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Dietary lipid and gross energy affect protein utilization in the rare minnow *Gobiocypris rarus**

WU Benli (吴本丽)[1, 2], XIONG Xiaoqin (熊小琴)[1, 2], XIE Shouqi (解绶启)[3], WANG Jianwei (王剑伟)[1, **]

[1] *Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institution of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

[2] *University of Chinese Academy of Sciences, Beijing 100049, China*

[3] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China*

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Abstract An 8-week feeding trial was conducted to detect the optimal dietary protein and energy, as well as the effects of protein to energy ratio on growth, for the rare minnow (*Gobiocypris rarus*), which are critical to nutrition standardization for model fish. Twenty-four diets were formulated to contain three gross energy (10, 12.5, 15 kJ/g), four protein (20%, 25%, 30%, 35%), and two lipid levels (3%, 6%). The results showed that optimal dietary E/P was 41.7–50 kJ/g for maximum growth in juvenile rare minnows at 6% dietary crude lipid. At 3% dietary lipid, specific growth rate (SGR) increased markedly when E/P decreased from 62.5 kJ/g to 35.7 kJ/g and gross energy was 12.5 kJ/g, and from 75 kJ/g to 42.9 kJ/g when gross energy was 15.0 kJ/g. The optimal gross energy was estimated at 12.5 kJ/g and excess energy decreased food intake and growth. Dietary lipid exhibited an apparent protein-sparing effect. Optimal protein decreased from 35% to 25%–30% with an increase in dietary lipid from 3% to 6% without adversely effecting growth. Dietary lipid level affects the optimal dietary E/P ratio. In conclusion, recommended dietary protein and energy for rare minnow are 20%–35% and 10–12.5 kJ/g, respectively.

Keyword: rare minnow; *Gobiocypris rarus*; protein to energy ratio (E/P); crude lipid; growth

1 INTRODUCTION

Dietary protein content is a key nutritional factor affecting the growth performance of fish (Jauncey, 1982; Al Hafedh et al., 1999). However, any consideration of nutritional requirements in fish must take into account the balance of a range of nutrients, which can interact with significant outcomes (Smith, 1989; De Silva et al., 1991). Therefore, in addition to protein, it is also important to determine the dietary content of carbohydrates and lipids, which are used as non-protein energy sources. Imbalance in dietary nutrients may decrease growth, nutrient utilization, and body lipid deposition (Garling Jr and Wilson, 1976). Both under- and over-nutrition could have adverse effects on fish growth and physiology (Kaushik, 1995). The optimum dietary protein content for good performance depends on the energy content of food (Cowey, 1979; Salhi et al., 2004). An optimal dietary protein to energy ratio (E/P) is important because any excess or deficiency of non-protein energy results in lower protein and energy utilization and may also depress fish growth performance (Shiau and Peng, 1993; Lupatsch et al., 1998; Ali and Jauncey, 2005; Tibbetts et al., 2005). The E/P ratio has been studied for several species such as channel catfish (Garling and Wilson, 1976), Nile tilapia (El-Sayed and Teshima, 1992), Asian seabass (Catacutan and Coloso, 1995), and grouper (Shiau and Lan, 1996). Dietary lipid is a good energy source for fish

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** Corresponding author: wangjw@ihb.ac.cn

and exhibits a protein-sparing effect (Reinitz et al., 1978; Kim and Lee, 2005).

The rare minnow (*Gobiocypris rarus*) is a small cyprinid fish endemic to Sichuan Province, China. It has been cultured for use as a potential model fish under laboratory conditions since 1990 (Cao and Wang, 2003). However, little research relating to its nutrition has been conducted to date. Previous experiments have shown that at the specific energy level of 17.0 kJ/g, the optimal protein and lipid requirements are 32.6% and 7.07%, respectively (Wu et al., unpublished data). An energy level of 17.0 kJ/g in that trial was probably and overestimation because of relatively low feed consumption. Lower consumption of high-energy food may lead to a reduction in growth resulting from a deficiency of necessary nutrients (Lovell, 1979; Daniels and Robinson, 1986). The present study was conducted to detect the optimal protein and energy levels, as well as the effects of E/P on growth for rare minnows, to provide a nutrition standard for the small model fish.

