, total hardness and chloride were within the acceptable range throughout the experiment as reported by Boyd (2000). Significantly lower (P<0.05) excretion of metabolites such as ammonia, nitrite, nitrate and phosphate was observed by Jana *et al*. (2006) for 40% protein level than other treatments. Their results are comparable with our results. The mean values of ammonia, nitrite, nitrate and phosphate were significantly lower (P<0.05) in 40% protein level fed diet (PL_5) than other diets (PL_2, PL_3 and PL_4) except PL_1 which may be due to the low protein availability for growth and body metabolism.

In this study highest (P/E) ratio was found to be 22.3 (100g MJ^{-1}) for 40% protein level diet. Similar protein energy ratio on the bases of best growth performance was observed in 40% protein level by Hossain *et al*. (2002) 20.9 (100g MJ^{-1}) for *Tor Putitora*; Hossain *et al*. (2005) 21.7 (100g MJ^{-1}) for *Mystus Cavasius* and Mohanta *et al*. (2008b) 19.80 (100g MJ^{-1}) for *Puntius gonionotus.*

According to Sugiura *et al*. (2001) it is not the feed formulae or feed ingredients required by the living body but nutrients which make the chemical composition of these diet components and feed formulae. For maximum growth in fish, with a minimum level of protein, a balance of essential amino acids should be provided in order to fulfill the basic body requirements. This can be achieved when FM is used as main source of protein. In the present study PL_5 diet having maximum

FM showed maximum growth. This may be due to sufficient amino acids availability for fish growth and body mechanism. PL_1 diet with minimum amount of fish meal showed minimum growth which may be due to lack of proper amino acids balance because Lim and Webster, (2006) reported that fry and fingerlings require high protein diet than adult fish. As PL_5 showed maximum growth in grass carp fry, therefore, PL_5 was found to be best feed in terms of growth.

Table 1. Proximate composition of feed ingredients used in formulation of different diets

Ingredients	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)	Energy (Kcal/100g)
Fish Meal	57.00±0.001	11.78±0.001	2.40±0.002	21.24±0.002	9.78±0.001	453.80±0.02
Rice Polish	16.00±0.001	13.37±0.002	1.92±0.001	9.42±0.002	8.76±0.002	444.60±0.02
Corn Flour	10.75±0.001	8.40±0.002	1.55±0.002	1.26±0.001	7.34±0.001	426.50±0.03
Wheat Flour	6.00±0.002	2.76±0.001	1.10±0.002	1.08±0.002	7.98±0.001	419.00±0.02
Gluten	27.00±0.002	1.55±0.001	7.17±0.001	8.34±0.002	10.90±0.002	413.80±0.02

Table II. Ingredient composition (g/100) of experimental feeds fed to fingerlings *Ctenopharyngodon idella*

Ingredients (g $100g^{-1}$)	PL_1	PL_2	PL_3	PL_4	PL_5
Fish Meal**	25.00	35.00	40.00	45.00	50.00
Rice Polish***	9.00	-	-	1.00	-
Corn Flour****	12.10	9.00	9.00	-	-
Wheat Flour*****	47.90	45.50	28.80	18.50	2.50
Gluten******	0.50	5.00	16.70	30.00	42.00
Cellulose	0.50	0.50	0.50	0.50	0.50
Ascorbic Acid	0.50	0.50	0.05	0.05	0.05
Carboxymethyl Cellulose	2.00	2.00	2.00	2.00	2.00
Vitamin Premix*	1.00	1.00	1.00	1.00	1.00
Cod Liver Oil	2.00	2.00	2.00	2.00	2.00
Total	100.20	100.25	100.15	100.15	100.05

*1 g vitamin mix (choline chloride 500 mg; thiamine HCl 5 mg; riboflavin 20 mg; pyridoxine HCl 5 mg; nicotinic acid 75 mg; calcium pantothenate, 50 mg; inositol, 200 mg; biotin 0.50 mg; folic acid 1.50 mg; ascorbic acid 100 mg; menadione (K) 4 mg; a-tocopheryl acetate (E) 40 mg; cyanocobalamine (B12) 0.0001 mg
** Pakfish Pure Karachi
*** Bari Rice Mill Mureidke
**** Faisalabad Trading Company Okara
***** Sunny Flour Mill Lahore
******Rafhan Company Faisalabad

Table III. Chemical composition (g/100g), digestible energy value (MJ/100g) and protein energy ratio (100g/ MJ) of experimental feeds (dry matter basis)

