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Effect of Two Dietary Protein Levels on Body Weight and Composition in *Channa punctatus* (Bloch.) Fingerlings

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Abstract

Low-protein (33%) and high protein (40%) diets were formulated to feed the fingerlings of *Channa punctatus*. Seven feeding treatments were maintained and the fingerlings were fed at 3% BW d^{-1} for 45 days. Studies have revealed that regular feeding on low protein (LP) diet resulted in significantly ($P<0.05$) low growth, while feeding on 1L/3H diets resulted in good growth performance and was equal to the fish fed continuously on high protein (HP) diet. Nutrient retention (SGR, GCE, APD) and FCR values were similar in fingerling fed either continuously on high protein diet or on a feeding schedule consisting of 1L/3H. These studies have indicated the possibility of protein saving without compromising the growth and nutrient retention in C. *punctatus.* Based on total protein input 12.36% protein can be saved without affecting growth, by adopting the 1L/3H feeding schedule as compared to daily feeding on high protein diet.

Keywords: *Channa punctatus*, low protein diet, high protein diet, protein sparing.

Channa punctatus (Bloch.) Yavrularında İki farklı Proteinin Vücut Ağırlığı ve Kompozisyonu Üzerine Etkisi

Özet

Channa punctatus yavrularını beslemek için düşük (%33) ve yüksek protein (%40) içeren yemler hazırlanmıştır. Yedi farklı beslenme şekli ile yavru balıklar 45 gün boyunca vücut ağırlığının %3'ü oranında beslenmiştir. Yapılan çalışmalar sonucu düşük proteine (LP) sahip yemlerle beslenen balıklarda büyüme oranı düşükken, yüksek proteine sahip yemlerle (1L/3H) beslenen yavru balıklarda büyüme performansı yüksek çıkmış ve sürekli olarak yüksek protein (HP) diyetiyle beslenen balıklarınkine eşit bulunmuştur. Besin (SGR, GCE, APD) ve FRC değerleri; hem sürekli yüksek proteinli yemle beslenen hemde 1L/3H'den oluşan yemle beslenen yavru balıklarda benzer çıkmıştır. Sonuç olarak, C. *punctatus* için büyüme ve besin değerleri riske edilmeden protein tasarrufu yapılabilir. Toplam protein girdisi temel alındığında, günlük yüksek proteine sahip yemle yapılan beslenmeyle karşılaştırıldığı zaman 1L/3H beslenme programı sayesinde büyüme etkilenmeksizin %12,36 protein tasarrufu sağlanabilir.

Anahtar Kelimeler: *Channa punctatus*, düşük proteinli diyet, yüksek proteinli diyet, protein koruyucu.

Introduction

Fish preferentially utilize proteins for energy purposes, although conventional energy sources, carbohydrates and lipids may also to some extent satisfy their needs (Keshavanath *et al*., 2001). To maximize nutrient utilization and minimize the solid and soluble waste load, it is essential to provide cultured fish with the optimum levels of protein (Cho, 1993). Generally nutrients absorbed in excess of requirements may be excreted as ammonia and urea (Kibria *et al*., 1998).

When food waste is high and nutrient retention and assimilation is poor, a major portion of the nitrogen is added to the culture system which may ultimately pollute the environment. The aim of aquaculture should, therefore, be to provide sufficient and optimum protein for good growth through balanced feed. Keeping in view the findings of De Silva (1985), who conclusively proved that feeding the fish everyday with the same level of protein is not economical. Therefore, the main objective of this experiment is to establish whether the frequent feeding or alternating diet of low and high protein can increase the use of energy from carbohydrate and fat, leading to more efficient protein utilization (protein sparing).