2 MATERIAL AND METHOD

2.1 Fish rearing

All rare minnows were from a closed colony (IHB, Institute of Hydrobiology, CAS) and kept in a 1 000-L quadrate resinous tank before grouping. The larvae were fed with *Artemia* nauplii, which were gradually replaced by red worms (Chironomus larva) for 45 days. Healthy individuals of similar size were allocated to several polycarbonate tanks (40 cm×20 cm×25 cm). Water was recycled at 0.6–1.0 L/min for each tank, and 1/3 of the tank's volume was replaced with freshwater daily. Thirty-five individuals were stocked per tank, which were covered by nets to prevent the fish from jumping out. The fish were acclimated with mixed experimental diets at least 7 days before being moved to small test tanks. The fish were fed with designed feeds for 15 min to apparent satiation at 10:00 and 16:00 daily. Three tanks were used for each diet and the amount fed was recorded daily. Uneaten food was siphoned and dried to calculate food consumption. During the 8-week feeding trial, light period was artificially controlled at 14 h from 08:00 to 22:00 (80–100 lx). The water temperature, pH, and dissolved oxygen were monitored daily. The temperature was 25.0−26.0°C, pH was 7.8−8.5, and dissolved oxygen was 7.5−8.5 mg/L (HQ30d, Hach, Loveland, Co., USA). The NH_4-N, NO_2-N, and hardness were determined weekly (APHA, 1992).

2.2 Diet preparation

For nutrient sources, casein and gelatin were used for protein (4:1), fish and soybean oils for lipids (1:1), and dextrin for carbohydrates. All raw materials were crushed, passed through a sieve (60 mesh, 250 μm) and manually mixed. Water (30%) was added to the homogeneous compounds and the mix was placed into a household noodle maker (LM-20, Limai, China) with a perforated metal plate (0.5 mm) to produce pellets that could be consumed by small fish. All diets were stored at -4°C before use. Twenty-four diets were designed based on preliminary experiments, which determined the suitable ranges of protein and energy. Crude lipid (CL) was fixed at 3% and 6%, crude protein (CP) was 20%–35%, and the gross energy (GE) levels were regulated by carbohydrates at 10.0 kJ/g, 12.5 kJ/g, and 15.0 kJ/g (Table 1).

2.3 Sampling and chemical analysis

The fish were anesthetized by MS-222 (100×10^{-6}) and measured after 24 h of fasting for each tank. The initial and final fish body weight and length were measured individually. Six individuals from each tank were dissected to obtain the hepatosomatic index (HSI), visceral-somatic index (VSI), and length of intestinal tract to body length index (DSI). Twenty individuals were collected after measuring for final body composition analysis (AOAC, 2005). Dry matter and ash in diets and fish carcasses were determined gravimetrically after drying for 10 h at 105°C in an oven and after combustion for 24 h at 550°C in a muffle furnace. Crude protein (N×6.25) was determined according to the Kjeldahl method (Kjeltec Auto Analyzer 2300, Foss, Eden Prairie, MN, USA). Crude lipid was determined gravimetrically in the samples following ether extraction (Soxtec system HT 1043, Tecator, Extraction Unit, Hoganas, Sweden). The energy value was acquired by a Phillipson microbomb calorimeter (Gentry Instruments Inc., Aiken, SC, USA).

2.4 Statistical analysis

All statistical analysis was carried out in SPSS Version 19.0 (SPSS, Chicago, IL, USA). The measured data were subjected to one, two, and three-way analysis of variance (ANOVA). Significant differences among treatments were tested using Tukey's multiple range tests and results of $P<0.05$ were deemed statistically significant. Duncan's multiple comparison was carried out to determine the differences among groups.

