

Dietary Protein Requirement of Giant Snakehead, *Channa marulius* (Ham., 1822) Fry and Impact on Growth Indices

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Received: 9 April 2012/Revised: 5 June 2012/Accepted: 12 July 2012/Published online: 31 July 2012
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Abstract Six semi-purified diets (energy 17.97–18.47 kJ g⁻¹) containing protein levels of 300 g kg⁻¹(diet-A), 360 g kg⁻¹(diet-B), 420 g kg⁻¹(diet-C), 480 g kg⁻¹(diet-D), 540 g kg⁻¹ (diet-E) and 600 g kg⁻¹(diet-F) were estimated for the protein requirement of *Channa marulius* fry (length 4.11 ± 0.59 cm) in a completely randomized experiment design in triplicate set. The fry were reared in 18 FRP tanks at a stocking density of 40 fry m³ and fed @ 8–5 % bw. The diets A, B, C and D showed significantly ($p < 0.05$) low survival levels of 46.6, 46.6, 46.6 and 53.3 in comparison to diets E (88.3 %) and F (85.0 %) after 28th day of rearing. The net biomass, SGR and per day weight gain were found significantly ($p < 0.05$) higher and FCR low with diets E and F in comparison to diets A, B, C and D. The proximate analysis of carcass showed that the fish fed diets E and F had significantly ($p < 0.5$) higher deposition of protein and lipids in the tissue. The study revealed that the protein requirement of *C. marulius* fry is around 540–600 g kg⁻¹ and the fry could be reared to fingerling size on formulated diets.

Keywords *Channa marulius* · Protein requirement · Survival · Growth

Introduction

Giant snakehead, *Channa marulius* (Hamilton) belonging to family Channidae is one of the important food,

ornamental and game fish of India, Pakistan, China, Thailand and Cambodia [1]. They are found in rivers, canals, lakes, swamps, marshes and rice fields [2] and grow to a maximum length of 183 cm [3] and weight of 30 kg [4]. The snakeheads are in great demand as food fish due to their appealing flavor [5], fewer muscular spines, medicinal importance [6–11] and air-breathing nature [9] that facilitates high density culture and easy transport in live condition to the markets. However, due to environmental degradation and absence of aquaculture technology, the population of this species has declined tremendously during last 3–4 decades in natural waters and there is a need to sustain their population through aquaculture.

The snakehead (striped snakehead: *C. striatus*) culture is widely popular in Thailand and on limited scale in India, Philippines and Taiwan [9, 12–15]; by and large very little work has been carried out on culture of *C. marulius* due to poor understanding of breeding and larval rearing requirements that could facilitate mass seed availability under captive conditions. The species has been observed to breed in almost all type of aquatic systems including rivers, reservoirs, rice-fields, ponds and small cement tanks and their seed in large quantities can be procured in the form of eggs (floating eggs), yolk-sac larvae, fry and fingerlings from such water bodies. The major problem associated with snakehead culture is its predatory and cannibalistic habit that starts from the larval stage [16] and continues till adult stage consuming prey fish more than half to two-third of its length [9, 16–18].

Several studies have demonstrated that although cannibalistic aggression in fishes is difficult to be stopped, even after feeding the fish to satiation level but it can be reduced by increasing natural food availability [19–22] or by weaning the fish to accept formulated feed [17, 18, 22, 23]. Successful larval rearing of snakehead, therefore, depends more on the

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understanding of dietary requirements at the first-feeding and during growth. Therefore, the aim of the present study was to evaluate the dietary protein requirement of giant snakehead fry so that a nutritionally-balanced diet could be developed for raising fingerling in captivity which could provide stocking material for culture in ponds to meet need-based diversified aquaculture commodity in India.

Material and Methods

Procurement of Test Fish

The early fry of *C. marulius* were procured from the farm facilities of NBFGR, Lucknow, India where they bred naturally in May 2011. The fry were washed thoroughly and disinfected with formalin (15 mg L^{-1}) and reared initially in two fibre reinforce plastic (FRP) tanks of 1,125 L (size $1.5 \times 1.0 \times 0.75 \text{ m}$) for 30 days. The fry were initially given zooplankton as the main food for 3 days followed by a combination of zooplankton and egg custard for next 7 days, subsequently zooplankton was withdrawn from their diet. The fry were then acclimatized to synthetic diet 'D' (protein 480 g kg^{-1}) for 5 days prior to start the experiment.

