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# Optimization of Dietary Protein Requirement for the Growth, Survival and Feed Utilization of *Osteobrama belangeri* (Valenciennes, 1844) Fingerling

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## ABSTRACT

In order to optimize dietary protein requirement of *Osteobrama belangeri* fingerling an experiment was conducted by using six semi-purified diets with graded protein levels (20, 25, 30, 35, 40 and 45 %) using casein and gelatin as main protein source. Six treatments in triplicates (18 circular fiber reinforced plastics tanks; 1000 l capacity each) were used to conduct the experiment for 75 days in the wet laboratory. Ten fingerlings with mean body weight of 4.23±0.08g were stocked in each tank and fed twice daily. The mean weight gain and specific growth rate was recorded highest in fish fed with 25% protein diet. Similarly, net biomass gain, body weight gain and daily weight gain was highest in fish fed with 25% protein diet. 30% and 35% protein diets gave 100% fish survival. The lowest feed conversion ratio (FCR) and highest feed conversion efficiency (FCE) was also recorded in 25% protein diet. Moisture, crude protein, crude lipid, crude ash contents of *O. belangeri* did not differ significantly among the treatments. Thus, growth performance and feed utilization was optimum in fingerling of *O. belangeri* while they were fed semi-purified diet containing 25% protein.

**Key words:** Feed utilization, Growth, *Osteobrama belangeri*, Protein requirement, Semi-purified diets

## INTRODUCTION

*Osteobrama belangeri*, also known as Pengba, is a highly esteemed fish in India. Formerly, it was widely distributed in rivers and lakes but has got extinct in the wild in some parts of India due to construction of barrage in the early 1990's in Manipur state of India (Dinesh and Mema, 2012; Singh *et al.,* 2016). Despite its culture potential, systematic attempts were not made to culture and propagate this species in India. Among the other carps available *O. belangeri* priced almost double than any other carps consumed by the people and thus has high commercial value.

Intensified culture of fish needs provision of nutritionally balanced diet. So, for obtaining optimum growth for the fish, it is important to know the optimum protein requirement while formulating a balanced diet, because protein is the major nutrient promoting growth and other metabolic activities (Abdel-Tawwab *et al.,* 2010). Very limited information is available on the nutritional requirement of *O. belangeri*. Therefore, this study was carried out to determine the effects of graded dietary protein levels on growth performance, feed utilization and carcass composition of *O. belangeri.*

## MATERIALS AND METHODS

The present experiment was carried out for 75 days during February to May, 2017 in the wet laboratory of the College of Fisheries, Central Agricultural University, Lembucherra, Tripura, India. The experiment consisted of 18 circular fiber reinforced plastics tanks (FRP) (1000 l each) of six treatments in triplicate groups and the experiment was conducted by following completely randomized design. The tanks were equipped with sponge filter for providing aeration and filtration of water. About 320 (mean weight of 4.23g) pengba fish were collected from the college pond and kept in two circular FRP tanks for 3 days for conditioning.

The experimental diets consisted of six different protein levels *viz.* 20% ($T_1$), 25% ($T_2$), 30% ($T_3$), 35% ($T_4$), 40% ($T_5$) and 45% ($T_6$). The moisture free ingredients were grounded, sieved, weighed and thoroughly mixed in a container and added water at 300 to 400 ml $kg^{-1}$ diet. The ingredients composition of the diets is shown in Table 1. Dissolved gelatin were

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**Table 1. Composition of diets (% dry matter basis)**

| Ingredients | Protein levels (%) | | | | | |
|---|---|---|---|---|---|---|
| | 20% | 25% | 30% | 35% | 40% | 45% |
| [1]Fish meal | 17 | 17 | 17 | 17 | 17 | 17 |
| Corn flour | 16 | 16 | 16 | 16 | 16 | 16 |
| [1]Broken wheat | 20 | 20 | 20 | 20 | 20 | 20 |
| [2]Casein | 9 | 14 | 19 | 24 | 29 | 34 |
| [2]Gelatin | 2 | 3 | 4 | 5 | 6 | 7 |
| [2]Dextrin | 30 | 24 | 18 | 12 | 6 | 0 |
| [2]CMC (Carboxymethylcellulose) | 1 | 1 | 1 | 1 | 1 | 1 |
| [3]Vegetable oil | 3 | 3 | 3 | 3 | 3 | 3 |
| [4]Vitamin + mineral | 2 | 2 | 2 | 2 | 2 | 2 |

