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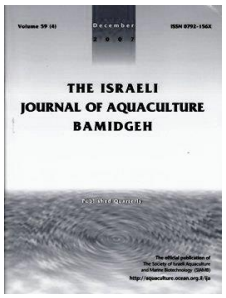

Effects of Dietary Protein and Energy Levels on Growth, Feed utilization and Body Composition of Skin Carp *Hemibarbus labeo*

LV Yao-Ping [1] HU Ze-Hui[2*] LU Jun[3] DAI Qing-Min[1] and JIN Jing[4]

[1] *College of ecology, Lishui University, Lishui 323000 China;*

[2] *Key Laboratory of Mariculture and Enhancement of Zhejiang Province, Marine Fisheries Research Institute of Zhejiang, Zhoushan 316021 China;*

[3] *Lishui Fishery Technology Promotion Station of Zhejiang Province, Lishui 323000 China;*

[4] *College of Ocean, Ningbo University, Ningbo 315211 China*

Keywords: *Ctenopharyngodon idella;* Alpha-ketoglutarate; growth performance; serum biochemical parameters

Abstract

A 12-week feeding trial was conducted to assess the effects of dietary protein and energy levels on the growth, feed utilization, and body composition of skin carp *Hemibarbus labeo.* Ten experimental diets with five levels of crude protein (CP) and two levels of crude lipid (CL) were designated as 32%, 36%, 40%, 44%, 48% and 18 MJ/kg,19.5MJ/kg, respectively. Final body weight (FBW) and weight gain rate (WGR) increased, whereas the feed intake (FI) and feed conversion ratio (FCR) decreased, with increasing dietary protein level from 32% to 48%. Fish fed the diets containing 44% CP and CL providing 19.5MJ/kg exhibited higher FBW and WGR, but lower FI and FCR. At the end of the feeding trial, the intraperitoneal fat ratio (IPFR) decreased with the increase of CP levels. Significant differences (P<0.05) were found in the carcass composition of fish fed different levels of CP and CL. No noticeable trend was observed for moisture and ash in the carcass. The optimum CP and CL level were found to be 44% and 19.5MJ/kg respectively for skin carp reared in net cages.

Corresponding author. Tel: +86-580-2299-817, Fax: +86-580-2299-866, E-mail:deg813@126.com

Introduction

Protein and lipids are essential in formulated diets, as they supply energy for catabolism in all animals including fish (NRC 1993). Dietary protein requirements of fish are closely related to dietary energy levels, as supplied by lipids in fish feeds (Cui *et al*. 2011). Dietary protein and lipid deficiency can inhibit the growth of fish. High dietary protein content causes excess emission of ammonia into culture water, thereby polluting the environment (Boyd, 1990). It is important to determine the optimal requirements of protein and energy for aquatic animals (Han *et al*. 2011). The requirement of crude protein (CP) and crude lipid (CL) has been established for many fish species, such as *Ctenopharyngodon idella* (Lin D et al.1980), *Sarotherodon mosambicus* (Jauncey, 1982), *Mystus nemurus* (Khan et al,1993), *Carassius auratus* (Lochmann et al,1994), *Barbode altus* (Elangovan et al,1997) and *Mylopharyngodon piceus* (Chen et al,2014).

Skin carp *Hemibarbus labeo* is a cyprinid bottom-dwelling insectivorous fish which can be found in streams throughout East Asia. It is a tasty, popular, and commercially important freshwater species. According to the statistics, production of carp, barbel and other cyprinids has reached 1.53 million tons, representing 3 percent of world food fish aquaculture production in 2012 (FAO yearbook). There are few reports on the requirements of protein and energy for *H. labeo*. The present study was designed to examine the interaction between dietary protein and energy levels on the growth, feed utilization, and body composition of juvenile *H.labeo* and determine their dietary protein and lipid requirements.

Materials and methods

Preparation of feeds. A 5×2 factorial design was established, including 5 levels of crude protein (32%, 36%, 40%, 44%, and 48%) and 2 levels of crude lipid (providing 18MJ/kg or 19.5MJ/kg). A total of 10 feed treatments were examined. The crude protein and crude lipid contents in the formulated feeds were estimated using published digestible coefficients (Bureau *et al.* 1999).The formulation, chemical composition, and energy content of the feeds are shown in Table 1.

