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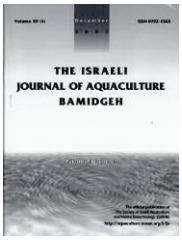
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

The Israeli Journal of Aquaculture - Bamidgeh, IJA_70.2018.1551, 9 pages



Effects of Dietary Protein and Lipid Levels on Growth Performance, Feed Utilization and Biochemical Parameters of Barbless Carp (*Cyprinus pellegrini*)

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Keywords: barbless carp *Cyprinus Pellegrini*; feed utilization; growth performance; protein to lipid ratio

Abstract

An 8-week feeding trial was conducted to evaluate the effects of varying dietary protein (35%, 40%, 45%) and lipid (4%, 8%, 12%) levels with protein to energy (P/E) ratios ranging from 17.04 to 23.58 g protein/MJ on growth performance, feed utilization, and biochemical parameters of barbless carp (*Cyprinus pellegrini*). Fish fed diets with 40% protein exhibited higher thermal growth coefficient (TGC) and energy retention compared with fish fed the diets with 35% protein, higher protein efficiency ratio (PER), and protein retention, compared with fish fed diets with 35% and 45% protein. TGC and energy retention were significantly lower in fish fed the diets with 4% lipid compared to fish fed diets with 8% and 12% lipid. Fish fed the diet with 40% protein and 12% lipid had similar TGC as those fed the diets containing 8% and 12% lipid with 45% protein, but showed relatively better PER. Further, fish fed the diet with 40% protein and 12% lipid exhibited relatively lower plasma γ-glutamyl transferase, aspartate aminotransferase, and alanine aminotransferase activities, and total protein and blood urea nitrogen contents compared with fish fed the other diets. These results indicate that a diet containing 40% protein and 12% lipid with P/E of 19.38 g protein/MJ would be suitable for growth and health of barbless carp.

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Introduction

Protein accounts for the largest portion of total feed cost, and a sufficient level is necessary to ensure healthy growth of fish (Shapawi *et al.*, 2014). However, excess dietary protein intake should be avoided due to increased feed costs and nitrogen emission into the environment (NRC, 2011). To prevent this from happening, lipid can be utilized as a dietary energy source, and thereby spare protein for growth and provide essential fatty acids (Lee *et al.*, 2002). Excess of dietary lipid can also result in side effects including body lipid deposition and fatty liver disease (Gao *et al.*, 2009). Thus, it is important to optimize dietary protein to energy (P/E) ratio for high-efficiency and environmentally-friendly compound feed. Several studies have been conducted to determine the optimal dietary P/E ratio for some cultured fish (Ai *et al*., 2004; Ali *et al*., 2008; Ebrahimi *et al*., 2013; Wang *et al*., 2017).

Barbless carp *Cyprinus Pellegrini,* is a native species distributed only in the Xingyun and Qilu Lakes of the Yunnan-Guizhou plateau in China (Tang and Chen, 2012). Historically, barbless carp was an economically important fish species in these two lakes but their numbers have declined dramatically since the invasion of exotic species, overfishing, habitat destruction, and loss of spawning grounds (Shen *et al.*, 2009). Thus, barbless carp is legally protected and listed in China's Red Data Book of Endangered Animals (Yue and Chen, 1998). Conservation action plan is being carried out by national institutes to preserve this precious species, and artificial breeding of barbless carp has been successful (Zhang *et al*., 2010). Nowadays, barbless carp has been reintroduced into the Xingyun Lake where it is artificially reproduced and cultured as an excellent economic endemic species (Shen *et al.*, 2009). This precious species is expected to be restored and developed. However, limited information on the nutritional requirements of this species has been reported (Deng *et al.*, 2013). No information is available concerning the optimum dietary P/E ratio for barbless carp so far. The objective of the present study is to evaluate the effects of dietary P/E ratio on growth performance, feed utilization, and biochemical parameters of barbless carp.

Materials and Methods

Experimental diets.

Fish meal, fish protein concentrate, and soy protein concentrate were used as dietary protein sources, fish oil and soybean lecithin as lipid sources, and wheat flour and dextrin as carbohydrate sources. Nine semi-purified diets (P35L4, P35L8, P35L12, P40L4, P40L8, P40L12, P45L4, P45L8, P45L12) were formulated to contain three crude protein levels (35%, 40%, 45%), and each with three crude lipid levels (4%, 8%, 12%), to produce dietary P/E ratios ranging from 17.04 to 23.58 g protein/MJ (Table 1). Feed ingredients were ground into fine powder through a 320-μm mesh. After thoroughly mixing the dry ingredients, fish oil and soybean lecithin together with distilled water were added to produce dough. The dough was then extruded using a pellet feed maker (KS-180, Jiangsu Jingu Rice Mill Co., Ltd., Jiangsu, China) through a 2-mm die. The moist feed was dried in a forced air oven at room temperature and stored at -20°C until further use.

Experimental animals and conditions.

F_2-generation barbless carp were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). Prior to the start of the experiment, apparently healthy fish were acclimatized to the experimental tanks and fed a commercial carp diet (32% crude protein, 5% crude lipid; supplied by Tongwei Co., Ltd., Chengdu, China) for 2 weeks. After acclimatization, a total of 540 fish (initial average weight 40.8 g) were randomly distributed into 27 flow-through fiberglass tanks (1.0 × 0.7 × 0.8 m^3) with 20 juveniles per tank. Each tank was then randomly assigned to one of three replicates of the nine dietary treatments. Fish were hand-fed to apparent satiation twice (08:00, 18:00) daily for 8 weeks. Water was recirculated through a 4000-L biological and mechanical filtration system containing vertical quartz sand filter and activated carbon purifier to remove solid and nitrogenous wastes, and water temperature was maintained at 18–22°C. All rearing tanks were provided with continuous aeration and maintained under natural photoperiod.