Materials and Methods

Fingerlings of *C. punctatus* (Mean body weight 6.8 g) were obtained from the local suppliers of Hisar (lat. 29°10' N and long 75°46' E). Fish were maintained in transparent glass aquaria (60x30x30 cm) kept in an air-conditioned laboratory where the temperature was maintained at 25±1°C and the lighting schedule at 12 h of light (08^{00}-20^{00}h) alternating with 12 h of darkness (20^{00}-08^{00}). The average intensity of light inside the laboratory was approximately 1,000 lux. Fish were acclimated in the laboratory for a minimum period of seven days prior to the initiation of experimental treatments and were fed *ad libitum* on a feed containing 40 percent protein. The water in the aquaria was renewed daily with water which has been previously equilibrated to the desired temperature (25°C).

Two diets with low (33%) and high (40%) protein were prepared. Percentage composition of the ingredients and proximate composition of experimental diets are given in Table 1.

Fish were individually weighed and randomly distributed in each aquaria with two replicates for each treatment and were fed at 3% BWd^{-1} for the whole experimental duration of 45 days. Following seven feeding schedules were maintained.

Treatment 1 Feeding daily on low protein diet (LP)
Treatment 2 Feeding daily on high protein diet (HP)
Treatment 3 Feeding one day low alternating with one day high protein diet (1 LP/HP)
Treatment 4 Feeding one day low alternating with two days high protein diet (1 LP/2HP)
Treatment 5 Feeding one day low alternating with three days high protein diet (1 LP/3 HP)
Treatment 6 Feeding two days low alternating with two days high protein diet (2LP/2HP)
Treatment 7 Feeding two days low alternating with three days high protein diet (2LP/3 HP)

Treatment-1 and Treatment-2 were used as two controls. The amount of feed given was adjusted every 15th day after bulk weighing each group of fish. Fish were exposed to the diets continuously for 3h and thereafter uneaten feed was siphoned out and stored separately for drying and calculating FCR (Feed conversion efficiency). The faecal matter voided by the fish was collected separately from each aquarium. The pooled faecal samples from each treatment were dried in an oven at 60°C and were subsequently analysed for digestibility estimations following the method of Spyridakis *et al*. (1989). At the termination of experiment, the fish from all the treatments were weighed individually and processed for subsequent analysis.

Analytical Techniques

The feed ingredients, experimental diets, faecal samples and fish carcass (initial and final) were analysed following the procedure of AOAC (2000). Chromic oxide levels in the diets as well as in the faecal samples were estimated spectrophotometrically following the method of Furukawa and Tsukhara (1966).

Live weight gain (g), growth percent gain, specific growth rate (SGR, % d^{-1}), protein efficiency ratio (PER) and gross conversion efficiency (GCE)

Table 1. Percentage composition of ingredients and proximate composition of experimental diets fed to C. *punctatus*

Ingredients (%)	Diets	
	High protein (HP)	Low protein (LP
Groundnut oilcake [a]	60.00	10.00
Hydrothermically processed soybean [b]	20.00	10.00
Wheat flour[c]	9.00	39.00
Rice bran[d]	9.00	39.00
Chromic oxide (Cr_2O_3)[e]	1.00	1.00
Mineral premix and amino acids (MPA) [f]	1.00	1.00
Proximate composition (%)		
Crude protein	40.13±0.000	33.54±0. 293
Crude fat	10.70±0.057	11.467±0.088
Crude fibre	4.33±0.066	4.233±0.066
Ash	3.73±0.033	4.033±0.066
Nitrogen free extract (NFE)	41.12±0.33	46.72±0.442
Gross energy (KJ g^{-1})	20.12±0.033	20.47±0.0 14