[1]Obtained from College of Fisheries, CAU, Lembucherra, Agartala, India; [2]Himedia Laboratories Ltd., Mumbai, India; [3]Ruchi Soya Pvt. Ltd., Raighad, India; [4]Agrimin Forte, Mumbai, India; $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ corresponds to 20%, 25%, 30%, 35%, 40%and 45% crude proteinin diets, respectively.

added and again mixed it properly; vegetable oil, Carboxymethylcellulose (CMC) and vitamin-mineral premix were also added. Using pelletizer, 2 mm pellet size moist feed were prepared and dried in an oven at 50-55 ºC. The dried diets were then stored in air-tight containers with proper labeling until used. The proximate composition of all the used feed ingredients (Table 2) and the experimental diets (Table 3) and whole body composition of the experimental fish (Table 7) were measured according to the standard procedure of (AOAC, 2005).

Feeding was done twice a day at 2-3% of the total biomass in the morning (10 AM) and afternoon (4 PM). The average fish weight was recorded before stocking. The sampling of fish and water quality parameters were done fortnightly. Growth and mortality of the fish were recorded during sampling. Water samples were taken from each FRP tank during sampling and water quality parameters were analyzed according to the procedure given by (APHA, 2005).

The water quality parameters including dissolved oxygen (DO) and temperature were measured using Optical DO Probe (ProODO™, YSI Environmental). The digital pH meter (HI 991001, HANNA) was used to measure pH. Total ammonia-nitrogen ($NH_3$-N), nitrite-nitrogen ($NO_2$-N), nitrate-nitrogen ($NO_3$-N) and orthophosphate ($PO_4$-P) levels in water were measured by Continuous Flow Analyzer equipped with auto sampler (SA 1100, SKALAR). Alkalinity, total hardness and free carbon dioxide were analyzed by titremetric methods (APHA, 2005).

Sampling of fish was done fortnightly and adjusted

**Table 2. Proximate composition of feed ingredients (%)**

| Parameters | Fish meal | Broken wheat | Corn starch | Casein | Gelatin |
|---|---|---|---|---|---|
| **% DM** | | | | | |
| Dry matter | 12.19±0.41 | 9.25±0.29 | 5.95±0.12 | 8.92±0.14 | 5.88±0.07 |
| Crude protein | 48.38±0.46 | 11.83±0.09 | 0.37±0.03 | 77.73±0.56 | 93.03±0.20 |
| Crude fat | 7.75±0.06 | 4.31±0.16 | 0.10±0.008 | 0.16±0.01 | 0.06±0.01 |
| Crude fiber | 0.92±0.03 | 2.96±0.45 | 0.41±0.09 | 0.45±0.27 | 0.21±0.01 |
| Ash | 28.69±0.19 | 27.22±0.52 | 0.20±0.02 | 8.38±0.28 | 0.59±0.002 |
| NFE* | 2.04±0.72 | 68.91±1.32 | 92.93±0.12 | 4.32±0.83 | 0.20±0.15 |

Values are expressed as mean± S.E. (n=3); NFE* (Nitrogen free extract)

**Table 3. Proximate composition of experimental diets (%)**

| Parameters | Protein levels (%) | | | | | |
|---|---|---|---|---|---|---|
| | 20% | 25% | 30% | 35% | 40% | 45% |
| Moisture | 4.48±0.25 | 4.85±0.42 | 5.66±0.37 | 6.38±0.007 | 7.15±0.08 | 6.94±0.004 |
| **% DM** | | | | | | |
| Ash | 12.23±0.18 | 11.85±0.47 | 13.19±0.10 | 14.22±0.20 | 12.71±0.13 | 13.59±0.29 |
| Crude protein | 19.28±0.29 | 25.34±0.30 | 29.77±0.15 | 35.95±0.25 | 38.06±0.39 | 44.38±0.31 |
| Crude lipid | 1.79±0.12 | 1.41±0.09 | 1.66±0.16 | 1.01±0.04 | 1.03±0.03 | 1.10±0.09 |
| Crude fibre | 1.47±0.09 | 1.48±0.27 | 1.28±0.20 | 1.03±0.03 | 1.61±0.30 | 0.99±0.004 |
| NFE | 60.44±0.30 | 56.04±0.73 | 48.41±0.77 | 42.38±0.38 | 38.42±0.39 | 32.97±0.41 |
| Digestible energy (kcal $100^{-1}$g diet) | 33.61±0.38 | 33.43±4.56 | 32.77±2.74 | 31.85±0.47 | 31.92±0.79 | 31.93±1.68 |