Table 1. Formulation and proximate composition of diets for barbless carp, *Cyprinus pellegrini* (%, dry matter).

Dietary protein level (%)	*35%*			*40%*			*45%*		
Dietary lipid level (%)	*4%*	*8%*	*12%*	*4%*	*8%*	*12%*	*4%*	*8%*	*12%*
Fish meal[1]	21.00	21.00	21.00	25.00	25.00	25.00	29.00	29.00	29.00
Fish protein concentrate[2]	8.00	8.00	8.00	10.00	10.00	10.00	12.00	12.00	12.00
Soy protein concentrate[3]	14.00	15.00	16.00	17.00	18.00	19.00	20.00	21.00	22.00
Wheat flour[4]	41.17	36.17	31.17	32.77	27.77	22.77	24.37	19.37	14.37
Dextrin[5]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish oil[1]	1.20	5.20	9.20	0.60	4.60	8.60	0.00	4.00	8.00
Soybean lecithin (40%)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Choline chloride (50%)[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin C[6]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin (30%)[1]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix[7]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix[8]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proximate composition (%)									
Dry matter	92.50	93.76	93.27	91.11	93.26	94.02	92.93	94.36	94.65
Crude protein	35.76	35.78	35.49	40.38	40.35	40.67	45.70	45.44	45.75
Crude lipid	3.90	7.91	12.03	3.96	7.95	11.99	3.82	7.86	11.94
Ash	7.23	7.40	7.45	8.10	8.27	8.42	9.00	9.23	9.17
Gross energy (kJ/g)	19.04	19.93	20.82	19.23	20.09	20.98	19.38	20.23	21.17
P/E (g protein/MJ)	18.78	17.95	17.04	21.00	20.09	19.38	23.58	22.46	21.61

[1] Kunming Tianyuan Feed Co., Ltd., Yunnan, China.
[2] Fisheries Research Institute of Shanghai, Shanghai, China.
[3] Dongying Wonderful Vegetable Protein Science and Technology Co., Ltd., Shandong, China.
[4] Kunming Hongshan Flour Co., Ltd., Yunnan, China.
[5] Qufu Tianli Medical Supplements Co., Ltd., Shandong, China.
[6] L-Ascorbate-2-polyphosphate (35%).
[7] g/kg mixture: $MgSO_4 \cdot 7H_2O$, 180; KI, 1; $FeSO_4 \cdot H_2O$, 260; $ZnSO_4 \cdot H_2O$, 180; $CuSO_4 \cdot 5H_2O$, 25; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 180; $CoCl_2 \cdot 6H_2O$, 0.75.
[8] g/kg mixture: retinyl acetate (2800000 IU/g), 2; cholecalciferol, 0.03; DL-α-tocopheryl acetate, 30; menadione, 3; thiamine hydrochloride, 8; riboflavin, 11; pyridoxine hydrochloride, 8; vitamin B_{12}, 0.02; ascorbic acid, 50; folic acid, 1; biotin 0.1; niacin, 30; calcium D-pantothenate, 32; inositol, 25.

Sampling and chemical analyses.

At the end of the feeding trial, fish were fasted for 24 h before harvest. All fish were anesthetized with eugenol (1:12000) and weighed to calculate growth rate and feed utilization. A total of fifteen fish at initiation of feeding trial and five fish per tank at termination were randomly collected and stored at -20°C for proximate composition analyses. Another six fish per tank were sampled for analysis of biochemical parameters in plasma and liver. Plasma samples were collected from the caudal vein with a heparinized syringe and transferred into a heparinized tube. Plasma was recovered after centrifugation (6000 g, 10 min) and immediately stored at -80°C until analysis. Liver samples were removed and stored frozen (-80°C) for subsequent determination of γ-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities. To obtain adequate crude enzyme extract solution, the amount of physiological saline solution (0.9% NaCl) needed for addition to the wet liver was determined by a preliminary study. Wet liver plus the 4-fold volume (v/w) of ice-cold physiological saline solution were added to a 10 ml test tube and homogenized using an IKA homogenizer (IKA Works Asia, Bhd, Malaysia). The homogenate was centrifuged (9000 g for 30 min at 4°C) using a high-speed refrigerated centrifuge (MX-160, Tomy, Tokyo, Japan). The supernatant was diluted to an adequate volume with a physiological saline solution (if necessary) and used as a crude enzyme solution. All samples were pooled by tank for analysis. Analysis of dry matter (105°C, 24 h), crude protein (Kjeldahl nitrogen × 6.25), crude lipid (ether extraction by Soxhlet method), and ash (550°C, 18 h) in feed ingredients, experimental diets and whole-body samples were performed following standard laboratory procedures (AOAC, 1995). Gross energy content in experimental diets and whole-body samples were measured using a bomb calorimeter (Parr 1351; Parr Instrument Co., Moline, IL, USA). Plasma total protein, albumin, total amino acid (TAA), and blood urea nitrogen (BUN) contents, and

GGT, AST, ALT and ALP activities were determined by colorimetric enzymatic methods using commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). One unit of GGT is defined as the amount of enzyme that produced 1 mmol of p-nitroaniline per min at 37°C. One unit of AST is defined as the amount of enzyme that will generate 1 mmol of glutamate per min at 37°C. One unit of ALT is defined as the amount of enzyme that generates 1 mmol of pyruvate per min at 37°C. One unit of ALP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg phenol in 15 min at 37°C. The hepatic GGT, AST, ALT and ALP activities were determined using the same kits in plasma and expressed as enzyme activity per gram soluble protein. The soluble protein content in the liver samples was quantified by the Bradford method (Bradford, 1976) using a total protein quantification kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) with bovine serum albumin as the standard.