Parameters	PL_1	PL_2	PL_3	PL_4	PL_5
DM	92.06	92.23	92.95	93.71	94.45
CP	20.00	25.00	30.00	35.02	39.99
CL	08.64	08.41	08.62	08.51	08.61
Ash	06.87	08.54	10.31	12.35	14.15
Fiber	01.52	01.84	2.62	03.46	04.24
OM	85.19	83.69	82.63	81.35	80.30
T-CHO	56.55	50.28	44.01	37.82	31.70
NFE[1]	55.03	48.44	41.39	34.37	27.46
DE	01.79	01.79	01.79	01.79	01.79
P/E	11.17	13.93	16.73	19.50	22.30

DM Dry matter, CP Crude protein, CL crude lipid, OM organic matter, T-CHO total carbohydrate, DE digestible energy, P/E Protein energy ratio

Table IV. Growth response and survival of *Ctenopharyngodon idella* fry fed diets with varying protein levels

Parameters	Dietary treatments				
	PL_1	PL_2	PL_3	PL_4	PL_5
Initial body weight (g)/fish	0.576±0.004^a	0.577±0.001^a	0.577±0.001^a	0.577±0.003^a	0.576±0.000^a
Final Weight (g)/fish	1.800±0.058^a	2.203±0.104^b	2.254±0.132^b	2.240±0.001^b	2.616±0.012^{ab}
WG *(%)	212.500±8.850^a	281.282±17.80^b	289.948±23.40^b	288.215±6.240^b	354.166±2.08^{ab}
ADG (g / day)**	2.040±0.092^a	2.710±00.170^b	2.795±00.219^b	2.772±0.057^b	3.400±0.021^{ab}
SGR (%/day)***	1.900±0.092^a	2.232±00.156^b	2.270±0.198^b	2.270±0.049^b	2.523±0.021^{ab}
FCR****	2.980±0.035^a	2.150±0.033^b	2.040±0.283^b	2.010±0.049^b	1.730±0.021^{ab}
SR (%)*****	100^a	100^a	100^a	100^a	100^a

Values are expressed as Mean±SD of triplicate groups of ten fishes. Means with different superscript letters within a row are significantly different (P<0.05)

WG * = [Final body weight - Initial body weight] × 100/ Initial body weight - Ali (2001)

ADG (g/day) ** = [Final body weight - Initial body weight] ×100/ Culture period in days

SGR (%/day) *** = [l00 X (In final body weight - In initial body weight)] / Experimental duration - Giri *et al*. (2009)

FCR**** = Dry feed intake (feed consumed)/ Wet body weight gain - Goda *et al*. (2007)

SR (%) ***** = [Final number of fish - initial number of fish] × 100 Hernández *et al* . (2009)

Table V Nitrate, nitrite, phosphate and ammonia values (mg/L) for different dietary treatments

Dietary treatment	Ammonia	Nitrite	Nitrate	Phosphate
PL_1	0.054 ± 0.001^a	0.037 ± 0.003^a	5.100 ± 0.184^a	0.077 ± 0.000^a
PL_2	0.082 ± 0.001^b	0.042 ± 0.001^a	5.060 ± 0.071^a	0.088 ± 0.011^a
PL_3	0.080 ± 0.002^b	0.045 ± 0.001^a	4.965 ± 1.096^a	0.089 ± 0.000^a
PL_4	0.081 ± 0.002^b	0.063 ± 0.002^b	4.215 ± 0.757^b	0.110 ± 0.001^b
PL_5	0.062 ± 0.001^a	0.039 ± 0.002^a	3.440 ± 0.382^c	0.081 ± 0.016^a

Values are expressed as mean of triplicate samples± SD

Means with different superscript letters within a row are significantly different (P<0.05)

Conclusion: Present study revealed that the maximum growth of grass carp was observed when fed a diet PL_5 (40% protein level) with P/E ratio 22.3g protein MJ^{-1}. Individual WG, ADG and final WG was found to be highest for PL_5. Although growth of grass carp slightly declined in second half of the experiment due to temperature variations in all the treatments, however WG and SGR in PL_5 was observed maximum throughout the experiment. Diet was most efficiently utilized in PL_5 (40% protein level) showing lowest feed conversion ratio. FCR was found to be highest for PL_1 (20% protein level) showing poorly utilized diet. For PL_5 diet, protein in diet was efficiently used which resulted in minimum waste generation. Regardless of all the diets being isoenergetic and isolipidic diets, 40% protein level showed best results for the growth of grass carp fry.

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