Values are expressed as mean± S.E. (n=3); $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets respectively; Digestible energy (Kcal $100^{-1}$g) = (4 x Crude protein %) + (9 x Lipid %) + (4 x Carbohydrate %)

the quantity of feed according to the total biomass after each sampling. To evaluate the growth performance, following parameters were used

Body weight gain (%) = $[(w_t - w_0)/w_0] \times 100$

Where, $w_0$ and $w_t$ are initial and final live weight of the fish.

Specific growth rate (SGR) (% $day^{-1}$) = [(In $B_{Wf}$ – In $B_{Wi}$) /culture period] ×100

Where, $B_{Wi}$ and $B_{Wf}$ were initial and final body weights of the fish.

Survival (%) = [Total number of fish harvested / Total number of fish stocked] ×100

Daily weight gain (g $day^{-1}$) = [Total final weight – total initial weight] /culture period.

The following parameters were applied to evaluate the feed utilization performance of the experimental fish

Apparent FCR = Amount of dry feed intake (g) / fresh weight gain in fish (g)

Apparent FCE (%) = 1 / FCR×100;

Apparent protein efficiency ratio (PER) = Fresh weight gain in fish (g)/ Amount of protein fed (g)

Apparent protein conversion efficiency (PCE %) = protein gained (g)/ protein consumed *100.

The data was analyzed by using statistical method with Statistical Package for Social Sciences (SPSS, version 22.0). One way-ANOVA was performed to determine the differences between mean values and compared by Duncan's Multiple Range test (Duncan, 1955) at $P<0.05$ level. The broken line regression model was applied to find the optimum value of protein requirement using NLIN procedure (Robbins *et al.,* 1979) of SAS software (SAS, 1996) version 9.3 of the SAS system for windows (SAS Institute Inc., Cary, NC, USA). Approximate sampling errors for L, U and R were computed using the matrix of sums of squares and products of first derivatives and $Se^2$, given the least squares estimates of each parameter which were shown in Table 7 and Fig. 1, respectively.

## RESULTS AND DISCUSSION

The values of different parameters of the water quality including temperature (23.6-29.9 ºC), dissolved oxygen (5.02-10.13 mg $l^{-1}$), pH (5.5-7.68), alkalinity