Table 1 Formulation, chemical composition and energy content of the experimental feeds (% dry matter)

Ingredient	LE1	LE2	LE3	LE4	LE5	HE1	HE2	HE3	HE4	HE5
Fish meal	0.299	0.359	0.418	0.478	0.54	0.299	0.369	0.43	0.491	0.552
Soybean meal	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Wheat flour	0.200	0.200	0.200	0.200	0.170	0.200	0.200	0.200	0.200	0.200
Celite	0.169	0.139	0.110	0.080	0.050	0.116	0.080	0.050	0.019	
$CaHPO_4$	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Methionine	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Fish oil	0.092	0.062	0.032	0.002	0.000	0.145	0.111	0.080	0.050	0.008
[1] Vitamin premix	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
[2] Mineral premix	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Dry matter (g Kg^{-1})	924	920	915	911	910	923	918	914	909	905
Crude protein (g Kg^{-1})	320	361	401	439	479	320	361	401	440	481
Crude lipid (g Kg^{-1})	79	80	80	79	80	120	121	120	120	121
Ash (g Kg^{-1})	170	165	165	143	147	134	128	127	114	110
Gross energy (MJ kg^{-1})	17.5	17.7	17.9	18.1	18.2	19.5	19.8	20.0	20.3	20.1

[1] Vitamin premix contained the following vitamins per kg of feed: vitamin A, 2500 I U; vitamin D_3, 2000 I U; vitamin E, 50 I U; vitamin K, 1mg; thiamin, 1mg; riboflavin, 6mg; pyridoxine, 5mg; vitamin B_{12}, 0.02mg; D-calacium pantothenate, 20mg; choline, 1000 mg; niacin, 10 mg; biotin, 0.14mg; folacin, 1mg; ascorbic acid, 50mg.

[2] Mineral premix compositions (g Kg^{-1} diet): NaCl, 1200; $FeSO_4$, 13; $ZnSO_4$, 60; $MnSO_4$, 32; $CuSO_4$, 7; KI, 8.

The formulated feeds were made into slow-sinking pellets (3mm diameter, 7-10mm long) using a laboratory-scale single screw extruder. The pellets were dried at room temperature, and fish oil was sprayed on the surface of the pellets with a sprayer in a rotating stirring drum. The pellets were stored at -20ºC until used.

Feeding trial

H. labeo fingerlings were obtained from a commercial farm in Lishui, Zhejiang province, China. Two weeks prior to initiation of the feeding trial, all the animals were reared in net cages (2.5 m×2.5 m×1.5 m) and fed a diet containing 32% crude protein and 18MJ/ kg crude lipid to adapt to the experimental diet and culture conditions.

At the start of the feeding trial, fish were starved for 24 h. Similar sized healthy fish (mean initial weight: 7.57±0.11g) were then selected and randomly assigned to 30 cages (1 m×1 m×1.5m) at a density of 30 fish per cage. Each diet was fed to triplicate groups. The fish were fed to apparent satiation twice daily 08:00 and 16:00, respectively, for 12 weeks. During the feeding trial, the water temperature ranged from 22ºC to 30ºC. Dead fish were weighed, and accounted for in the calculation of the feed conversion ratio.

Sample collection and chemical analysis. At the end of the feeding trial the fish were starved for 24h. They were counted, weighed and weight gain rate (WGR) and survival rate (SR) was calculated. Then five fish from each pen were randomly selected and their whole-body composition determined. Moisture content, crude protein, crude lipid and ash were analyzed using standard procedures (AOAC 1995). Eight subsamples of 3 fish each were then randomly collected from the remaining acclimated fish and sacrificed to calculate the condition factor and hepatosomatic index.

Calculation and statistical analysis

The formulae for the parameters mentioned above are as follows:

Feed intake (FI), (%/day)=$100\times I/[(W_t+W_0)/2\times t]$

Weight gain rate (WGR, %)=$100\times(W_{t1}-W_{01})/\ W_{01}$

Feed conversion ratio (FCR/dry feed gain)=$I/(W_t-W_0+W_d)$

Protein efficiency ratio (PER, %) =$100\times G_P/C_P$

Hepatosomatic index (HSI, %) =$100\times W_l/W_s$

Intraperitoneal fat ratio (IPFR, %) =$100\times W_f/W_s$

Condition factor (CF, g cm^{-3}) =$100\times W_s/L_s^3$

where I (g) is the total amount of the consumed feed on a dry weight basis, W_t (g) is the total final body weight (FBW),W_0 (g) is the total initial body weight, t (day) is the duration of the experiment, W_{t1} is the average body weight of one fish at the end of the experiment and W_{01} at the start of the experiment, W_d (g)is the total body weight of the dead fish, G_p (%) is the protein growth content and C_p(%) is the protein intake content, W_s (g/fish) is the body weight of the fish dissected at the end of the experiment and L_s(cm) the total length, W_l (g) is the liver weight of the fish dissected at the end of the experiment, and W_f is the intraperitoneal fat weight of the fish dissected at the end of the experiment.