Statistical analysis.

All percentage data were subjected to arcsine transformation before statistical analysis. The data from each treatment were subjected to one-way analysis of variance (ANOVA), two-way ANOVA was also used to analyze the interactive effects of dietary protein and lipids levels. When overall differences were significant ($P < 0.05$), Tukey's multiple range test was used to compare the mean values. Statistical analysis was performed using the SPSS 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Growth performance and feed utilization.

During the 8-week feeding period, no fish mortality was observed in any of the dietary treatment groups. Dietary protein and lipid levels either individually or in combination had significant effects on feed intake (FI), weight gain (WG), and thermal growth coefficient (TGC) of fish (Table 2).

Table 2. Growth performance of barbless carp *C. pellegrini* fed diets with different protein and lipid levels for 8 weeks.

Diet	*Initial body weight (g)*	*Final body weight (g)*	*Feed intake[2] (g/kg ABW[1]/day)*	*Weight gain[3] (%)*	*TGC[4]*	*FCR[5]*	*PER[6]*
P35L4	40.83 ± 0.12	74.76 ± 0.63^{a}	20.91 ± 0.45^{cd}	83.09 ± 1.13^{a}	0.69 ± 0.01^{a}	2.00 ± 0.06^{c}	1.40 ± 0.04^{ab}
P35L8	40.87 ± 0.09	76.67 ± 0.86^{ab}	21.47 ± 0.35^{d}	87.60 ± 2.12^{ab}	0.72 ± 0.01^{ab}	1.98 ± 0.05^{c}	1.42 ± 0.04^{ab}
P35L12	40.83 ± 0.12	74.25 ± 0.82^{a}	19.92 ± 0.11^{bc}	81.84 ± 2.46^{a}	0.68 ± 0.02^{a}	1.92 ± 0.03^{c}	1.47 ± 0.03^{abc}
P40L4	40.69 ± 0.12	81.15 ± 1.63^{bc}	18.43 ± 0.30^{a}	99.43 ± 4.41^{b}	0.79 ± 0.03^{bc}	1.56 ± 0.02^{ab}	1.59 ± 0.02^{c}
P40L8	41.00 ± 0.06	83.10 ± 1.86^{c}	19.88 ± 0.20^{bc}	102.68 ± 4.66^{b}	0.82 ± 0.03^{c}	1.64 ± 0.04^{ab}	1.51 ± 0.04^{abc}
P40L12	40.63 ± 0.15	89.52 ± 1.26^{d}	20.66 ± 0.10^{cd}	120.35 ± 3.88^{c}	0.92 ± 0.02^{d}	1.54 ± 0.05^{ab}	1.60 ± 0.03^{c}
P45L4	40.87 ± 0.03	81.90 ± 1.04^{bc}	19.16 ± 0.27^{ab}	101.00 ± 2.17^{b}	0.80 ± 0.02^{bc}	1.61 ± 0.05^{ab}	1.36 ± 0.04^{a}
P45L8	40.80 ± 0.06	94.05 ± 0.86^{d}	20.26 ± 0.13^{bcd}	130.51 ± 2.20^{c}	0.99 ± 0.01^{d}	1.44 ± 0.02^{a}	1.53 ± 0.02^{bc}
P45L12	40.77 ± 0.09	90.48 ± 1.45^{d}	20.11 ± 0.18^{bc}	121.92 ± 3.08^{c}	0.94 ± 0.02^{d}	1.49 ± 0.03^{ab}	1.47 ± 0.03^{abc}
Protein level (%)							
35	40.84 ± 0.06	75.22 ± 0.54^{u}	20.77 ± 0.28^{v}	84.18 ± 1.32^{u}	0.69 ± 0.01^{u}	1.97 ± 0.03^{v}	1.43 ± 0.02^{u}
40	40.78 ± 0.08	84.59 ± 1.50^{v}	19.66 ± 0.34^{u}	107.49 ± 3.90^{v}	0.85 ± 0.02^{v}	1.58 ± 0.02^{u}	1.57 ± 0.02^{v}
45	40.81 ± 0.04	88.81 ± 1.89^{v}	19.84 ± 0.20^{u}	117.81 ± 4.56^{v}	0.91 ± 0.03^{v}	1.51 ± 0.03^{u}	1.45 ± 0.03^{u}
Lipid level (%)							
4	40.80 ± 0.06	79.27 ± 1.28^{x}	19.50 ± 0.41^{x}	94.51 ± 3.21^{x}	0.76 ± 0.02^{x}	1.72 ± 0.07	1.45 ± 0.04
8	40.89 ± 0.05	84.60 ± 2.62^{y}	20.54 ± 0.27^{y}	106.93 ± 6.49^{y}	0.84 ± 0.04^{y}	1.69 ± 0.08	1.49 ± 0.03
12	40.74 ± 0.07	84.75 ± 2.70^{y}	20.23 ± 0.13^{y}	108.04 ± 6.75^{y}	0.85 ± 0.04^{y}	1.65 ± 0.07	1.51 ± 0.03
Two-way ANOVA (*P* value)							
Protein	0.700	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lipid	0.222	<0.001	<0.001	<0.001	<0.001	0.138	0.128
Protein × Lipid	0.304	<0.001	<0.001	<0.001	<0.001	0.067	0.025

*Values in a column with different superscripts significantly differ.