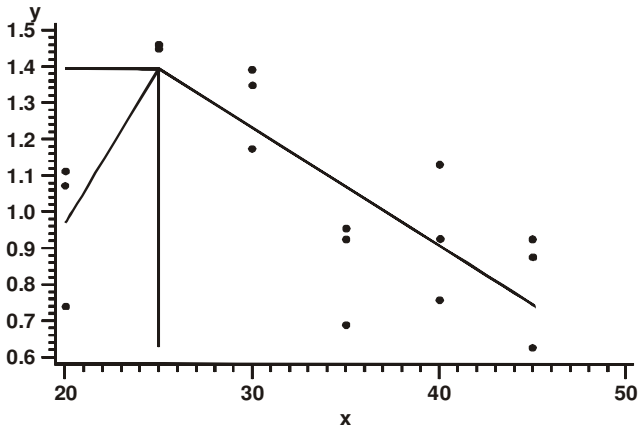


**Fig. 1. The broken line curve for SGR (optimum value) at 24.6303**

**Table 4. Growth performance of the experimental fish**

| Parameter | Protein levels (%) | | | | | | P value |
|---|---|---|---|---|---|---|---|
| | 20% | 25% | 30% | 35% | 40% | 45% | |
| Initial mean weight (g) | 4.41±0.12 | 4.3±0.05 | 4.03±0.06 | 4.25±0.14 | 4.23±0.01 | 4.18±0.13 | 0.195 |
| Mean final weight (g) | $9.2^{ab}$±0.56 | $12.6^{c}$±0.57 | $10.8^{bc}$±0.44 | $8.5^{a}$±0.74 | $8^{a}$±0.72 | $7.2^{a}$±0.068 | 0.002 |
| Net weight gain (g) | $4.7^{a}$±0.67 | $8.3^{b}$±0.1 | $6.8^{b}$±0.44 | $3.8^{a}$±0.6 | $3.5^{a}$±0.71 | $3.2^{a}$±0.73 | 0.002 |
| Specific growth rate (%/day) | $1.03^{a}$±0.12 | $1.53^{b}$±0.02 | $1.39^{b}$±0.07 | $0.88^{a}$±0.07 | $1^{a}$±0.11 | $0.88^{a}$±0.09 | 0.012 |
| Body weight gain (%) | $109^{a}$±17.6 | $193^{b}$±7.91 | 168.7±12.01 | $90.64^{a}$±11.64 | $82.55^{a}$±16.54 | $95.56^{a}$±19.9 | 0.031 |

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

(40-122 mg $l^{-1}$), hardness (34-94 mg $l^{-1}$) and total ammonia (0.14-0.46 mg $l^{-1}$) were in acceptable range without indicating any remarkable pattern during the whole duration of the experiment.

Date pertaining to growth performances of *O. belangeri* during the culture period are presented in Table 4. The initial mean weights of pengba for different treatments ranged between 4.03 to 4.41 g whereas variations from 7.2 to 12.6 g mean weights were observed in the final sampling. The highest mean weight gain (12.6±0.57 g) was observed in 25% protein diet which exhibited better performance compared with other treatments. The specific growth rate (% $day^{-1}$) of the fish were recorded at diminishing order from $T_2$, $T_3$, $T_1$, $T_5$, $T_4$ and $T_6$ groups respectively. The highest specific growth rate (1.53±0.02) was measured in group fed with 25% protein diet and the lowest value (0.88±0.07) was exhibited by fish fed on 40% protein diet.

Dietary protein is considered as important in fish nutrition and feeding, thus adequate dietary protein supply is required to improve their overall health and growth Lovell (1989) and Shang *et al.* (2018). Davies and Gouveia (2010) reported feed mixtures with 25% to 45% of raw protein are required by the fish. Numerous studies on dietary protein requirement have been conducted for different important aquaculture fish species by many workers, Mohanta *et al.* (2004); Chakraborty and Mirza (2007); Paul *et al.* (2009) and Paul and Giri (2015) and they found out that the dietary protein requirement for fish varied from one species to another species, which is due to their feeding habit, size of fish and water temperature. The protein requirement of fish decreases with the increasing size and age of fish NRC (2011). In another study, the dietary protein requirement for Indian major carps was stated to be 35% for spawn and fry and 25% for grown out and broodstock BIS (2013).

**Table 5. Yield parameters of the experimental fish**

| Parameter | Protein levels (%) | | | | | | P value |
|---|---|---|---|---|---|---|---|
| | 20% | 25% | 30% | 35% | 40% | 45% | |
| Initial biomass (g) | 44.16±14 | 43.0±0.6 | 40.0±1.3 | 42.5±0.16 | 42.33±1.36 | 41.83±0.44 | 0.195 |
| Final biomass (g) | $92.0^{ab}$±5.6 | $126.0^{c}$±1.57 | $107.0^{bc}$±3.7 | $81.0^{a}$±7.4 | $86.0^{a}$±6.6 | $77.0^{a}$±7.2 | 0.003 |
| Net biomass gain (g) | $47.83^{a}$±6.7 | $83.0^{b}$±1.04 | $67^{b}$±4.3 | $38.83^{a}$±6.05 | $43.67^{a}$±6.8 | $35.5^{a}$±6.04 | 0.001 |
| Daily biomass gain (g $day^{-1}$) | $2.39^{a}$±0.007 | $2.42^{ab}$±0.007 | $2.41^{b}$±0.008 | $2.39^{ab}$±0.005 | $2.34^{ab}$±0.002 | $2.35^{ab}$±0.009 | 0.195 |
| Survival (%) | $90^{ab}$±3.33 | $90^{ab}$±3.33 | $100^{b}$±00 | $100^{b}$±00 | $80^{ab}$±5.77 | $70^{a}$±8.81 | 0.048 |