Experiment Design

Feed trial was conducted in triplicate set with six variable diets in 18 FRP tanks of 1,125 litre (size $1.5 \times 1.0 \times 0.75 \text{ m}$) kept under an open shade at the NBFGR farm facility during July 2011. The tanks were filled with 500 L bore well water and covered with green house netting (75 % light cutting). Twenty fry (mean length $4.11 \pm 0.59 \text{ cm}$) of test fish were stocked in each of the 18 designated tanks. The average initial biomass of stocked fry of each of the tank was recorded before placing them in the tank which did not differ significantly ($p > 0.01$) among six treatments. Feed was given once a day (11.00 a.m.) initially @ 8 % of bw for first 10 days and then corrected to 5 % of bw for rest of the period. The tanks were cleaned on every third day through siphoning when all the debris was removed and 50 % tank water was replaced with fresh chlorine-free bore well water.

Preparation and Application of Test Diets

Six semi-purified test diets A, B, C, D, E, and F containing 300, 360, 420, 480, 540 and 600 g kg^{-1} protein levels were prepared using casein as a protein supplement. The other feed ingredients mixed in the various diets are given in Table 1. The feed ingredients after proper mixing were steamed in a pressure cooker for 10 min and after cooling divided into four blocks and stored in a deep freezer ($-20 \text{ }^\circ\text{C}$) in a sealed polythene bag. One by one these feed

blocks were taken out from the deep freezer, kept under normal cooling (temperature around $5\text{--}6 \text{ }^\circ\text{C}$) condition in a refrigerator and gradually used for feeding the fish. The feed was graded to small particle size with the help of a hand held metal grater and spread in circular fine meshed plastic tray (diam 22 cm) hanged in the water.

Collection of Survival and Growth data

Mortality was counted every day in the morning and evening and the dead fish were removed at the earliest. Cannibalism was estimated from the difference between initial number of fish stocked and sum of survivors plus dead fish removed. All the fishes were removed on 28th day, measured for length and weight and five samples from each of the replicates was packed in sealed polythene sackets and kept in deep freezer ($-20 \text{ }^\circ\text{C}$) for proximate analysis of carcass.

Proximate Analysis

Proximate composition of diets and fish carcasses was analyzed following methods of AOAC [24]. All samples were analyzed in triplicate. Dry matter was calculated by drying in an oven at $105 \text{ }^\circ\text{C}$ for 24 h; crude protein (N X 6.25) by the Kjeldahl method after an acid digestion method and crude lipid by ether extraction after acid hydrolyses. Nitrogen free extract was estimated by subtraction method.

Water Quality

Water samples for the analysis of temperature, pH, total alkalinity, dissolved oxygen, and free carbon dioxide were tested on every third day. The temperature was measured with a centigrade thermometer, pH with digital electronic meter (Eutec), total alkalinity, dissolved oxygen and free carbon dioxide by titration methods following the standard methods [25].

Data collection

All the surviving samples of fishes were measured for total length (L) and weight (W) at the end of the experiment. Survival rates (S), net biomass, specific growth rates (SGR), per day weight gain, feed conversion ratio (FCR) and length-weight relationship (W) were calculated using the following formulae:

$$S = 100 \times (n_t/n_0)$$

where, S is survival rate (%), n_t is the number of fishes survived at time t and n_0 is the number of fishes at the commencement of the experiment.