All values are mean ± S.E. of mean of 3 observations
a and d- used as basic feed ingredients
b. Used as main protein source of plant origin
c. Used as a binder to make the diets water stable
e. Used as an external digestibility marker
f. Used to fortify the diets with minerals and amino-acids. Each Kg contains
Copper – 312 mg; Cobalt – 45 mg; Magnesium - 2.114 g; Iron -979 mg; Zinc - 2.13 g; Iodine 156 mg; DL-Methionine - 1.92 g; L-lysine mono hydrochloride - 4.4 g; Calcium 30% and Phosphorous- 8.25%

were calculated using standard methods (Steffens, 1989). Apparent nutrient digestibility (APD) of the diets was calculated according to Cho *et al.* (1982) as follows:

$$APD = 100 - \frac{100 \times \%\ Cr_2O_3 \text{ in diet} \times \%\ \text{nutrient in faeces}}{\%\ Cr_2O_3 \text{ in faeces} \times \%\ \text{nutrient in diet}}$$

The energy contents of the diets and fish were calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364 KJg^{-1} for lipid, carbohydrate and protein respectively (Henken *et al.*, 1986).

Water Quality Parameters

Water samples for the determination of water quality parameters were obtained in replicates from each treatment at 20 days interval. Water temperature (°C) was recorded daily using digital thermometer. pH, conductivity and dissolved oxygen were monitored using multiline F-set-3 (E. Merck Ltd., Germany). All other parameters were determined following APHA (1998).

Statistical Analysis

Duncan's multiple range test and multivariate analysis was applied to find out the significant differences between different treatments.

Results and Discussion

Water Quality Parameters

The effects of 7 different dietary treatments are very clearly reflected on the physico-chemical characteristics of aquaria water (Table 2). There was not much variation in the measured parameters of water between different treatments except the two controls (LP and HP diets regularly).The pH remained alkaline (7.2 to 7.4). The water temperature fluctuated between 25.0 to 26.5°C.

Although DO levels remained at optimum levels, yet low DO values in aquaria where the fish were fed on HP daily and 1 LP/3 HP dietary treatments clearly indicated its utilization by the growing fish. These results are in agreement with those of Jindal (2008) and Jindal *et al.* (2008a; 2008b).

Significantly ($P>0.05$), not much variation in the NH_4-N excretion and o-PO_4 production was observed in all dietary treatments except the groups of fish fed on LP daily, where a slightly higher level of NH_4-N excretion and o-PO_4 production was observed. Further, when all the dietary treatments were compared then low levels of NH_4-N excretion and o-PO_4 production were observed in the groups of fish fed on dietary treatments HP daily and 1LP/3HP. These results are in agreement with those of Jindal *et al.* (2007; 2009).

Growth and Survival

Feeding results have revealed low mortality in all dietary treatments.

The growth response of C. *punctatus* fingerlings fed on seven different dietary schedules is shown in Table 3. Live weight gain, growth percent gain in body weight and SGR% d^{-1} of fish fed on seven different dietary schedules revealed a highly varied pattern of growth performance. Regular feeding on low protein diet (LP) resulted in significant ($P<0.05$) low growth, while feeding on ILP/3HP diets resulted in good growth performance and was almost equal to the fingerlings fed continuously on high protein diet (Figure 1).

Feed utilization efficiency was measured in terms of FCR. Significant ($P<0.05$) variations in this

Table 2. Water quality parameters of different aquariums stocked with *C. punctatus* fingerlings fed on two dietary treatments LP and HP in different sets of treatments