[1] ABW: average body weight (kg) = (initial body weight [kg] + final body weight [kg])/2.

[2] *Feed intake*: feed consumption (g)/(ABW (kg) × feeding days).

[3] Weight gain: (final body weight (g) – initial body weight (g))/initial body weight (g).

[4] TGC: thermal growth coefficient = ((final body weight$^{1/3}$ (g) – initial body weight$^{1/3}$ (g))/Σ(temperature (°C) × days) × 1000.

[5] FCR: feed conversion ratio = dry feed fed (g)/weight gain (g).

[6] PER: protein efficiency ratio = weight gain (g)/protein fed (g).

The highest values of WG (130.51%) and TGC (0.99) were observed in fish fed the P45L8 diet, whereas the lowest values (81.84%, 0.68) were recorded in fish fed the P35L12 diet, respectively. The best feed conversion ratio (FCR), and protein efficiency ratio (PER), were found in fish fed the P45L8, and P40L12 diets, respectively. With respect to dietary protein and lipid levels, WG and TGC were lower in fish fed the diets with 35% protein compared to fish fed the diets with 40% and 45% protein, and lower in fish fed the diets with 4% lipid compared to fish fed the diets with 8% and 12% lipid. Conversely, FCR was higher in fish fed the diets with 35% protein compared to fish fed the diets with 40% and 45% protein. PER was higher in fish fed the diets with 40% protein compared to fish fed the diets with 35% and 45% protein. However, FCR and PER were not affected by dietary lipid levels.

Protein and energy retention.

Dietary protein and lipid levels either individually or in combination had significant effects on protein intake, energy intake, protein gain, energy gain, protein retention, and energy retention of fish (Table 3).

Table 3. Protein and energy utilization of barbless carp Cyprinus pellegrini fed diets with different protein and lipid levels for 8 weeks.

Diet	*Protein intake (g/kg ABW[1]/day)*	*Energy intake (MJ/kg ABW[1]/day)*	*Protein gain (g/kg ABW[1]/day)*	*Energy gain (MJ/kg ABW[1]/day)*	*Protein retention[2] (%)*	*Energy retention[3] (%)*
P35L4	7.48 ± 0.16^{ab}	0.40 ± 0.01^{bc}	1.89 ± 0.05^{a}	0.08 ± 0.00^{a}	25.43 ± 1.26^{ab}	20.17 ± 0.85^{a}
P35L8	7.68 ± 0.13^{bc}	0.43 ± 0.01^{de}	2.08 ± 0.02^{abc}	0.09 ± 0.00^{ab}	27.03 ± 0.56^{abc}	21.40 ± 0.17^{ab}
P35L12	7.07 ± 0.06^{a}	0.41 ± 0.00^{cde}	1.97 ± 0.11^{ab}	0.09 ± 0.00^{ab}	27.83 ± 1.47^{abc}	21.23 ± 0.73^{ab}
P40L4	7.44 ± 0.12^{ab}	0.36 ± 0.01^{a}	2.34 ± 0.14^{cd}	0.09 ± 0.01^{ab}	31.43 ± 1.30^{c}	24.53 ± 0.84^{bc}
P40L8	8.02 ± 0.08^{cd}	0.40 ± 0.01^{cd}	2.30 ± 0.05^{bcd}	0.11 ± 0.00^{cd}	28.67 ± 0.43^{abc}	27.33 ± 0.20^{cde}
P40L12	8.40 ± 0.04^{de}	0.43 ± 0.01^{e}	2.54 ± 0.06^{de}	0.13 ± 0.00^{ef}	30.20 ± 0.75^{bc}	30.23 ± 0.74^{e}
P45L4	8.76 ± 0.12^{ef}	0.37 ± 0.01^{ab}	2.09 ± 0.08^{abc}	0.10 ± 0.00^{bc}	23.97 ± 1.27^{a}	26.50 ± 0.84^{cd}
P45L8	9.20 ± 0.06^{f}	0.41 ± 0.00^{cde}	2.86 ± 0.02^{e}	0.14 ± 0.01^{f}	31.03 ± 0.43^{c}	33.97 ± 1.08^{f}
P45L12	9.19 ± 0.08^{f}	0.43 ± 0.00^{de}	2.61 ± 0.06^{de}	0.12 ± 0.00^{de}	28.37 ± 0.58^{abc}	29.00 ± 0.95^{de}
Protein level (%)						
35	7.41 ± 0.11^{u}	0.41 ± 0.01^{v}	1.98 ± 0.04^{u}	0.09 ± 0.00^{u}	26.77 ± 0.68^{u}	20.93 ± 0.38^{u}
40	7.96 ± 0.15^{v}	0.40 ± 0.01^{u}	2.39 ± 0.06^{v}	0.11 ± 0.01^{v}	30.10 ± 0.60^{v}	27.37 ± 0.89^{v}
45	9.05 ± 0.09^{w}	0.40 ± 0.01^{uv}	2.52 ± 0.12^{w}	0.12 ± 0.01^{v}	27.79 ± 1.11^{u}	29.82 ± 1.19^{v}
Lipid level (%)						
4	7.89 ± 0.23^{x}	0.37 ± 0.01^{x}	2.11 ± 0.08^{x}	0.09 ± 0.00^{x}	26.94 ± 1.31^{x}	23.73 ± 1.03^{x}
8	8.30 ± 0.24^{y}	0.41 ± 0.01^{y}	2.41 ± 0.12^{y}	0.11 ± 0.01^{y}	28.91 ± 0.63^{y}	27.57 ± 1.84^{y}
12	8.22 ± 0.31^{y}	0.42 ± 0.01^{y}	2.37 ± 0.11^{y}	0.11 ± 0.01^{y}	28.80 ± 0.62^{y}	26.82 ± 1.46^{y}
Two-way ANOVA (*P* value)						
Protein	<0.001	0.011	<0.001	<0.001	0.002	<0.001
Lipid	<0.001	<0.001	<0.001	<0.001	0.046	<0.001
Protein × Lipid	<0.001	0.001	<0.001	<0.001	0.002	<0.001

