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Effect of Dietary Protein Levels and Lipid Sources on Growth Performance and Body Composition of Striped Catfish, *Pangasianodon hypopthalmus* (Sausage 1878) Advanced Fingerling

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Abstract Nine practical diets were formulated with three dietary protein levels (25%, 30% and 35%) and three lipid sources fish oil (FO), soybean oil (SBO) and equal combination of fish oil and soybean oil. Fish (initial average weight 30.5 g fish) were fed with practical diet for 60 days. The diet formulated with 30% dietary protein level and equal combination of FO and SBO as lipid source showed the highest weight gain percent, WGP (197.2) and it was found lowest (132.56) for the diet formulated with 25% dietary protein and SBO as lipid source. Specific growth rate, SGR also follows same pattern as weight gain percent. The two way ANOVA results showed that individual effect of protein level significantly affect the feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER). No individual effect of lipid source and interactive effect was found for FCR, FER and PER. Dietary protein level significantly affects the crude protein, ether extract and ash content of fish muscle but no significant difference was found between the treatment fed with 30% and 35% dietary protein. Our results suggest that diet formulated with 30% protein and combination FO and SBO as lipid source give better growth and cost effective feed formulation for *Pangasianodon hypothalamus*.

Keywords Growth, *Pangasianodon hypopthalmus*, Lipid source, Protein levels.

Introduction

Aquaculture is a feed hased industry with over 60% of the operational cost coming from feed source alone. The increasing cost of fish feed has given the attention of researchers on reducing the cost of the feed through the proper feed formulation in commercial feed. The cost of feed is mainly influenced by level and source of protein as it is an expensive compo-

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Table 1. Ingredients used and proximate composition of the diet. Composition of vitamin mineral mix (PREEMIX PLUS) (quantity/kg).

	Dietary protein levels (%)								
	25			30			35		
	Dietary lipid sources								
	FO	SO	FO+SO	FO	SO	FO+SO	FO	SO	FO+SO
Ingredients (g kg^{-})									
Fish meal	200	200	200	310	310	310	420	420	420
GNOC	99	99	99	99	99	99	99	99	99
MOC	100	100	100	70	70	70	40	40	40
Wheat flour	250	250	250	180	180	180	110	110	110
Rice bran	270	270	270	260	260	260	250	250	250
FO	60	–	–	60	–	–	60	–	–
SBO	–	60	–	–	60		–	60	–
FO+SBO	–	–	30+30	–`	–	30+30			30+30
Vitamin mix	20	20	20	20	20	20	20	20	20
BHT	1	1	1	1	1	1	1	1	1
Proximate composition (g 100 g^{-}) on dry matter basis									
Moisture	8.57	8.87	8.8	8.51	8.45	8.32	8.6	8.74	8.85
Crude protein	25.57	25.7	25.46	30.5	30.25	30.38	35.49	35.23	35.12
Ether extract	11.25	11.12	11.45	11.38	11.42	11.65	11.28	11.72	11.65
Ash	10.12	10.1	10.1	11.25	11.18	11.35	11.5	11.34	11.6
NFE	39.21	38.76	39.09	34.52	34.94	34.6	29.67	29.47	29.18
CF	5.28	5.45	5.1	3.84	3.76	3.7	3.46	3.5	3.6
GE (KJ/100 g)	18.10			18.22			18.26		

nent of the feed. The dietary protein is an important factor affecting growth performance of fish and also an important source of energy [1]. Transforming the dietary protein to the protein deposited in the fish muscle is the major aspects of fish culture. The excess amount of dietary protein or excess dietary energy may lead to nitrogenous pollution, abnormal high lipid deposition in the body, adverse effect on fish health and flesh quality. Therefore, the requirement of an optimal dietary protein and energy requirements of fish is essential for developing the nutrient balanced and cost effective fish diet.