Table 1 Composition of experimental diets and proximate composition

Ingredient	Feed ingredients used in different test feeds (g kg ⁻¹)					
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Casein ¹	300.0	360.0	420.0	480.0	540.0	600.0
Starch ²	434.0	374.0	314.0	254.0	194.0	134.0
Cellulose ³	180.5	180.5	180.5	180.5	180.5	180.5
Cod liver oil ⁴	45.0	45.0	45.0	45.0	45.0	45.0
Ascorbate ⁵	0.50	0.50	0.50	0.50	0.50	0.50
CMC ⁶	20.0	20.0	20.0	20.0	20.0	20.0
VM + MM ⁷	20.0	20.0	20.0	20.0	20.0	20.0
GE. (kg ⁻¹)	4,279	4,303	4,327	4,351	4,375	4,399
KJ g ⁻¹	17.95	18.07	18.17	18.27	18.37	18.47
Proximate Composition						
Protein	294.3*	349.0*	402.9*	475.7*	528.6*	586.6**
NFE	597.6**	551.6*	487.0*	426.0*	361.3*	307.6*
Lipid	44.0*	43.3*	41.6**	42.2**	44.5*	43.4*
Moisture	50.2	46.0	65.4	52.3	62.5	54.5
GE kg ⁻¹	4,279	4,303	4,327	4,351	4,375	4,399
KJ g ⁻¹	17.97	18.07	18.17	18.27	18.37	18.47

¹ HiMedia, Mumbai Lot No: 0000042681² HiMedia, Mumbai, Lot No: 0000028340³ Cellulose (HiMedia, Mumbai, Lot No: 0000040304)⁴ Cod Liver Oil, Manufacturer Universal Medicare Pvt. Ltd., Mumbai Batch No. R0109 J⁵ L-Ascorbate-2 triphosphate Ca salt-HiMedia, Mumbai, Lot No. 000000517⁶ HiMedia, Mumbai, Lot No. 0000042121⁷ Each kg of Vitamin and mineral mixture named ('Agrimin Forte') contains Vit. A 700000 IU, Vit. D₃ 70000 IU, Vit. E 250 mg, Nicotinamide 1,000 mg, Co 150 mg, Cu 1,200 mg, I 325 mg, Fe 1,500 mg, Mg 6,000 mg, Mn 1,500 mg, K 100 mg, Se 10 mg, Na 5.9 mg, S 0.72 %, Zn 9,600 mg, Ca 25.5 %, P 12.75 % Manufacturer Brindavan Phosphates Pvt. Ltd, 48 N, Doddaballpur Ind. Area, Doddaballpur-561 203, India and marketed by Virbac Animal Health India Pvt. Ltd., Andheri-Kurla Road, Andheri, Mumbai-400 059, India. Batch No. BFA-611 September 2010

*Values are significantly different at 95 % confidence limit for two replicates of each diet

**Values are significantly different at 99 % confidence limit for two replicates of each diet

$$NB = GB - IB$$

where, NB is net biomass (g) of all the surviving fishes, GB is measured gross biomass (g) of all the surviving fishes at the time of final harvesting and IB is the measured initial biomass (g) of all the stocked fishes.

$$SGR = 100(\ln W_t - \ln W_0)/(t_2 - t_1)$$

where, SGR is specific growth rate; W_t is the total weight at time t_2 , W_0 is initial weight at the time t_1 .

$$FCR = TF/NB$$

where, FCR is food conversion ratio, TF is total quantity of given feed (g), NB is net biomass (g).

$$W = aL^b$$

where, W is assumed weight of fish, 'a' and 'b' are constant and 'L' is length of fish.

$$PDWG = NB/t.$$

where, PDWG is per day weight gain, NB net biomass and 't' number of days.

Data Analysis

Analysis of variance was used to determine the significance levels between different production attributes by SPSS version 16.0 software. Student's *t* test was performed to analyze significance levels for diets. Regression graphs were plotted for comparing the length-weight relationship at final harvesting and 'Y' (simple linear regression) and 'R²' (correlation) were recorded using M.S. Excel (Version 2007) and 'W' was calculated by the formula ($W = aL^b$) in M.S. Excel spread sheet. The data are expressed as mean \pm standard deviation. Significant levels were

considered at $p < 0.05$ and $p < 0.01$ and means were compared using Duncan multiple range test.

