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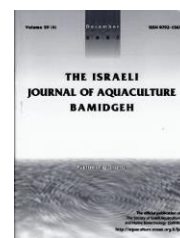


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## Effects of Dietary Protein and Lipid Levels on Growth Performance, Feed Utilization and Biochemical Parameters of Barbless Carp (*Cyprinus pellegrini*)

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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  $\gamma$ -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- $\mu$ m 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<sub>2</sub>-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<sup>3</sup>) 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.













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.

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