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

The mean weight gain was increasing with increasing level of dietary protein up to optimum level followed by decreasing trend in the present study. The tropical fishes generally require moderate temperature between 25-32 ºC (Solomon and Ezigbo, 2010). The growth of Indian major carps was affected when the temperature was lower than 20 ºC and which has been considered to be lower for fish to perform metabolic and other activities. The difference in temperature would hinder the intake of feed by fish and hence their growth rate. This outcome was demonstrated in an experiment conducted under controlled system in aquarium Zenebe *et al.* (2003). The present study is in agreement with the work of Bahnasawy *et al.* (2009) who reported that the weight gain increased significantly with increasing level of dietary protein from 17% to 30% with no significant increase with the diet of 35% protein in Nile tilapia. Lall and Tibbetts (2009) have revealed that the dietary protein requirement for many herbivorous and omnivorous fishesranged from 25% to 30% and is also in agreement with the results of the present study. Also, the optimum protein requirements for bighead carp (*Aristichthys nobilis*) were recorded as 30% (Santiago and Reyes, 1991).

Significant difference was found in SGR of pengba among the treatments. Although fish fed with 25 and 30% proteingave same statistical value the maximum SGR found in the fish fed with 25% (1.53±0.02) protein diet in comparison to other treatments. Similar results were reported by several authors including Nandeesha *et al.* (1994) on stunted yearlings of *L. rohita*, Swamy (2004) on stunted fingerlings of *Cirrhinus mrigala*, Kumar *et al.* (2011) on stunted fingerlings of *L. rohita* and Ramaswamy *et al.* (2013) on stunted fingerlings of *Catla catla*. Kumar *et al.* (2011) found that 25% protein diet as the best for specific growth rate of stunted fingerlings of *L. rohita* which is in agreement with the present study. Ramaswamy *et al.* (2013) also revealed that 25% protein diet gave best SGR values on stunted fingerlings of *C. catla*. Martinez-Palacios *et al.* (2007); Lee and Kim (2009) showed that specific growth rate is a good indicator of protein quality and decrease as fish increase in size. The decrease in growth rate of fingerlings of pengba with increasing level of protein above the satisfactory level in the present study is similar to those reported for catla by Dars *et al.* (2010). Therefore, 25% protein containing semi-purified diet gave the better growth performance of pengba. The growth rate was significantly decreased beyond the requirement level, especially at 35% protein diet. The reason behind this is that every fish species and fish size has a specific protein limit for the growth of fish and after that additional higher protein level could not be utilized efficiently. Wilson (1989) as well as El-Sayed and Teshima (1991) also found that dietary protein requirements decreased with increasing fish size and age.

**Table 6. Feed utilization parameters of the experimental fish**

| Parameter | Protein levels (%) | | | | | | P value |
|---|---|---|---|---|---|---|---|
| | 20% | 25% | 30% | 35% | 40% | 45% | |
| Apparent feed conversion ratio (FCR) | $2.37^{b}$±0.07 | $1.28^{a}$±0.06 | $1.78^{ab}$±0.12 | $1.84^{ab}$±0.16 | $2.18^{ab}$±0.5 | $2.02^{ab}$±0.31 | 0.127 |
| Apparent feed conversion efficiency (FCE %) | $43.2^{a}$±4.8 | $68.1^{b}$±4 | $56.6^{a}$±3.73 | $55.01^{a}$±4.62 | $50.12^{a}$±9.38 | $51.1^{a}$±6.8 | 0.022 |
| Apparent protein efficiency ratio (PER) | $0.087^{a}$±0.012 | $0.14^{b}$±0.002 | $0.12^{b}$±0.006 | $0.09^{a}$±0.012 | $0.078^{a}$±0.01 | $0.077^{a}$±0.01 | 0.005 |
| Apparent protein conversion efficiency (PCE %) | 35.16±9.2 | 57.87±7.15 | 55.85±7.8 | 45.58±10 | 40±2.62 | 31.76±9 | 0.313 |

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

Salim and Sheri (1999) also reported decreased growth rate of *Labeo rohita* after protein levels exceeded the optimal requirement. The reason behind this was the inability of the fish body to utilize the protein in the diet after reaching the optimum protein level in the diet. Li *et al.* (2000) observed a significant growth response in channel catfish (*Ictalurus punctatus*) when examined with different protein rich artificial diet and is in conformity with the result of the present experiment.