Table 1 Formulation and proximate chemical composition of experimental diets

	Ingredients (%, dry wt.)							Chemical composition			
Diets	Casein	Gelatin	Fish oil	Soybean oil	Dextrin	Cellulose	Others*	CP%	CL%	GE	E/P (kJ/g)
D(20, 6, 10)	18.0	4.5	2.8	2.8	18.0	45.4	8.6	20	6	10	50.0
D(20, 3, 10)	18.0	4.5	1.3	1.3	23.0	43.3	8.6	20	3	10	50.0
D(25, 6, 10)	22.8	5.7	2.7	2.7	10.0	47.5	8.6	25	6	10	40.0
D(25, 3, 10)	22.8	5.7	1.2	1.2	15.0	45.5	8.6	25	3	10	40.0
D(30, 6, 10)	27.6	6.9	2.6	2.6	3.0	48.7	8.6	30	6	10	33.3
D(30, 3, 10)	27.6	6.9	1.1	1.1	6.0	48.7	8.6	30	3	10	33.3
D(35, 6, 10)	32.0	8.0	2.6	2.6	0.0	46.2	8.6	35	6	10	28.6
D(35, 3, 10)	32.0	8.0	1.1	1.1	0.0	49.2	8.6	35	3	10	28.6
D(20, 6, 12.5)	18.4	4.6	2.8	2.8	32.0	30.8	8.6	20	6	12.5	62.5
D(20, 3, 12.5)	18.4	4.6	1.3	1.3	36.0	29.8	8.6	20	3	12.5	62.5
D(25, 6, 12.5)	22.8	5.7	2.7	2.7	25.0	32.5	8.6	25	6	12.5	50.0
D(25, 3, 12.5)	22.8	5.7	1.2	1.2	29.0	31.5	8.6	25	3	12.5	50.0
D(30, 6, 12.5)	27.6	6.9	2.7	2.7	16.0	35.5	8.6	30	6	12.5	41.7
D(30, 3, 12.5)	27.6	6.9	1.2	1.2	20.0	34.5	8.6	30	3	12.5	41.7
D(35, 6, 12.5)	32.0	8.0	2.6	2.6	9.0	37.2	8.6	35	6	12.5	35.7
D(35, 3, 12.5)	32.0	8.0	1.1	1.1	13.0	36.2	8.6	35	3	12.5	35.7
D(20, 6, 15)	18.0	4.5	2.8	2.8	47.0	16.3	8.6	20	6	15	75.0
D(20, 3, 15)	18.0	4.5	1.3	1.3	51.0	15.3	8.6	20	3	15	75.0
D(25, 6, 15)	22.8	5.7	2.7	2.7	39.0	18.5	8.6	25	6	15	60.0
D(25, 3, 15)	22.8	5.7	1.2	1.2	43.0	17.5	8.6	25	3	15	60.0
D(30, 6, 15)	27.6	6.9	2.6	2.6	31.0	20.7	8.6	30	6	15	50.0
D(30, 3, 15)	27.6	6.9	1.2	1.2	35.0	19.5	8.6	30	3	15	50.0
D(35, 6, 15)	32.0	8.0	2.6	2.6	23.0	23.2	8.6	35	6	15	42.9
D(35, 3, 15)	32.0	8.0	1.1	1.1	27.0	22.2	8.6	35	3	15	42.9

"Others*" contained vitamin premix (mg/kg diet): thiamin 20, riboflavin 20, pyridoxine 20, cyanocobalamin 0.02, pantothenic acid 50, folic acid 5, inositol 100, niacin 100, biotin 0.1, ascorbic 100, vitamin A 110, vitamin D 20, vitamin E 50, vitamin K 10, starch 645.2; mineral premix (mg/kg diet): NaCl 500, $MgSO_4 \cdot 7H_2O$ 8 155.6, $NaH_2PO_4 \cdot 2H_2O$ 12 500, KH_2PO_4 16 000, $Ca(H_2PO_4)_2 \cdot 2H_2O$ 7 650.6, $FeSO_4$ 1 250, $C_6H_{10}CaO_6 \cdot 5H_2O$ 1 750, $ZnSO_4 \cdot 7H_2O$ 178, $MnSO_4 \cdot 4H_2O$ 61.4, $CuSO_4 \cdot 5H_2O$ 15.5, $CoSO_4 \cdot 6H_2O$ 34.5, KI 114.8, starch 753.7; choline chloride (1.1%); spirulina (1%); dimethylpropiothetin (DMPT , 0.1%) and carboxyl methyl cellulose (CMC, 1%).