Parameters	Different treatments						
	LP	HP	1LP/1HP	1LP/2HP	1LP/3HP	2LP/2HP	2LP/3HP
Dissolved oxygen (DO) mg/L	5.0±0.001	4.5±0.003	4.8±0.002	4.9±0.008	4.3±0.004	4.8±0.004	4.8±0.002
pH	7.20	7.30	7.30	7.20	7.40	7.40	7.30
Water temperature (°C)	25.00	26.00	25.30	25.50	26.50	25.30	25.50
Conductivity micro (µ) mhos cm^{-1}	0.50±0.005	0.49±0.003	0.49±0.000	0.51±0.002	0.48±0.007	0.47±0.002	0.46±0.007
Free Carbon dioxide (Free CO_2) mg/L	16.9±0.000	17.0±0.002	16.8±0.001	16.7±0.004	16.8±0.002	16.3±0.000	16.4±0.002
Total alkalinity (mg/L)	215.0±0.002	225.0±0.000	215.0±0.001	216.0±0.003	214.0±0.001	220.0±0.005	225.0±0.003
Total hardness (mg/L)	213.0±0.005	212.0±0.004	219.0±0.001	218.0±0.003	228.0±0.002	216.0±0.002	218.0±0.007
Ammonical nitrogen (mg/100 g BW of fish)	0.553±0.008	0.410±0.006	0.421±0.004	0.420±0.004	0.416±0.006	0.453±0.003	0.433±0.005
Ortho phosphate (mg/100 g BW of fish)	0.066±0.003	0.036±0.003	0.043±0.003	0.045±0.002	0.040±0.000	0.050±0.000	0.046±0.002

* All values are mean ± S.E. of mean of 3 observations

Table 3. Growth performance of fish *Channa punctatus* fed on two experimental diets LP- 33% and D_{30} - 40% protein in different sets of treatment

Name of the treatment	Survival (%)	Weight gain (g)	Growth % gain in Body Weight	Growth/ day (%) Body Weight	Specific Growth Rate (SGR)	Apparent protein digestibility (APD)	Food Consumption /day (%) Body Weight	Feed Conversion Ratio (FCR)	Gross Conversion Efficiency (GCE)
Low protein (LP) daily	85	$5.980^{A} \pm 0.005$	$80.494^{A} \pm 0.403$	$0.748^{A} \pm 0.006$	$0.755^{A} \pm 0.006$	$87.25^{A} \pm 0.005$	$1.915^{A} \pm 0.014$	$2.560^{A} \pm 0.002$	$0.391^{A} \pm 0.000$
High protein (HP) daily	90	$6.910^{B} \pm 0.133$	$95.686^{B} \pm 0.839$	$0.841^{B} \pm 0.012$	$0.851^{B} \pm 0.012$	$89.64^{B} \pm 0.004$	$1.952^{B} \pm 0.009$	$2.321^{B} \pm 0.045$	$0.431^{B} \pm 0.008$
1LP/1HP	88	$6.520^{C} \pm 0.035$	$84.155^{C} \pm 0.396$	$0.831^{BC} \pm 0.005$	$0.812^{BC} \pm 0.006$	$88.34^{AB} \pm 0.002$	$1.886^{AC} \pm 0.024$	$2.346^{B} \pm 0.012$	$0.426^{BC} \pm 0.002$
1LP/2HP	88	$6.573^{BC} \pm 0.153$	$83.744^{C} \pm 1.022$	$0.797^{BDE} \pm 0.015$	$0.806^{BD} \pm 0.015$	$88.00^{AB} \pm 0.003$	$1.905^{AD} \pm 0.007$	$2.391^{BC} \pm 0.056$	$0.418^{BDE} \pm 0.009$
1LP/3HP	92	$6.856^{BC} \pm 0.263$	$96.348^{B} \pm 2.171$	$0.849^{B} \pm 0.032$	$0.831^{B} \pm 0.033$	$89.89^{B} \pm 0.002$	$1.972^{CDEFG} \pm 0.007$	$2.288^{B} \pm 0.094$	$0.438^{B} \pm 0.017$
2LP/2HP	90	$6.210^{AC} \pm 0.109$	$81.207^{AC} \pm 0.724$	$0.759^{ACD} \pm 0.011$	$0.766^{ACD} \pm 0.011$	$87.50^{AB} \pm 0.003$	$1.892^{AF} \pm 0.005$	$2.492^{AC} \pm 0.044$	$0.402^{ACDE} \pm 0.007$
2LP/3HP	90	$6.716^{BC} \pm 0.068$	$85.075^{C} \pm 0.698$	0.817 ± 0.010^{B}	$0.826^{B} \pm 0.010$	$87.50^{AB} \pm 0.003$	$1.883^{AG} \pm 0.004$	$2.305^{B} \pm 0.023$	$0.410^{D} \pm 0.004$