The mean initial and final biomass of different treatments and other yield parameters including net gain in biomass, daily biomass gain and survival percentage for all the treatments have been shown in Table 5. Overall survival (%) of the fish varied from about 80 to 100% in different treatments. No significant difference (P>0.05) was recorded in overall survival (%) among the treatments. The final biomass measured was highest for treatment $T_2$ (126±1.57 g) followed by $T_3$ (107±3.7 g) whereas, lowest value was found in treatment $T_6$ (77±7.2 g). Accordingly, $T_2$ showed significantly higher net gain in biomass (83.03±1.04 g) as compared to $T_3$, $T_1$, $T_5$, $T_4$ and $T_6$. Similarly, relatively higher mean daily biomass gain was recorded for $T_2$ (2.42±0.007 g day$^{-1}$) as compared to rest of the treatments and $T_5$ recorded lowest mean daily weight gain (2.34±0.002 g day$^{-1}$). Kumar *et al.* (2011) worked on stunted fingerlings of *L. rohita* and Ramaswamy *et al.* (2013) on stunted fingerlings of *C. catla* and found that 25% protein level in diets gave the best weight gain of fish.

The feed utilization parameters including apparent FCR, apparent PER, apparent FCE and apparent PCE were measured for all treatments and are presented in Table 6. The FCR value did not differ significantly (P>0.05) among the treatments, but the lowest (1.28) and highest (2.37) FCR values were measured in $T_2$ and $T_1$, respectively. The highest value of FCE (60.1%) was observed in $T_2$ and lowest FCE (43.2%) in $T_1$. The PCE also showed no significant variations among treatments. The highest PCE (57.87 %) was recorded in $T_2$ followed by $T_3$ (55.85%) and the lowest in $T_6$ (31.76 %). Similarly, the apparent PER was highest in $T_2$ (0.14), followed by $T_3$ (0.12), while the lowest apparent PER value of 0.077 was observed in treatment $T_6$.

It may be notable that pengba is an omnivorous fish with inclination to consume macrophytes and inclusion of high protein in diet might have problem in feed utilization and leads to poor growth and feed utilization. The apparent FCR, PER and PCE showed no significant difference (P>0.05) among the treatments. Apparent FCR value decreased with increasing dietary protein level up to 25% diet and thereafter, increased continuously except in 45% dietary protein group. Similar FCR values were also observed by Khattab *et al.* (2000) but Paul *et al.* (2009) observed the best FCR value in fish fed 30% dietary protein for *Labeo bata*

**Table 7. Proximate composition of the experimental fish (g kg$^{-1}$ dry matter)**

| Parameter | Protein levels (%) | | | | | | P value |
|---|---|---|---|---|---|---|---|
| | 20% | 25% | 30% | 35% | 40% | 45% | |
| Moisture | 69.49±0.69 | 69.9±0.45 | 70.0±0.56 | 67.9±0.61 | 69.37±0.68 | 69.59±0.61 | 0.432 |
| **% DM** | | | | | | | |
| Ash | 8.8±0.51 | 8.59±0.39 | 8.41±0.43 | 8.85±0.22 | 8.9±0.42 | 9.1±0.16 | 0.505 |
| Protein | 54.42$^{ab}$±0.91 | 56.73$^{b}$±0.47 | 55.31$^{ab}$±0.82 | 54.25$^{ab}$±0.89 | 53.02$^{a}$±0.26 | 54.1$^{a}$±0.78 | 0.032 |
| Lipid | 34.85±0.23 | 35.59±0.21 | 35.46±0.25 | 34.41±0.36 | 34.82±0.11 | 35.44±0.27 | 0.169 |
| Fiber | 0.28±0.041 | 0.29±0.038 | 0.36±0.037 | 0.38±0.058 | 0.35±0.031 | 0.33±0.059 | 0.210 |
| NFE (Nitrogen free extract) | 4.62$^{bc}$±0.92 | 3.69$^{a}$±0.86 | 2.81$^{ab}$±0.96 | 3.21$^{ab}$±0.49 | 5.21$^{c}$±0.19 | 4.32$^{bc}$±0.78 | 0.009 |

Mean value having different superscript in the same row differs significance (P<0.05) (mean± S.E.) (n=3); $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ corresponds to 20%, 25%, 30%, 35%, 40% and 45% crude protein diets, respectively.