Results and Discussion

A significantly ($p < 0.05$) higher survival of 88.3 and 85.0 % respectively in diets E (protein 540 g kg⁻¹) and F (protein 600 g kg⁻¹) in the present study in comparison to diets A (protein 300 g kg⁻¹), B (protein 360 g kg⁻¹), C (protein 420 g kg⁻¹) and D (protein 480 g kg⁻¹) revealed that the protein requirement of *C. marulius* is quite high at fry stage (length 4.11 ± 0.59 cm) (Table 2). This was well corroborated with the work of Mohanty and Samantaray [26] who obtained highest growth performances (survival was not defined by the authors) in *C. striata* fry fed formulated diet (made from natural ingredients) containing 550 g kg⁻¹ protein (energy 4,320 kcal kg⁻¹) fed at the rate of 10 % bw day⁻¹. Though highly significant variations ($p < 0.05$) in the survival rates between diets A–D with that of diets E and F were observed, the average length and weight were insignificant ($p > 0.05$) with all the six diets pointing the poor survivals in diets A–D may be due to higher rate of cannibalism or cannibalism attempts that might have caused injury and subsequent mortality. It is well known that snakeheads observed great amount of cannibalism at all stages of life and it is one of the major reasons of low survival during their culture [16]. In the process of cannibalism although shooters are able to prey

on fish measuring 2/3 in length [17] or 63–80 % [18] to predator size in case of *C. striatus*, no information as to predator–prey ratio is available for *C. marulius* though the species is known to grow larger in size, more predatory and cannibalistic in nature in comparison to earlier species. *C. striatus* in the process of cannibalism ingested comparatively smaller numbers (≥ 10 %) of prey and a large number of them die due to injury, shock and spread of diseases [23]. This phenomenon was observed in the present study also whereby hardly 15–20 % of the populations were found missing in the tanks and rest were collected in dead condition showing signs of injury in different parts of the body, more prominently on the caudal fin, which was eaten up either in toto or in part.

It has been demonstrated in several of the studies that application of formulated diets had improved survival greatly in fishes that observed great amount of cannibalism [23, 27–31]. However, it is also more important that formulated feed should meet the nutritional requirement of fish in general. The poor survival with isocaloric diets A, B and C containing protein levels of 300, 360 and 420 g kg⁻¹ respectively, has revealed that nutritionally deficient diets tend to aggravate cannibalism as feed applied to all the treatments was in uniform quantity and was totally consumed everyday within a short span of time. The higher size of minimum length and weight of fishes in all the treatments at the time of termination of experiment with that of initial length and weight also confirmed that the feed was accepted in all the treatments, however, it is the

Table 2 Growth parameters of *Channa marulius* fed with different protein levels in the diet

Parameter	Feed A	Feed B	Feed C	Feed D	Feed E	Feed F
Initial no.	60	60	60	60	60	60
Survival (%)	46.6 ^b	46.6 ^b	46.6 ^b	53.3 ^b	88.3 ^a	85.0 ^a
Initial avg. length (cm)	4.11 ± 0.59 ^a	4.11 ± 0.59 ^a	4.11 ± 0.59 ^a	4.11 ± 0.59 ^a	4.11 ± 0.59 ^a	4.11 ± 0.59 ^a
Final avg. length (cm)*	6.34 ± 0.76 ^a	6.40 ± 0.79 ^a	6.50 ± 0.89 ^a	6.40 ± 0.90 ^a	6.23 ± 0.61 ^a	6.49 ± 0.6 ^a
Initial av. weight (g)	0.78 ^a	0.82 ^a	0.83 ^a	0.86 ^a	0.80 ^a	0.81 ^a
Final average weight (g)*	1.62 ± 0.57 ^a	1.68 ± 0.69 ^a	1.89 ± 0.74 ^a	1.71 ± 0.70 ^a	1.51 ± 0.46 ^a	1.72 ± 0.53 ^a
Initial biomass (g)* [#]	47.5 ^a	49.0 ^a	50.5 ^a	49.5 ^a	48.5 ^a	49.5 ^a
Gross biomass (g)*	46.4 ^c	47.2 ^c	53.0 ^c	62.8 ^{bc}	80.5 ^{ab}	88.6 ^a
Net biomass (g d ⁻¹)*	-1.1 ^c	-1.8 ^c	2.5 ^c	14.5 ^{bc}	33.0 ^{ab}	39.1 ^a
SGR % d ⁻¹ *	-0.0805 ^c	-0.0661 ^c	0.0748 ^c	0.3627 ^{bc}	0.771 ^{ab}	0.875 ^a
Weight gain per day (g)*	-0.01 ^c	-0.021 ^c	0.02 ^c	0.17 ^{bc}	0.39 ^{ab}	0.46 ^a
FCR*	2.37 ^{cd}	2.48 ^d	2.09 ^c	1.87 ^b	1.35 ^a	1.26 ^a
'y' values**	0.0709 _x – 2.8474	0.0883 _x – 3.9726	0.0795 _x – 3.2779	0.0785 _x – 3.2300	0.0745 _x – 3.1314	0.0780 _x – 3.3771
'w' values**	0.0062*L ^{3.0000}	0.0057*L ^{3.0000}	0.0066*L ^{3.0000}	0.0064*L ^{3.0000}	0.0061*L ^{3.0000}	0.0063*L ^{3.0000}
'R ² ' values**	0.878	0.940	0.951	0.943	0.939	0.853