Data were analyzed using the variance of analysis for factorial layout. When overall differences were significant at $P<0.05$, Tukey's test was used to compare the mean values between individual treatments using SPSS 13.0. The optimal dietary protein contents based on FBW and dietary protein level were estimated using the broken-line model (Robbins *et al*. 1979).

Results

Growth and feed utilization. During the experiment, survival rate was 98% for all treatments. No significant differences between the treatments were detected. Dietary crude protein levels significantly affected FBW, FI, WGR, FCR and PER of fish fed the formulated feeds ($P<0.01$). FBW, WGR and PER depended on the dietary crude protein level, but were not affected by the dietary crude lipid level (Table 2).

Table 2 Summary of analysis of variance in variable among fish fed the formulated feeds

Parameters	*Sources of variance in this experiment*		
	Dietary lipid level	*Dietary protein level*	*Lipid level×protein*
df	1	4	4
FI, FCR	*P*<0.05	*P*<0.01	NS
FBW, WGR	NS	*P*<0.01	NS
PER	NS	*P*<0.01	NS
CF, HSI	NS	NS	NS
IPFR	NS	*P*<0.05	NS
Moisture	*P*<0.05	*P*<0.01	*P*<0.01
Crude	NS	*P*<0.01	*P*<0.01
Lipid	*P*<0.01	*P*<0.01	*P*<0.01
Ash	NS	*P*<0.05	*P*<0.05

FI, feed intake; FCR, feed conversion ratio; FBW, final body weight; WGR, weight gain rate; PER: protein efficiency
rate; CF, Condition factor; HSI, Hepatosomatic index; IPFR, Intraperitoneal fat ratio.

FI and FCR were affected by both crude protein and crude lipid levels (Table 2). FI and FCR tended to decrease with the increase of crude protein and crude lipid. A further increase of dietary crude protein to 44% led to a slight rise in FI and FCR. Fish fed diets containing 44% crude protein and crude lipid providing 19.5MJ/kg energy, exhibited better FCR ($P<0.05$), whereas fish fed diets containing 32% crude protein and 18MJ/kg crude lipid had the highest FCR ($P<0.05$, Table 3). When dietary crude protein was increased from 32% to 44%, protein utilization rose. Protein utilization decreased when crude protein levels where higher than 44% (Table 3).

Dietary crude protein levels significantly affected WGR, FBW and PER of fish fed experimental diets (Table 2). WGR, FBW and PER of fish fed diets with the same crude lipid content increased with the increase of crude protein from 32% to 44%. A further increase of dietary crude protein beyond 44% resulted in a slight decline in WGR, FBW and PER. Fish fed a diet containing 44% crude protein and 19.5MJ/kg energy exhibited the highest WGR, FBW and PER among fish fed the experimental diets ($P<0.05$, Table 3).

Body composition. At the end of the experiment, moisture and crude lipid content in fish carcasses were affected by both crude protein and crude lipid levels, while crude protein and ash contents in the carcass were affected only by crude protein levels (Table 2). Crude protein and ash content in the carcass of fish fed diets with identical crude lipid levels increased with the increase in crude protein from 32% to 44% (Table 4).

Body indices. IPFR was dependent on crude protein levels (Table 2). IPFR decreased with the increase of crude protein from 32% to 44% at the same crude lipid level (18MJ/kg), however there was no effect on IPFR with a crude lipid level of 19.5MJ/kg. Levels of crude protein and crude lipid in the formulated feeds did not significantly affect HSI and CF ($P>0.05$, Table 2 and 5). The HSI and IPFR of fish fed 44% crude protein and 19.5MJ/kg crude lipid were the lowest.

Dietary protein requirement analysis Based on the fish growth in this experiment, analysis of the broken-line regression for FBW indicated that the optimal dietary protein content was 44% (Fig.1).