**Table 8. Parameter estimates for broken line regression model for SGR**

| Parameter | Estimate | Approx Std Error | Approximate 95% Confidence Limits | |
|---|---|---|---|---|
| L | 1.4029 | 0.0690 | 1.2558 | 1.5500 |
| U | -0.0200 | . | . | . |
| V | -0.0323 | 0.00631 | -0.0458 | -0.0189 |
| R | 24.6303 | 0.5854 | 23.3825 | 25.8781 |

fry. Therefore, relatively lowest FCR was observed in $T_2$ (1.28), fed with 25% diet. On the other hand, relatively higher apparent protein efficiency ratio (PER) was observed (0.14) in $T_2$ followed by $T_3$ (0.12). Present results is in agreement with Ramaswamy *et al.* (2013), who studied the effects of dietary protein levels on growth, survival, protein efficiency ratio, feed conversion ratio and specific growth rate for *C. catla* during grow-out phase and reported optimal dietary protein level of 25%. Li *et al.* (2006) observed that 24% and 36% protein diet provided the same growth and feed conversion efficiency. Then they finally recommended that 28% protein containing diet would be useful for the growth of channel catfish fingerlings.

The mean final proximate composition of *O. belangeri* in different treatments is given in Table 7. The moisture, crude ash, crude lipid and crude fibre content of *O. belangeri* did not show any significant variations (P>0.05) among the treatments. However, significantly (P>0.05) higher protein level was recorded in $T_2$ (56.73±0.47%) compared to other treatments and the lowest protein level of pengba (53.02±0.26%) was obtained in treatment $T_5$. The nitrogen free extract was found significantly (P<0.05) higher in $T_5$ (5.21±0.19%) followed by $T_1$ (4.62±0.92%) and the lowest value in $T_3$ (2.81±0.96%).

There was no higher significant differences (P>0.05) reported in the proximate compositions of experimental fish except crude protein and nitrogen free extract. Minor variations were observed in mean values of moisture, ash, crude lipids and crude fibre. But, the changes in whole body proximate composition were less consistent. The highest crude protein content was found in 25% protein fed group while lowest ash was obtained in 20% protein fed group. Similar results were also reported by Mohanta *et al.* (2013); Ma *et al.* (2014) and Wang *et al.* (2017). Lipid levels of *O. belangeri* under all the treatments were found to be markedly higher as compared to other carps in the range of 34.82- 35.59%. Proximate composition of fish is influenced by various factors including age, sex, maturity, geographical location, and experimental feeding conditions. The result of the present study showed that pengba is a fatty fish. The protein and lipid contents of the fish were found to be increased with increasing level of optimum protein inclusion in the diet, which is also in agreement with study conducted by Paul *et al.* (2009).

Approximate sampling errors for the two-slope broken-line model of L, U and R were calculated using the matrix of sums of squares and products of first derivatives and $S_e^2$, given the least squares estimates of each parameter (Table 8 and Fig. 1). The optimum value using broken line regression for SGR is 24.63 % protein for *O. belangeri* fingerlings. From regression analysis [$Y = 1.4029–0.0200\ X\ (246.303 – x) –0.0323X\ (x – 246.303)$; $R^2 = 0.96$, where Y = SGR and x = protein level in the diet], it was found that the requirement of protein of *O. belangeri* is 24.63 %.

## CONCLUSIONS

The results of the present study suggests that under laboratory condition the dietary protein level of 25% gives the optimum performance in terms of growth, survival, feed utilization and whole body/carcass composition of *O. belangeri*. From broken line regression analysis for specific growth rate, it was found that the optimum protein requirement of *O. belangeri* is 24.63%.

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