[#] Mean values within same rows and with different superscripts are significantly different at ($p < 0.05$) and ($p < 0.01$)

*n Values of all the surviving fishes

**n = 28 fishes

quality of feed which in the instant case is the level of protein that mattered for low and higher survival in different treatments (Table 2). It is better explained in case of diets E and F as the survival is better in these diets. It reveals that optimum dietary protein levels reduce the tendency of cannibalism. Qin and Fast [32] also have reported that when snakeheads begin feeding on formulated feed, the progressive size variation as fish grows does not necessarily provoke cannibalism when an adequate amount of suitable food is available.

The tanks showing higher rate of mortality also found to have greater size variations both in length and weight (Table 2 and Figs. 1, 2, 3, 4, 5, 6) that could have been occurred due to cannibalism in these tanks. Qin and Fast [23] and Qin et al. [33] observed that *C. striatus* in all treatments of feed application rates was found to cannibalize most small individuals and all treatments had a few large individuals at the end. This situation did not arise in the present study due to short-term experiment; however, greater differences in minimum and maximum size were observed in treatments showing poor survival provided with diets of low levels of protein. The wide range in initial size distribution enabled large individuals to cannibalize small ones and hence resulting into low survival.

Although acceptability of feed was recognized as stated above, significant differences ($p < 0.05$) existed amongst treatments in case of net biomass production, SGR, weight gain per day and FCR. The feeds A and B, however, showed negative values for net biomass production, SGR and per day weight gain due to higher rate of mortality/cannibalism and low protein levels in these diets. These values were, however, significantly higher in diets C ($p > 0.05$) and D ($p < 0.05$) which were also likely to happen due to reasons stated above. However, no significant ($p < 0.05$) change in net biomass, SGR and weight gain per day was observed in diets E and F provided with higher protein levels in the present study that indicated that the fish may efficiently consume protein up to 600 g kg^{-1} and protein level of 540 g kg^{-1} would be economical for its culture (Table 2). This finding was partly favoured in

respect that protein level of 540 g kg^{-1} in diet performed best and economical but protein level of 600 g kg^{-1} also showed more or less similar growth in fishes which was in contrast to the findings of Mohanty and Samantaray [26], who observed depressed growth at protein levels higher than 550 g kg^{-1} in *C. striatus*. Similar observations have