All values are mean ± S.E. of mean
Mean with same letter in the same row are not significantly (P>0.05) different.
Data were analysed by Duncan's Multiple Range Test; Duration of experimental period – 45 days

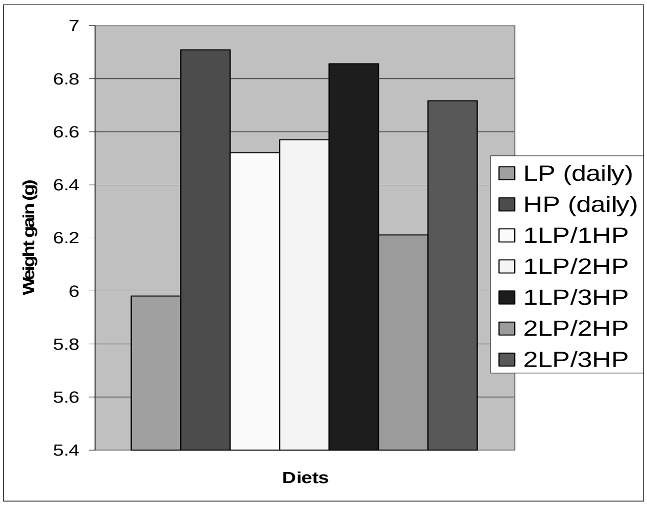

Figure 1. Weight gain (g) of *Channa punctatus* fed on two experimental diets LP and HP in different sets of treatments.

parameter were observed among different feeding schedules. FCR values remained low in fingerlings when fed continuously on high protein diet (HP) or on 1 LP/3 HP feeding schedule. On the other hand, GCE and APD values were enhanced in fingerlings fed on a feeding schedule consisting of l LP/3 HP, which were not significantly (P>0.05) different from the fish fed continuously on high protein diet (Table 3).

The results of present studies on growth performance and nutrient retention have revealed that it is economically beneficial and biologically more productive to feed C. *punctatus* alternatively on one day low followed by three days high (ILP/3HP) protein diets instead of feeding the fish continuously and only on high protein (HP) diets. These results are in agreement with those of Saroha *et al.* (2004) and Saroha and Garg (2007). It appears this fish does not require the same protein input every day like common carp (Srikanth *et al.*, 1989) and Nile Tilapia (De Silva, 1985). APD and FCR values were similar in fingerlings fed either continuously on high protein diet or on a feeding schedule consisting of 1LP/3HP. On the other hand, no well defined patterns in growth and digestibility parameters were observed by De Silva (1985) in Tilapia *(Oreochromis niloticus*) and Srikanth *et al.* (1989) and Nandeesha (1990) in common carp.

Proximate Carcass Composition

Protein and energy contents increased while those of moisture and ash decreased with each increase in the high protein ratio (1 LP/1 HP, 1 LP/2 HP, 1 LP/3 HP) (Table 4). No significant (P>0.05)

Table 4. Proximate carcass composition (% fresh weight) of fish *Channa punctatus* fed on two diets LP and HP in different sets of treatments