*Values in a column with different superscripts significantly differ.

[1] ABW: average body weight (kg) = (initial weight [kg] + final weight [kg])/2.

[2] Protein retention (%): 100 × (protein gain [g/kg ABW/day]/protein intake [g/kg ABW/day]).

[3] Energy retention (%): 100 × (energy gain [g/kg ABW/day]/energy intake [g/kg ABW/day]).

The highest values of protein intake (9.20 g/kg ABW/day), energy intake (0.43 MJ/kg ABW/day), protein gain (2.86 g/kg ABW/day), energy gain (0.14 MJ/kg ABW/day), protein retention (31.43%) and energy retention (33.97%) were observed in fish fed the P45L8, P40L12, P45L8, P45L8, P40L4 and P45L8 diets, respectively. Conversely, the lowest values of protein intake (7.07 g/kg ABW/day), energy intake (0.36 MJ/kg ABW/day), protein gain (1.89 g/kg ABW/day), energy gain (0.08 MJ/kg ABW/day), protein retention (23.97%), and energy retention (20.17%) were recorded in fish fed the P35L12, P40L4, P35L4, P35L4, P45L4 and P35L4 diets, respectively. With respect to dietary protein and lipid levels, protein intake, and gain of fish gradually increased with increasing dietary protein levels. Fish fed the diets with 35% protein had the highest energy intake, the lowest energy gain and retention. However, the best protein retention was observed in fish fed diets with 40% protein. Protein intake, energy intake, protein gain, energy gain, protein retention, and energy retention were lower in fish fed diets with 4% lipid compared to fish fed diets with 8% and 12% lipid.

Biochemical parameters in plasma and liver.

GGT, AST, ALT and ALP activities in plasma and liver of fish were significantly affected by the dietary treatments (Table 4). However, plasma ALT and ALP, and hepatic AST, and ALP activities were not affected by dietary protein level, plasma ALP and hepatic GGT activities were not affected by dietary lipid level. The highest values of plasma GGT, AST, ALT and ALP, and hepatic GGT, AST, ALT, and ALP activities were observed in fish fed the P45L4, P35L8, P45L4, P35L12, P40L4, P35L12, P45L12 and P40L4 diets, whereas the

lowest values were recorded in fish fed the P40L8, P40L8, P35L12, P35L8, P45L4, P35L8, P35L8 and P35L12 diets, respectively. With respect to dietary protein and lipid levels, plasma GGT and hepatic ALT activities were higher in fish fed the diets with 45% protein compared to fish fed the diets with 35% and 40% protein. However, plasma AST activity was higher in fish fed the diets with 35% protein compared to fish fed the diets with 40% and 45% protein. Hepatic GGT activity was higher in fish fed diets with 40% protein compared to fish fed diets with 35% and 45% protein. Plasma GGT and AST, and hepatic ALP activities gradually decreased with increasing dietary lipid level. Plasma ALT activity was higher in fish fed diets with 4% lipid compared to fish fed diets with 8% and 12%. However, the highest hepatic AST and ALT activities were found in fish fed diets with 12% lipid.

Table 4. The γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in plasma and liver of barbless carp Cyprinus pellegrini fed diets with different protein and lipid levels.