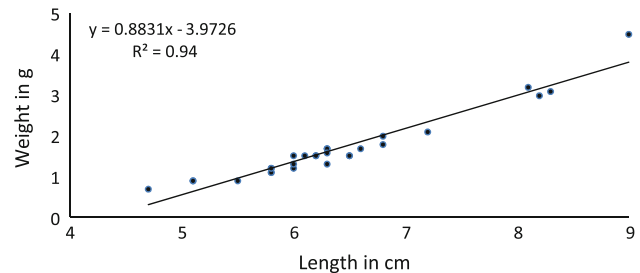


Fig. 2 Length-weight relationship of *C. marulius* fed diet B

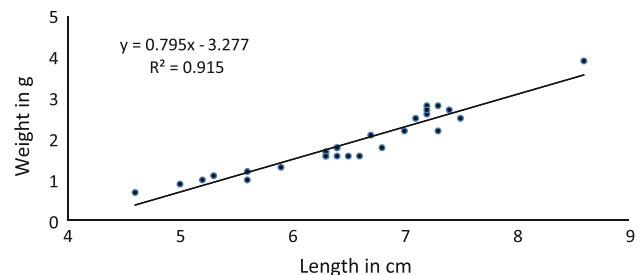


Fig. 3 Length-weight relationship of *C. marulius* fed diet C

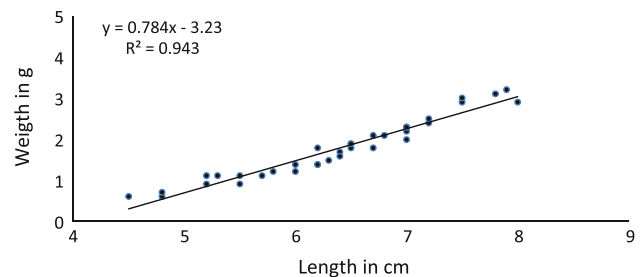


Fig. 4 Length-weight relationship of *C. marulius* fed diet D

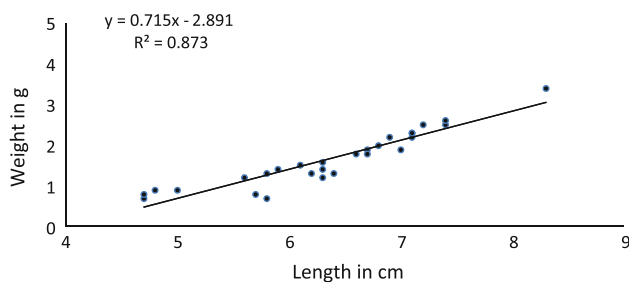


Fig. 1 Length-weight relationship of *C. marulius* fed diet A

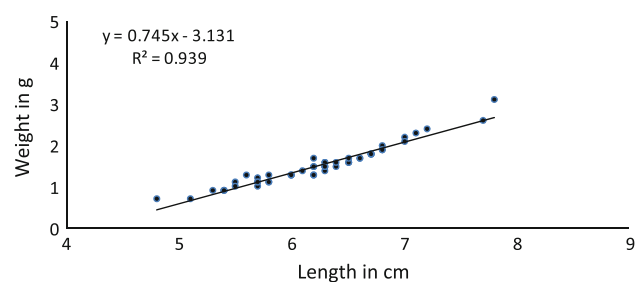


Fig. 5 Length-weight relationship of *C. marulius* fed diet E

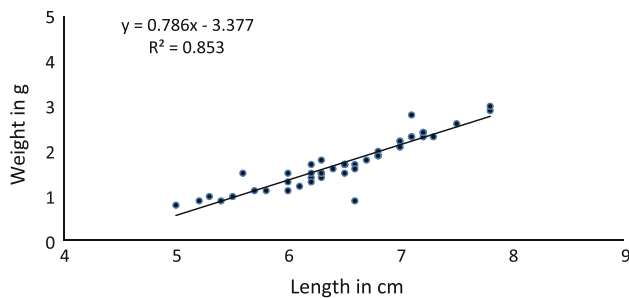


Fig. 6 Length-weight relationship of *C. marulius* fed on diet E

also been made in case of juvenile *C. striata* [34], *C. micropeltes* [35], *Chanos chanos* [36], *Epinephelus tauvina* [37], *Cyprinus carpio* [38], *Ictalurus punctatus* [39] and *Sarotherodon mossambicus* [40].

The 'W' values were found to follow Fulton's cube law with all the diet treatments indicating well being of the fish and was better than the adult natural stock [41, 42]. The 'R²' values (0.853–0.943) were also comparable with the natural population [43] that also confirmed satisfactory growth on all experimental artificial feeds (Table 2). These facts illustrate that the cause of mortality in fish in different treatments is not because of poor feed acceptability, instead it is the quality of feed that mattered for higher survival and growth.