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Table 3 Growth performance and feed utilization of juvenile Hemibarbus labeo fed the experimental diets with different levels of protein and lipid for 12 weeks (Mean±S.E., n=3)

	Diet treatment									
Parameters	LE1	LE 2	LE 3	LE 4	LE 5	HE1	HE 2	HE 3	HE 4	HE 5
IBW[1](g)	7.48±0.18	7.53±0.11	7.56±0.18	7.61±0.10	7.59±0.13	7.67±0.09	7.61±0.11	7.51±0.08	7.59±0.05	7.52±0.11
FBW[2](g)	17.34±0.27^{a}	18.82±0.34^{bc}	20.39±0.58^{df}	22.12±1.10^{g}	19.66±0.36^{cef}	18.07±0.16^{ab}	19.54±0.69^{cef}	21.49±1.66^{dg}	23.83±0.80^{h}	20.15±0.28^{cd}
FI[3]（%/d）	1.578±0.025^{g}	1.484±0.015^{ef}	1.401±0.034^{cd}	1.318±0.050^{b}	1.437±0.009^{de}	1.516±0.003^{fg}	1.429±0.041^{de}	1.340±0.075^{bc}	1.230±0.033^{a}	1.394±0.022^{cd}
WGR[4]（%/d）	0.934±0.009^{a}	1.018±0.027^{b}	1.102±0.035^{c}	1.185±0.041^{d}	1.058±0.039^{bc}	0.953±0.022^{a}	1.047±0.025^{bc}	1.167±0.077^{d}	1.271±0.035^{e}	1.095±0.004^{c}
FCR[5]	1.81±0.04^{g}	1.58±0.04^{ef}	1.41±0.08^{cd}	1.23±0.07^{b}	1.49±0.06^{de}	1.71±0.06^{fg}	1.48±0.09^{de}	1.27±0.15^{bc}	1.07±0.05^{a}	1.39±0.05^{cd}
PER[6]（%）	25.62±0.58^{bc}	26.41±1.08^{bcd}	28.81±1.85^{cd}	28.89±1.50^{de}	20.65±0.92^{a}	23.24±1.19^{ab}	27.05±2.52^{bcd}	32.67±4.75^{e}	36.24±2.03^{f}	21.50±0.69^{a}

Table 4 Carcass composition of juvenile *H. labeo* fed the experimental diets with different levels of protein and lipid for 12 weeks (Mean±S.E., n=3)

		Diet treatment									
Parameters	initial	LE1	LE 2	LE 3	LE 4	LE 5	HE1	HE 2	HE 3	HE 4	HE 5
Moisture (%)	76.41±0.33	77.21±0.06^{a}	76.38±0.05^{b}	75.57±0.31^{c}	75.07±0.05^{d}	75.87±0.23^{c}	79.44±0.12^{e}	77.19±0.18^{a}	74.48±0.47^{f}	73.33±0.25^{g}	77.75±0.21^{h}
Protein (%)	16.88±0.07	14.56±0.01^{b}	15.05±0.35^{b}	15.98±0.15^{c}	15.97±0.08^{c}	14.76±0.05^{b}	13.08±0.29^{a}	14.71±0.62^{b}	16.71±0.38^{d}	17.38±0.16^{e}	14.55±0.08^{b}
Lipid (%)	3.13±0.06	5.20±0.07^{a}	5.04±0.07^{b}	4.60±0.05^{c}	4.15±0.02^{d}	4.45±0.02^{e}	4.75±0.01^{f}	5.13±0.04^{ab}	5.73±0.05^{g}	6.05±0.12^{h}	4.27±0.12^{i}
Ash (%)	4.12±0.01	2.82±0.13^{ab}	3.18±0.15^{de}	3.16±0.07^{de}	2.96±0.04^{bc}	3.17±0.08^{de}	2.70±0.11^{a}	2.81±0.05^{ab}	3.03±0.08^{cd}	3.36±0.21^{e}	2.86±0.08^{abc}

Values in the same column sharing a common superscript letter were not significantly different (P>0.05).

Table 5 The body indices of juvenile *H. labeo* fed the experimental diets with different levels of protein and lipid for 12 weeks (Mean±S.E., n=3)

	Diet treatment									
Parameters	LE1	LE 2	LE 3	LE 4	LE 5	HE1	HE 2	HE 3	HE 4	HE 5
CF[1]（% g cm^{-3}）	0.86±0.07^{a}	0.81±0.03^{ab}	0.82±0.02^{ab}	0.82±0.01^{ab}	0.86±0.02^{a}	0.82±0.05^{ab}	0.82±0.01^{ab}	0.80±0.00^{ab}	0.84±0.04^{ab}	0.79±0.04^{b}
HSI[2]（%）	1.27±0.18	1.28±0.23	1.15±0.26	1.25±0.32	1.23±0.25	1.30±0.17	1.13±0.25	1.20±0.23	1.07±0.15	1.18±0.06
IPFR[3]（%）	2.29±0.34^{de}	1.50±0.30^{abc}	1.14±0.52^{a}	1.07±0.16^{a}	1.35±0.06^{ab}	2.61±0.45^{e}	1.96±0.17^{bcde}	2.15±0.23^{cde}	0.99±0.38^{a}	1.85±0.48^{bcd}

Values in the same column sharing a common superscript letter were not significantly different (P>0.05).