Name of the treatment	Moisture	Crude protein	Crude Fat	Total Ash	Nitrogen Free Extract	Gross Energy (KJ/g)
Low protein (LP) daily	74.352±0.056^{A}	15.563±0.227^{A}	4.512±0.001^{A}	3.015±0.000^{A}	2.557±0.219^{A}	5.902±0.020^{A}
High protein (HP) daily	73.400±0.275^{B}	16.206±0.003^{B}	4.655± 0.002^{B}	3.222±0.001^{B}	2.514±0.270^{A}	6.103±0.047^{B}
1LP/1HP	73.405±0.245^{B}	16.006±0.003^{BC}	4.582±0.001^{C}	3.123±0.001^{BE}	2.882±0.244^{A}	6.090±0.041^{BC}
1LP/2HP	73.980±0.000^{AC}	16.120±0.001^{BD}	4.607±0.002^{D}	3.164±0.000^{C}	2.127±0.000^{A}	5.997±0.000^{ACD}
1LP/3HP	73.387±0.217^{B}	16.181±0.001^{BE}	4.622±0.002^{E}	3.179±0.000^{C}	2.630±0.220^{A}	6.103±0.036^{B}
2LP/2HP	73.484±0.288^{BC}	15.980±0.00^{CDEF}	4.506±0.001^{F}	3.038±0.030^{AD}	2.936±0.318^{A}	6.084±0.054^{BD}
2LP/3HP	73.579±0.243^{BC}	16.051±0.001^{BF}	4.595±0.002^{G}	3.144± 0.002^{CE}	2.629± 0.247^{A}	6.062±0.041^{BD}

All values are mean ± S.E. of mean
Mean with same letter in the same row are not significantly (P>0.05) different.
Data were analysed by Duncan's Multiple Range Test

differences in protein accumulation in fish carcass, fat deposition and gross energy levels were seen in the fish groups fed on HP diet daily and 1 LP/3 HP dietary treatments.

The fish fed continuously on low protein diets had low carcass protein, while fingerlings fed on high protein diets had only slightly higher values of carcass protein than fingerlings fed on 1 LP/3HP diet. Srikanth *et al.* (1989) also observed high deposition of protein in common carp. Carcass moisture and lipid appeared to be inversely related. These results appear to be in agreement to those of Shearer (1994) on trout and Saroha and Garg (2007) on *C. mrigala*.

In the absence of sufficient/optimal levels of protein contents in the diets, fishes either use the energy of lipids or of carbohydrates. Lipids are well utilized as energy sources but only restricted amounts can be used because a large supply of dietary fat affects carcass composition, which is undesirable from the standpoint of higher processing losses, storage problems and consumer acceptance and also brings about technological problems while preparing diets. Since the fat contents in the diets were fixed at 10 to 11 percent and the crude fat contents of the carcass varied between 4.5-4.6; therefore, these values are considered to be not high, enough to degrade the quality of fish flesh.

Role of Carbohydrate Energy in Protein Sparing

The differential response in growth performance of fish fed on diets may be attributed to the difference in protein levels. Since alternate administration of high/low protein diets has influenced the growth performance of fish independently of the mean dietary protein input, this may be attributed to a rhythm in certain basic metabolic activities (De Silva, 1985). Similar response of fish fed on high protein diet or 1 LP/3HP schedule may indicate that the fingerlings might have used the necessary nutrients and energy from the carbohydrates of the diets which were about 20 percent high in low protein diet.

According to Falge *et al.* (1978), the increase in carbohydrate content of the diet actually reduces the activity of the proteolytic enzymes. Thus, results of present study revealed that digestibility of the protein is really affected by carbohydrate contents of the diets.

Since growth performance was not significantly different from those obtained on feeding the fish either continuously on high protein diet or on a feeding schedule consisting of 1 LP/3 HP, therefore it is possible to save 12.36 percent protein by adopting 1 LP/3 HP feeding schedule. De Silva (1985), Srikanth *et al.* (1989) and Nandeesha (1990) also found 1 LP/3HP schedule best for the farmed fishes. This again supports our view that the growth is independent from the mean dietary protein provided to the fish which could be due to rhythmicity of certain basic metabolic activities (De Silva, 1985).

Conclusion

Studies have revealed the possibility of protein saving without compromising the growth and nutrient retention. Although present studies provide some indication on the growth pattern, there is still a need to confirm these findings through large scale field trials and to standardize the techniques for adoption in culture ponds in which natural food also contributes through fertilization and play a major role in fish production.

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