Protein efficiency studies on snakehead body tissue have been performed in good number of cases both from capture and culture stocks [11, 26, 44–46], however, there is dearth of such literature on *C. marulius*. Barring the study of Zuraini et al. [11], the level of protein in body tissues in case of *C. striatus* has been reported to be 230 g kg⁻¹ [11] to 449.0 g kg⁻¹ [45] in natural stocks whereas in experimental culture, protein level as high up to 713 g kg⁻¹ has been reported when fish fed dietary protein level 450 g kg⁻¹ along with a lipid level of 65 g kg⁻¹ [44]. The latter, therefore support the present findings in which protein levels in body carcass of *C. marulius* was recorded from 560.9 to 666.3 g kg⁻¹ (Table 3) when *C. marulius* was fed semi-purified diets containing dietary protein levels of 300 g kg⁻¹

(energy 17.97 kJ g⁻¹) to 600 g kg⁻¹ (18.47 kJ g⁻¹). The availability of protein in body carcass greatly depends on species, size, age, season, protein quality, dietary level of energy, water quality and presence of natural food and culture management [45, 47].

Protein efficiency in *C. marulius* was found almost directly proportional to the dietary protein levels as all treatments had significantly ($p < 0.05$) different carcass protein with highest protein in diet F (Table 3). These results were similar to the work of Aliyu-Paiko et al. [44] and Mohanty and Samantray [26]. Higher amount of carcass protein in comparison to the dietary protein in all the treatments revealed that this fish has high sparing capacity of metabolizable NFE to protein [44]. This also fits well in case of lipid levels with diets E and F, where carcass lipid levels were found significantly ($p < 0.05$) higher than the dietary lipid levels (Table 3). According to Gam et al. [45], carcass protein level depends on availability of natural food in water in case of *C. striatus* and found highest during rainy season when quantity of natural food is at maximum. The reason of higher deposition of carcass protein in the fish in the present study and that of findings of Aliyu-Paiko et al. [44] therefore, may be due to feeding higher amount of dietary protein in the present instance. The moisture content was also found significantly ($p < 0.05$) low with diet F in comparison to other diets. The average values of water quality was found in normal range having temperature 28.3 ± 1.15 °C, pH 7.5 ± 0.07 , total alkalinity 138.4 ± 2.06 mg L⁻¹ and DO 6.84 ± 0.27 mg L⁻¹ respectively whereas, free CO₂ was absent in all the tanks.

Conclusion

On the basis of survival, growth and protein efficiency indices recorded in the present study, the dietary protein requirement of *C. marulius* fry was assessed to be around 54–60 g kg⁻¹ at energy value of 18.4 kJ g⁻¹ when fed @ 8–5 % of bw day⁻¹. It was also evaluated that this fish has

Table 3 Proximate composition of the carcass of *C. marulius* fed on different diets

Parameter	Initial	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Protein (A) (g kg ⁻¹)	651.5 ^a	560.9 ^d	576.9 ^{cd}	583.3 ^c	627.6 ^b	586.6 ^b	666.3 ^a
NFE (B) (g kg ⁻¹)	123.0 ^a	114.0 ^{bc}	102.2 ^d	117.0 ^{ab}	111.3 ^{bc}	108.2 ^{cd}	108.1 ^d
Lipids (C) (g kg ⁻¹)	54.4 ^c	43.0 ^d	44.6 ^d	34.6 ^f	38.5 ^e	58.6 ^b	66.8 ^a
Moisture (%)	72.04 ^d	80.30 ^c	81.77 ^{bc}	84.26 ^a	82.78 ^{ab}	81.95 ^{bc}	72.04 ^e
Dry matter (%)	27.95	19.70	18.23	15.74	17.22	18.045	27.96
Total (A + B + C) (DM basis) (g kg ⁻¹)	828.9	718.0	723.8	734.9	777.4	798.1	841.2
GE kg ⁻¹	393.19	338.36	342.15	341.94	363.10	381.82	404.98
KJ g ⁻¹	16.51	14.21	14.37	14.36	15.25	16.03	17.00

Values in a row are significantly different at 95 % confidence limit

high sparing capacity of utilizing metabolizable NFE in protein and lipid replacement. However, this needs to be confirmed with natural feed ingredients in future studies.

Acknowledgments The first and third authors are thankful to Uttar Pradesh Council of Agricultural Research, Lucknow for funding the part of this work. The facilities provided by the Director, NBFGR, Lucknow for carrying out this work is also greatly acknowledged.

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