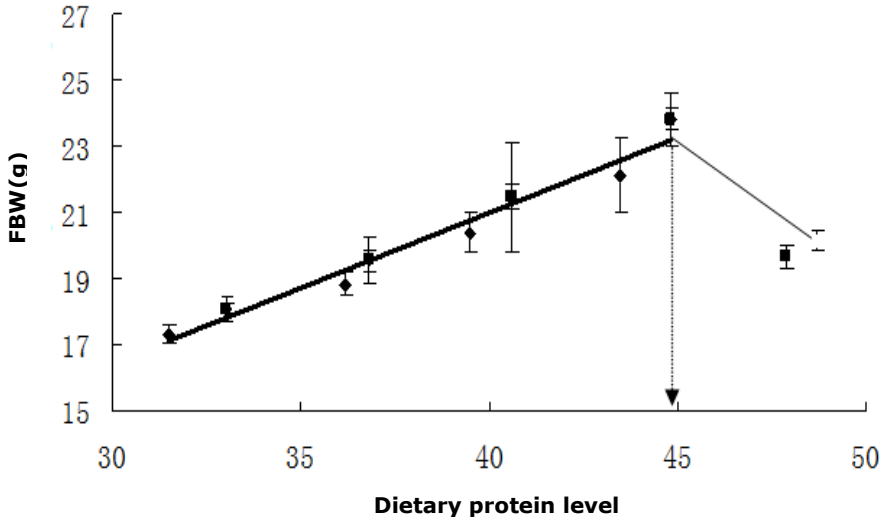

Figure 1 Broken-line analysis of the relationship between dietary protein level and final body weight (FBW) indicates that the optimal dietary protein content was 44%. Each point represents the mean of three replicates.

Discussion

In the present study, the experimental results showed a correlation between growth, FCR of *H. labeo* and protein in the diet. When there was insufficient dietary protein growth was inhibited, resulting in high FCR. When diet protein levels increased, *H.labeo* growth improved and FCR decreased, however when dietary protein level exceeded the optimal level fish growth and FCR did not improve. Other protein requirement studies on Nile tilapia *Oreochromis nilotica* × *O. aureus*, *Channa striata*, *Maccullochella peeliii*, *Zacco barbata*, *Bidyanus Bidyanus*, *Spinibarbus hollandi* and *Mylopharyngodon piceus* have shown similar correlations between growth response and protein level. In our experiment, analysis of weight gain data by a broken line regression model showed that the optimum dietary protein level required for the maximum growth of *H. labeo* was 44% of dry diet. This protein requirement was higher than for carp (http://www.fao.org/fishery/affris/species-profiles/common-carp/nutritional-requirements/en/), indicating that *H.labeo*, like other fish, need less dietary protein.

In the present study, FI decreased as crude lipid levels increased at the five tested crude protein levels. This result was similar to that reported for *Cyprinus carpio var. Jian* (Li et al., 2012). At the same dietary crude protein level, there was an increase in growth and reduction of FCR in fish fed the diet providing 19.5MJ/kg energy from crude lipid. Ratio of protein to energy (P/E) in diets is an important consideration in the formulation of cost-effective and environmentally friendly fish feed. Dietary P/E varies among fish species, particularly between cold water and warm water fish. Coldwater fish utilize high levels of dietary lipids for energy, and they require lower dietary P/E. similarly to P/E values for rainbow trout (22g/MJ) (Lee and Putnam 1980) and Atlantic salmon (18 g/MJ) (Hillestad and Johnsen 1994) are typical. In contrast, P/E in warm water fish is relatively high, e.g., 28 g/MJ for Duchenne amberjack (Jover *et al*. 1999), and 28 g/MJ for red drum (McGoogan and Gatlin 1999). In the present study, FBW, PER and FCR, of *H. labeo* improved when fed a diet containing 44% crude protein and 19.5MJ/kg energy from crude lipid with P/E value of 22g/MJ. This indicates that the optimal P/E for *H.labeo* may be slightly lower than other warm water carnivorous fish. An appropriate increase of dietary crude lipid level could reduce the dietary crude protein necessary for *H. labeo*.

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