Diet	*Serum*				*Liver*			
	GGT (U/L)	*AST (IU/L)*	*ALT (IU/L)*	*ALP (U/dl)*	*GGT (U/g protein)*	*AST (U/g protein)*	*ALT (U/g protein)*	*ALP (U/g protein)*
P35L4	13.96 ± 1.33^{a}	30.60 ± 2.08^{ab}	7.33 ± 0.52^{bc}	3.91 ± 0.30^{a}	6.24 ± 0.10^{bcd}	95.14 ± 6.26^{ab}	7.15 ± 0.58^{a}	24.67 ± 1.30^{ab}
P35L8	20.93 ± 1.09^{ab}	34.95 ± 2.47^{b}	6.04 ± 0.57^{abc}	2.56 ± 0.42^{a}	4.46 ± 0.15^{abc}	78.47 ± 5.81^{a}	4.99 ± 0.75^{a}	23.91 ± 1.91^{ab}
P35L12	13.95 ± 0.72^{a}	30.13 ± 2.14^{ab}	3.71 ± 0.52^{a}	8.87 ± 0.75^{c}	3.34 ± 0.14^{ab}	137.12 ± 2.34^{b}	6.86 ± 0.42^{a}	15.93 ± 1.94^{a}
P40L4	20.93 ± 1.09^{ab}	30.78 ± 1.13^{ab}	6.30 ± 0.52^{abc}	4.96 ± 0.35^{a}	10.02 ± 0.35^{e}	112.90 ± 2.30^{ab}	6.65 ± 0.46^{a}	26.84 ± 1.08^{b}
P40L8	11.63 ± 1.06^{a}	17.85 ± 2.18^{a}	5.78 ± 0.52^{ab}	4.21 ± 0.60^{a}	5.92 ± 0.17^{abcd}	106.54 ± 5.27^{ab}	5.06 ± 0.55^{a}	17.55 ± 2.33^{ab}
P40L12	16.28 ± 1.15^{a}	19.03 ± 1.84^{a}	5.52 ± 0.26^{ab}	3.61 ± 0.60^{a}	8.82 ± 0.14^{de}	120.08 ± 6.89^{ab}	6.92 ± 0.84^{a}	16.14 ± 2.38^{ab}
P45L4	30.24 ± 1.03^{b}	26.53 ± 1.52^{ab}	8.88 ± 0.47^{d}	5.41 ± 0.42^{ab}	3.03 ± 0.11^{a}	98.21 ± 1.68^{ab}	7.96 ± 0.38^{ab}	25.03 ± 1.96^{ab}
P45L8	20.93 ± 0.86^{ab}	22.04 ± 2.02^{ab}	4.49 ± 0.27^{ab}	8.42 ± 0.62^{bc}	7.52 ± 0.25^{cde}	110.87 ± 6.86^{ab}	6.83 ± 0.45^{a}	22.00 ± 1.56^{a}
P45L12	17.44 ± 1.16^{a}	21.39 ± 2.20^{ab}	4.75 ± 0.39^{ab}	3.01 ± 0.30^{a}	4.72 ± 0.33^{abc}	122.08 ± 9.74^{ab}	11.98 ± 0.29^{b}	26.16 ± 2.62^{b}
Protein level (%)								
35	16.28 ± 1.59^{u}	31.89 ± 1.77^{v}	5.69 ± 0.52	5.11 ± 1.23	4.68 ± 0.59^{u}	103.57 ± 2.02	6.33 ± 0.52^{u}	21.50 ± 1.88
40	16.28 ± 1.90^{u}	22.55 ± 2.73^{u}	5.87 ± 0.25	4.26 ± 0.81	8.25 ± 0.80^{v}	113.17 ± 3.92	6.21 ± 0.51^{u}	20.18 ± 2.31
45	22.87 ± 2.58^{v}	23.32 ± 1.34^{u}	6.04 ± 0.69	5.61 ± 1.04	5.09 ± 0.88^{u}	110.38 ± 5.35	8.92 ± 1.04^{v}	24.39 ± 1.07
Lipid level (%)								
4	21.71 ± 3.10^{y}	29.30 ± 1.28^{y}	7.50 ± 0.53^{y}	4.76 ± 1.30	6.43 ± 1.29	102.08 ± 5.90^{u}	7.25 ± 0.39^{uv}	25.51 ± 1.66^{v}
8	17.83 ± 2.14^{xy}	24.94 ± 3.40^{xy}	5.43 ± 0.39^{x}	5.06 ± 1.16	5.97 ± 0.63	98.63 ± 7.08^{u}	5.62 ± 0.55^{u}	21.15 ± 1.43^{uv}
12	15.89 ± 1.93^{x}	23.52 ± 2.50^{x}	4.66 ± 0.39^{x}	5.16 ± 1.21	5.63 ± 1.07	126.43 ± 4.63^{v}	8.58 ± 0.44^{v}	19.41 ± 2.33^{u}
Two-way ANOVA (*P* value)								
Protein	0.002	0.002	0.718	0.052	<0.001	0.387	0.002	0.059
Lipid	0.009	0.042	<0.001	0.687	0.275	0.005	0.002	0.005
Protein × Lipid	0.004	0.064	0.012	<0.001	<0.001	0.080	0.080	0.016

*Values in a column with different superscripts significantly differ.

Total protein, albumin, TAA, and BUN contents in plasma of fish were significantly affected by the dietary treatments (Table 5).

Table 5. Total protein, albumin, total amino acid (TAA) and blood urea nitrogen (BUN) contents in plasma of barbless carp Cyprinus pellegrini fed diets with different protein and lipid levels.

Diet	*Total protein (g/L)*	*Albumin (g/L)*	*TAA (mol/L)*	*BUN (g/L)*
P35L4	28.42 ± 1.31^{a}	18.30 ± 1.00^{abc}	0.49 ± 0.02^{d}	4.74 ± 0.72^{a}
P35L8	26.30 ± 1.21^{a}	21.17 ± 0.72^{bc}	0.43 ± 0.01^{d}	5.49 ± 1.10^{abc}
P35L12	34.16 ± 1.82^{ab}	18.30 ± 1.44^{abc}	0.31 ± 0.01^{c}	7.13 ± 0.52^{abc}
P40L4	34.16 ± 1.72^{ab}	22.25 ± 1.08^{bc}	0.14 ± 0.02^{ab}	8.07 ± 1.02^{abc}
P40L8	39.45 ± 0.76^{b}	16.50 ± 0.63^{ab}	0.13 ± 0.01^{a}	5.38 ± 0.46^{ab}
P40L12	30.08 ± 1.67^{ab}	17.58 ± 0.72^{abc}	0.10 ± 0.01^{a}	5.23 ± 1.06^{ab}
P45L4	33.71 ± 0.46^{ab}	20.81 ± 1.08^{bc}	0.16 ± 0.02^{ab}	9.93 ± 0.69^{bc}
P45L8	29.47 ± 1.87^{a}	13.64 ± 0.80^{a}	0.12 ± 0.01^{a}	10.49 ± 0.49^{c}
P45L12	31.44 ± 1.51^{ab}	23.32 ± 1.44^{c}	0.20 ± 0.01^{b}	7.69 ± 0.49^{abc}
Protein level (%)				
35	29.62 ± 1.59^{u}	19.25 ± 0.73	0.41 ± 0.03^{w}	5.78 ± 0.68^{u}
40	34.56 ± 1.91^{v}	18.78 ± 1.17	0.13 ± 0.01^{u}	6.23 ± 0.71^{u}
45	31.54 ± 1.15^{uv}	19.25 ± 1.95	0.16 ± 0.02^{v}	9.37 ± 0.60^{v}
Lipid level (%)				
4	32.09 ± 1.37	20.45 ± 0.83^{y}	0.26 ± 0.07^{z}	7.58 ± 1.03
8	31.74 ± 1.64	17.10 ± 1.48^{x}	0.23 ± 0.07^{y}	7.12 ± 1.11
12	31.89 ± 1.06	19.73 ± 1.27^{y}	0.20 ± 0.04^{x}	6.68 ± 0.68
Two-way ANOVA (*P* value)				
Protein	0.019	0.830	<0.001	0.002
Lipid	0.968	0.011	<0.001	0.505
Protein × Lipid	0.006	0.001	<0.001	0.056