There are few study on dietary protein and energy requirements of catfish have been studied by authors [2—5]. Fish oil is an important component of fish feed but the rapid growth of aquaculture, stagnating catches from fisheries and rising demand for fish oil in pharmaceutical industry and direct human consumption are also factors responsible for the current increase in fish oil prices [6]. Therefore, there is a need for replacement of these feed ingredients with economically viable and sustainable alternatives. Global fish oil production was 1.2—1.4 million tons/year that will not be sufficient to meet the requirements of animal and aqua feeds industries [7]. Therefore, Vegetable oils (VO) are the suitable alternative to fish oil in fish diet due to their abundance and relatively stable prices [8]. At current prices, soybean oil and meal are many costs effective in aqua feed compare to fish meal and fish oil [9]. Some studies about the partial replacement or complete replacement of fish oil by vegetable oil have been successfully done [10, 11].

Asian catfish or iridescent shark, *Pangasianodon hypophthalmus* is an omnivorous, freshwater fish native of the Mekong Delta [5] and is widely cultured in ponds in the Southeast Asian countries. The fish was introduced in India from Thailand as freshwater aquaculture species and promoted for large-scale aquaculture production in India. At present, the culture production of this fish has been hampered in India due to, among others, lack of quality feed and high-cost production. Therefore, there is a tremendous need for optimizing protein and energy levels in the fish diet during feed formulation that

Table 2. Growth performances and feed utilization (dry matter) of *Pangasianodon hypopthalmus* advanced fingerling fed diet containing different protein and lipid source. All values are mean ±SD, obtained from three replicates with the help of two-way ANOVA, In. WT (g), initial body weight; FBW(g) final body weight gain; WG(g), weight gain; FCR, feed conversion ratio; SGR(%), specific growth rate; PER, protein efficiency ratio; HSI, hepatosomatic index; VSI, viscerosomatic index.

Dietary protein (%)	Dietary lipid source	In. WT	FBW	AWG	WT (%)	SGR
25	FO	30.78 ± 0.12	72.49 ± 0.36	42.4 ± 0.80	138.83 ± 3.71	1.42 ± 0.01
	SBO	30.85 ± 0.11	71.55 ±0.65	40.78 ± 0.73	132.56 ±3.01	1.40 ± 0.02
	FO+SBO	30.92 ± 0.07	74.22 ± 0.49	44.61 ± 0.55	147.72 ± 1.34	1.45 ± 0.01
30	FO	30.98 ± 0.24	89.49 ± 0.22	58.9 ± 0.85	191.16 ± 4.93	1.76 ± 0.01
	SBO	31.08 ± 0.15	87.35 ± 0.26	55.97 ± 0.92	179.15 ± 5.34	1.72 ± 0.05
	FO+SBO	31.12 ± 0.16	90.67 ± 0.52	60.3 ± 0.1.28	197.2 ± 6.84	1.78 ± 0.02
35	FO	30.99 ± 0.10	90.34 ± 0.41	59.02 ± 1.03	192.12 ± 3.85	1.78 ± 0.01
	SBO	30.8 ± 0.09	88.0 ± 0.35	57.12 ± 0.21	181.96 ± 2.12	1.74 ± 0.02
	FO+SBO	31.21 ± 0.16	91.73 ± 0.28	60.55 ± 0.0.68	198.91 ± 6.65	1.79 ± 0.03
Protein level (P)		NS	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
Lipid source (L)		NS	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
P × L		NS	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
R^2		NS	0.997	0.986	0.978	0.994

Table 2. Continued.