*Values in a column with different superscripts significantly differ.

However, plasma albumin content was not affected by dietary protein level, plasma total protein, and BUN contents were not affected by dietary lipid level. The highest values of plasma total protein, albumin, TAA and BUN contents were observed in fish fed the P40L8, P45L12, P35L4 and P45L8 diets, whereas the lowest values were recorded in fish

fed the P35L8, P45L8, P40L12 and P35L4 diets, respectively. With respect to dietary protein and lipid levels, fish fed the diets with 40% protein exhibited the highest plasma total protein content. Plasma albumin content was lower in fish fed the diets with 8% lipid compared to fish fed the diets with 4% and 12% lipid. The lowest plasma TAA content was found in fish fed the diets with 40% protein or 12% lipid. Plasma BUN content gradually increased with increasing dietary protein level.

Whole-body composition.

Dietary protein and lipid levels either individually or in combination had significant effects on whole-body moisture, protein, lipid, ash, and energy contents of fish (Table 6).

Table 6. Proximate composition (% wet weight) of the whole-body compositions of barbless carp *Cyprinus pellegrini* fed diets with different protein and lipid levels.

Diet	*Moisture (%)*	*Crude protein (%)*	*Crude lipid (%)*	*Ash (%)*	*Energy (kJ/g)*
Initial	76.12	15.28	3.67	3.98	5.07
P35L4	73.23 ± 0.24^{e}	16.55 ± 0.20^{a}	5.86 ± 0.06^{ab}	3.69 ± 0.05^{cd}	6.24 ± 0.06^{a}
P35L8	72.15 ± 0.18^{cde}	17.06 ± 0.10^{ab}	6.55 ± 0.19^{abc}	3.61 ± 0.15^{cd}	6.63 ± 0.09^{ab}
P35L12	72.85 ± 0.09^{de}	16.96 ± 0.48^{a}	6.57 ± 0.56^{abc}	3.34 ± 0.13^{abc}	6.61 ± 0.11^{ab}
P40L4	73.45 ± 0.32^{e}	17.50 ± 0.33^{ab}	5.20 ± 0.10^{a}	3.51 ± 0.08^{bcd}	6.20 ± 0.10^{a}
P40L8	71.60 ± 0.31^{bcd}	17.17 ± 0.10^{ab}	7.61 ± 0.16^{cd}	3.11 ± 0.05^{a}	7.07 ± 0.07^{bc}
P40L12	70.09 ± 0.47^{ab}	17.26 ± 0.10^{ab}	8.98 ± 0.49^{d}	3.70 ± 0.03^{cd}	7.64 ± 0.17^{d}
P45L4	72.54 ± 0.32^{cde}	16.42 ± 0.20^{a}	7.00 ± 0.12^{bc}	3.74 ± 0.03^{d}	6.66 ± 0.04^{ab}
P45L8	69.51 ± 0.57^{a}	18.10 ± 0.09^{b}	8.86 ± 0.40^{d}	3.19 ± 0.02^{ab}	7.90 ± 0.18^{d}
P45L12	70.95 ± 0.10^{abc}	17.49 ± 0.09^{ab}	7.98 ± 0.17^{cd}	3.21 ± 0.04^{ab}	7.30 ± 0.09^{cd}
Protein level (%)					
35	72.74 ± 0.18^{w}	16.85 ± 0.17^{u}	6.33 ± 0.21^{u}	3.55 ± 0.08^{v}	6.49 ± 0.08^{u}
40	71.71 ± 0.52^{v}	17.31 ± 0.11^{uv}	7.26 ± 0.57^{v}	3.44 ± 0.09^{uv}	6.97 ± 0.22^{v}
45	71.00 ± 0.48^{u}	17.34 ± 0.25^{v}	7.95 ± 0.30^{w}	3.38 ± 0.09^{u}	7.25 ± 0.17^{w}
Lipid level (%)					
4	73.07 ± 0.20^{y}	16.82 ± 0.21^{x}	6.02 ± 0.26^{x}	3.65 ± 0.04^{y}	6.37 ± 0.08^{x}
8	71.09 ± 0.45^{x}	17.44 ± 0.17^{y}	7.67 ± 0.36^{y}	3.30 ± 0.09^{x}	7.16 ± 0.18^{y}
12	71.29 ± 0.43^{x}	17.24 ± 0.17^{xy}	7.84 ± 0.41^{y}	3.42 ± 0.08^{x}	7.18 ± 0.16^{y}
Two-way ANOVA (*P* value)					
Protein	<0.001	0.029	<0.001	0.043	<0.001
Lipid	<0.001	0.011	<0.001	<0.001	<0.001
Protein × Lipid	<0.001	0.006	<0.001	<0.001	<0.001

*Values in a column with different superscripts significantly differ.

The highest values of whole-body moisture, protein, lipid, ash, and energy contents were observed in fish fed the P40L4, P45L8, P40L12, P45L4 and P45L8 diets, whereas the lowest values were recorded in fish fed the P45L8, P45L4, P40L4, P40L8 and P40L4 diets, respectively. With respect to dietary protein and lipid levels, whole-body moisture and ash contents gradually decreased with increasing dietary protein level, whereas whole-body protein, lipid and energy contents increased with increasing dietary protein level. Fish fed the diets with 4% lipid exhibited higher whole-body moisture and ash contents, but lower whole-body lipid and energy contents when compared with fish fed the diets with 8% and 12% lipid. Whole-body protein content was lower in fish fed the diets with 4% lipid compared to fish fed the diets with 8% lipid.

Discussion

The present study showed that growth rate and feed efficiency of barbless carp were significantly affected by dietary P/E ratio (17.04–23.58 g protein/MJ diet). The best growth performance was observed in fish fed the two diets containing 8% and 12% lipid at 45% protein level. However, fish fed the diet containing 40% protein and 12% lipid had similar growth rate as those fed the above two diets, but showed relatively better PER. Further, fish fed diets with 45% protein showed higher hepatic ALT activity and plasma BUN content compared with fish fed the diets with 35% and 40% protein, suggesting the dietary protein of 45% is an excess level for barbless carp. Thus, a diet containing 40% protein and 12% lipid with P/E ratio of 19.38 g protein/MJ is suitable for barbless carp. This protein requirement is in line with the reported level for this species (37.3–43.6%, Deng *et al.*, 2013).

It is well known that inadequate dietary P/E ratio may result in lower growth rate as well as lower protein and energy retention (Ai *et al.*, 2004). In this study, TGC and energy retention of barbless carp linearly improved with the increase of dietary lipid

content at 40% protein level, whereas those were relatively lower in barbless carp fed the lowest (4%) and highest (12%) contents of dietary lipid compared to medium (8%) content of dietary lipid at 35% or 45% protein level, which may be attributed to the imbalance of dietary P/E ratio. The determined P/E ratio (19.38 g protein/MJ) in this study is within the range reported for some other fish species. The optimal P/E ratio has been reported to be 18.96 g protein/MJ for Nile tilapia *Oreochromis niloticus* (Ali *et al*., 2008), 19.57 g protein/MJ for blunt snout bream *Megalobrama amblycephala* (Li *et al.*, 2010), and 19.22 g protein/MJ for kutum *Rutilus frisii kutum* (Ebrahimi *et al.*, 2013). However, it should be noted that dietary P/E ratio should not be used apart from dietary nutrient content although it has been used as an important criterion for feed formulation (Zhang *et al.*, 2017). In this study, the dietary P/E ratio of P35L4 and P40L12 groups was similar (18.78 versus 19.38 g protein/MJ), but they resulted in obvious differences in growth rate and feed utilization. By contrast, P40L12 and P45L8 diets had apparent differences in the P/E ratio (19.38 versus 22.46 g protein/MJ), but they had comparable growth rate and feed utilization. This phenomenon had also been observed in some other fish species (Lee *et al.*, 2002; Zhang *et al.*, 2017). Thus, dietary P/E ratio must be used together with absolute amounts of dietary protein and lipid levels.

Many studies with fish have revealed a protein-sparing effect of dietary lipid (Li *et al.*, 2010; Shapawi *et al.*, 2014), however it was not observed in this study based on PER, protein and energy retention values. Similarly, the protein-sparing effect of dietary lipid was not observed in previous studies (Jiang *et al.*, 2015). Based on whole-body lipid content results, the surplus lipid was mainly used to enhance fat deposition rather than metabolized for energy to reduce protein catabolism. In some cases, the degree of fat varied according to the size of the fish (Xu *et al.*, 2015)

Previous studies showed that high dietary energy inclusion levels may result in high fat deposition in fish (Millikin, 1983). Moreover, higher levels of dietary lipid usually resulted in some pathological damage in fish (Rueda-Jasso *et al.*, 2004). In this study, higher dietary lipid levels also caused higher lipid accumulation in the fish body. However, it is worth noting that depressed GGT, AST, ALT and ALP activities in plasma of fish fed the diets with 12% lipid compared with fish fed the diets with 4% and 8% lipid, suggests that dietary lipid of 12% did not cause obvious damage to barbless carp, at least during the experimental period. These results indicate that barbless carp may be a species with high-energy requirements and its ability to utilize dietary lipid is high. Thus, it is appropriate to feed a diet containing 40% protein and 12% lipid with P/E ratio of 19.38 g protein/MJ to barbless carp without compromising growth and health.

In conclusion, the results of the present study demonstrated that a diet containing 40% protein and 12% lipid with P/E ratio of 19.38 g protein/MJ is suitable for optimum growth rate, feed utilization, and health of barbless carp.

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (Grant numbers 31460693 and 31760761); Natural Science Foundation of Yunnan Province (2018FA018); and Foundation of Tongwei (TA2016A001). There are no conflicts of interest.

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