Dietary protein (%)	Dietary lipid source	FCR	FER	PER	HSI	VSI
25	FO	2.07 ± 0.02	0.48 ± 0.01	1.91 ± 0.01	1.40 ± 0.02	3.99 ± 0.08
	SBO	2.08 ± 0.01	0.47 ± 0.01	1.87 ± 0.02	1.38 ± 0.08	4.05 ± 0.04
	FO+SBO	2.05 ± 0.06	0.48 ± 0.00	1.92 ± 0.02	1.37 ± 0.04	4.02 ± 0.01
30	FO	1.62 ± 0.05	0.61 ± 0.02	2.01 ± 0.01	1.19 ± 0.01	4.04 ± 0.06
	SBO	1.68 ± 0.02	0.60 ± 0.00	1.96 ± 0.02	1.20 ± 0.03	4.06 ± 0.02
	FO+SBO	1.62 ± 0.04	0.61 ± 0.0	2.04 ± 0.0.03	1.17 ± 0.08	4.05 ± 0.01
35	FO	1.61 ± 0.01	0.61 ± 0.01	1.72 ± 1.06	1.20 ± 0.01	4.10 ± 0.06
	SBO	1.66 ± 0.02	0.59 ± 0.02	1.68 ± 0.01	1.19 ± 0.05	4.13 ± 0.03
	FO+SBO	1.6 ± 0.04	0.62 ± 0.02	1.74 ± 0.0.02	1.20 ± 0.04	4.12 ± 0.04
Protein level (P)		$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
Lipid source (L)		NS	NS	NS	NS	NS
P × L		NS	NS	NS	NS	NS
R^2		0.986	0.987	0.971	0.970	0.716

promote fish growth as well as minimize nitrogenous output but also reduce the cost of feed. The aim of this study is to investigate the interactive effect of dietary protein and lipid sources on growth and body composition of *P. hypopthalmus* advanced fingerlings and consequently determine the best dietary protein and lipid combination for optimum growth.

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Materials and Methods

Formulations and preparation of experimental diets

The practical based diet was formulated by using the feed ingredients such as fish meal, mustard oil cake, groundnut oil cake, wheat flour, rice bran, soybean oil, fish oil, vitamin and mineral mixture (PREEMIX PLUS), (Table 1). Nine experimental diets were formulated with three levels of crude protein (CP) 25%, 30% and 35% and three sources of lipid viz. Fish oil (FO), Soybean oil (SBO) and equal combination of fish oil and soybean oil (FO+SBO).

Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; 1, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L-lysine, 10 g; DL-Methionine, 10 g; Selenium, 125 mg.

Rearing of fish

The experiment was conducted over a period of 60 days at the wet lab of the Department of Fish Nutrition and Biochemistry and Physiology, ICAR- Central Institute of Fisheries Education, Mumbai. Advanced fingerlings were procured from Murband fish farm, Kalyan, Mumbai and were acclimatized for fifteen days in circular tank (5000 L) till their use in the feeding trial during which fish were fed with practical diet with 25% crude protein.

Experimental design

The experiment was set up in 9 distinct experimental groups (3×3), each group having 3 replicates in 27 uniform size circular plastic tubs of 500 L capacity. The tubs were arranged following a Completely Randomized Design (CRD). Fifteen fish were stocked in each plastic tub filled with 400 L chlorine free bore well water. Throughout the experimental period total volume of the water (400 L) and proper aeration was maintained in all experimental tubs.

Chemicals and glasswares

Neutral glasswares of Borosil were used throughout the experiment. Chemicals of various companies viz. Sigma, SRL, Hi-media, Qualigens, Merck, were used.

Feeding rate

Feeding rate was adjusted based on daily observation of the feed intake of the animal. Daily ration was divided into two equal parts, one part was given at 07:00 am and the rest at 7:00 pm.

Growth parameter

The growth parameters of the *Pangasianodon hypophthalmus* advanced fingerlings were assessed by taking their body weight at an interval of 15 days. The animals were kept starved overnight before body weight measurement. The growth performance was assessed using the following formulae:

Weight gain percent (WG %)

$$\text{Weight gain (\%)} = \frac{\text{Final weight–Initial weight}}{\text{Initial weight}} \times 100$$

Specific growth rate (SGR)

$$\text{SRG} = \frac{\text{Log}_e \text{ Final weight–Log}_e \text{ Initial weight}}{\text{Number of days}} \times 100$$

Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Food given (Dry weight)}}{\text{Body weight gain (Wet weight)}}$$

Feed efficiency ratio (FER)

$$\text{FER} = \frac{\text{Net weight gain (Wet weight)}}{\text{Feed given (Dry weight)}}$$

Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Net weight gain (Wet weight)}}{\text{Protein Fed}}$$

Hepato-somatic index (HSI)

$$\text{HSI} = \frac{\text{Liver weight (g)}}{\text{Weight of fish (g)}} \times 100$$

Viscerosomatic index (VSI))

$$\text{VSI} = \frac{\text{Viscera weight (g)}}{\text{Weight of fish (g)}} \times 100$$

Proximate analysis of tissue and diets

Proximate analysis of fish samples and diets were car-

Table 3. Proximate composition of muscle (dry matter basis) of *Pangasianodon hypopthalmus* advanced fingerling fed diet containing different protein and lipid source. All values are mean ±SD, obtained from three replicates with the help of two-way ANOVA.

Dietary protein (%)	Dietary lipid source	Moisture	Crude protein	Ether extract	Ash
25	FO	74.24 ± 0.65	60.51 ± 0.35	24.06 ± 0.85	8.76 ± 0.67
	SBO	73.06 ± 0.78	60.24 ± 0.89	24.91 ± 0.62	9.40 ± 0.43
	FO + SBO	74.50 ± 0.40	60.55 ± 0.29	24.52 ± 0.44	9.08 ± 0.68
30	FO	74.70 ± 0.62	61.89 ± 0.78	23.11 ± 0.53	9.30 ± 0.50
	SBO	73.29 ± 0.55	61.42 ± 0.34	23.63 ± 0.55	10.53 ± 0.43
	FO + SBO	74.37 ± 0.68	61.73 ± 0.86	23.49 ± 0.43	10.08 ± 0.62
35	FO	74.54 ± 0.33	62.66 ± 0.51	21.80 ± 0.62	10.03 ± 0.47
	SBO	73.75 ± 0.30	63.72 ± 0.48	22.79 ± 0.64	10.19 ± 0.52
	FO + SBO	74.52 ± 0.75	62.55 ± 0.95	22.52 ± 1.14	10.16 ± 0.56
Protein level (P)		NS	p<0.05	p<0.05	p<0.05
Lipid source (L)		NS	NS	NS	p<0.05
P × L		NS	NS	NS	p<0.05
R^2		0.60	0.813	0.737	0.981

ried out following standard methods.

Gross energy (GE)

The gross energy value of experimental diets was calculated on the basis of standard physiological values (22) as per the following formula.

$$GE\ (KJ/g) = CP\ (\%)\times 23.6 + EE\ (\%)\times 39.5 + TC\ (\%)\times 17.2$$

Statistical analysis

Growth performance, body composition and indices were analyzed using two way analysis of variance (ANOVA) at P<0.05 via SPSS 16.0 for Windows. Duncan's multiple range test was used for post hoc comparison of mean (p<0.05) between different acclimation temperatures. All data presented in the text, figures and tables are means ± standard deviation and statistical significance for all statistical tests was set at p<0.05.

Results and Discussion

Growth performance

Growth performance parameters of *Pangasianodon hypopthalmus* fed with different formulated diet are summarized in Table 2. The two way ANOVA results showed that the individual and interactive effect of dietary protein levels and lipid source were found for the final body weight gain (FBW), average weight gain (AWG), weight gain percent (WGP) and specific growth rate (SGR) of *Pangasianodon hypopthalmus*. However, there was no significant difference (p>0.05) was found between the treatment fed with dietary protein of 30% and 35% respectively for above mentioned parameters.

In this study, the diet formulated with 30% dietary protein level and equal combination of FO and SBO as lipid source showed the highest WGP (197.2) and it was found lowest (132.56) for the diet formulated with 25% dietary protein and SBO as lipid source. No significant difference was found between the treatment fed with 30% and 35% dietary protein. SGR also follows same pattern with highest value 1.79 for diet formulated with protein level as 35% and lipid source as FO and SBO as lipid source and lowest 1.40 value for the diet containing 25% dietary protein and SBO as lipid source. This result is consistent with the other reported protein requirement studies such as 30% protein and 12% lipid for swordtails [12], 40% crude protein and 12% lipid for small size (3.5 g) *P. hypopthalmus* [1]. The protein requirement for small and medium size *Pangasius sanitwongsei* was reported 40% and 30% respectively [13].

The two way ANOVA results (Table 2) showed that individual effect of protein level significantly ($p<0.05$) affect the Feed conversion ratio (FCR), Feed efficiency ratio (FER) and Protein efficiency ratio (PER) and no individual effect of lipid source ($p>0.05$) and interactive effect of protein levels and lipid sources ($p>0.05$) was found for above mention growth parameters. FCR values recorded among treatments were within the range of 1.60—2.08. The improved FCR value 1.60 was found for the treatment group fed with 35% dietary protein and equal combination of FO and SBO as lipid source. A similar value 1.62 was recorded for the treatment group fed with 30% dietary protein and equal combination of FO and SBO as lipid source. This observation is in agreement with the result of FCR found for tambaqui (*Colossoma macropomum*) fingerling fed with to SBO and fish oil. In the present study, PER increased for the treatment fed with 25 and 30% dietary protein, subsequently significantly decreased ($p<0.05$) for the 35% protein diet. Similar findings have been reported for other fish species [14, 15]. This is probably because more dietary protein is used as energy when high protein diets are fed to fish [14—16].

FCR and PER value found similar for the diet formulated with same dietary protein level and different lipid source. The present study result indicate that 50% replacement of fish oil with soybean showed better growth rate for *P. hypopthalmus*. Fish fed with different plant based lipid diet showed as well as or better growth then FO [15]. It is reported that *P. hypopthalmus*, being a warm water fish is adapted to utilize more plant based diet especially SBO and results are also supported for the study of sharp snout seabream [15].

Body indices

The Hepatosomatic index (HSI) and Viscerosomatic index (VSI) were monitored as body indices of *Pangasianodon hypopthalmus*. The result showed that HSI decreased significantly ($p<0.05$) when dietary protein increased from 25% to 30% and insignificant changes above 30% dietary protein. The effect of dietary lipid sources on HSI was marginal ($p=0.04$). The study showed that viscerosomatic index was significantly ($p<0.05$) affected by protein level in significantly ($p>0.05$) by lipid sources. As for HIS, diet formulated with different lipid source had not significant effect and these results were consistent with other previous study where no significant difference was found when fish oil was substitutes with vegetable oil such as Atlantic salmon [17] and turbot. At same protein level diet, the VSI was found higher for the diet containing SBO as lipid source compare to other two lipid source but it was not significantly different. These results are supported by the studies such [1] and such [14].

Proximate composition

Data pertaining to the biochemical composition of the experimental fish are given in Table 3. Two-way ANOVA result showed that only dietary protein has significant individual effect on proximate composition of fish muscle except moisture content. At the end of experiment, the percentage of moisture content was varied from 73.29% to 74.54% and muscle protein content significantly increased as the dietary protein increased, being lowest (60.24%) for the diet formulated with 25% dietary protein and soybean oil as lipid source and highest (61.89%) for the diet formulated with 30% dietary protein and equal combination of FO and SBO. No significant difference was found in protein content of fish fed with diet containing 30% and 35% protein with different lipid source. Up to a limit increase in dietary protein content help in conversion and deposition of protein in the muscle. Ash content of carcass was found in increasing trend with increasing the dietary protein content and it has significant individual effect of dietary protein levels, lipid sources and interactive effect.

In conclusion, *P. hypopthalmus* fingerling showed optimum growth on a diet containing 30% dietary protein and 6% lipid with equal combination of fish oil and soybean oil. Increasing the protein level from 30% to 35% did not show any significant difference ($p>0.05$) in response to weight gain and specific growth rate. With reference to dietary lipid source, there is possibility of 50% substitution of fish oil with soybean oil in the diet of *P. hypopthalmus* fingerling with positive effect on growth.

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