3 RESULT

3.1 Growth performance

There was no mortality during the trial in all treatments. Final body weights were significantly different in fish fed with diets varying in crude protein (CP), crude lipid (CL), and gross energy (GE; $P<0.01$; Tables 2 and 3). When fed diets with 6% lipid, fish showed relatively better growth at D(20, 6, 10), D(25, 6, 12.5), and D(30, 6, 12.5) and the corresponding E/P was 50 kJ/g, 50 kJ/g, and 41.7 kJ/g. Minimum body weight gain occurred at D(35, 6, and 10), at which the E/P was 28.6 kJ/g. When lipid was reduced to 3%, fish fed on D(35, 3, 12.5) achieved a final body weight of 0.348 g and an SGR of 2.95%/d, with an E/P of 35.7 kJ/g; whereas fish fed on D(20, 3, 15) exhibited minimum growth with an E/P of 75 kJ/g. Generally, feed with a gross energy of 12.5 kJ/g was sufficient for rearing rare minnows and surplus dietary energy could improve growth and decrease food intake. Crude protein levels of 20%–35% in diets could meet the requirement for juvenile growth in rare minnows when the energy or lipid levels are appropriate. CF, VSI, HSI, and DSI were higher in higher body weight groups. FCR fluctuated among groups and was higher in 3% lipid diets compared with 6% lipid diets at identical protein and energy levels. Additionally, some individuals, especially those fed with D(30, 6, 12.5), reached sexual maturity during the experimental period and both male and female gonads were well-developed.

Table 2 Growth performance of fish fed with varying crude protein (CP), gross energy (GE), and crude lipid (mean±S.E.)

GE (kJ/g)	CP (%)	IBW (g/fish)	28 d BW(g/fish)	FBW (g/fish)	FCR	PRE (%)	CF (g/cm^3)	VSI (%)	HSI (%)	DSI (%)
10	20	0.069	0.181±0.005^{d}	0.291±0.003^{d}	3.8±0.22^{c}	18.88±2.01^{d}	1.77±0.002^{c}	18.85±3.25^{d}	3.5±0.45^{c}	65±1.2^{b}
	25	0.069	0.134±0.004^{a}	0.202±0.004^{bc}	3.0±0.23^{b}	19.13±1.98^{d}	1.67±0.003^{ab}	15.63±2.11^{b}	2.5±0.28^{b}	64±0.9^{ab}
	30	0.068	0.148±0.003^{b}	0.206±0.005^{bc}	3.2±0.35^{b}	14.95±1.67^{ab}	1.69±0.002^{ab}	15.86±0.88^{b}	2.4±0.22^{b}	62±1.8^{a}
	35	0.068	0.127±0.005^{a}	0.152±0.006^{a}	3.1±0.31^{b}	13.23±1.55^{a}	1.62±0.001^{a}	14.87±2.01^{ab}	1.9±0.20^{a}	65±0.8^{b}
12.5	20	0.068	0.147±0.003^{b}	0.213±0.004^{c}	3.3±0.21^{b}	21.74±2.04^{e}	1.70±0.003^{b}	16.32±0.96^{c}	2.2±0.30^{ab}	67±1.3^{c}
	25	0.070	0.175±0.004^{d}	0.318±0.004^{d}	2.6±0.33^{a}	22.08±2.48^{e}	1.75±0.003^{bc}	19.23±3.06^{e}	3.3±0.23^{c}	66±1.0^{b}
	30	0.067	0.166±0.003^{c}	0.293±0.004^{e}	2.4±0.30^{a}	19.93±2.22^{de}	1.74±0.002^{b}	18.78±2.00^{d}	3.3±0.26^{c}	65±1.8^{b}
	35	0.067	0.155±0.003^{bc}	0.187±0.004^{b}	2.4±0.24^{a}	17.08±2.19^{bc}	1.67±0.001^{ab}	15.00±1.11^{ab}	1.8±1.98^{a}	62±1.4^{a}
15	20	0.065	0.146±0.003^{b}	0.184±0.003^{b}	3.7±0.19^{c}	19.39±2.41^{d}	1.78±0.002^{c}	14.19±1.18^{a}	2.0±0.32$^{.0}$	63±0.9^{a}
	25	0.063	0.139±0.004^{ab}	0.193±0.003^{bc}	3.2±0.33^{b}	17.94±2.07^{c}	1.71±0.002^{b}	14.88±1.77^{ab}	2.1±0.10^{ab}	63±2.0^{a}
	30	0.068	0.148±0.003^{b}	0.211±0.003^{c}	3.0±0.18^{b}	15.94±1.79^{b}	1.72±0.003^{b}	16.05±2.43^{bc}	2.6±0.11^{b}	63±2.0^{a}
	35	0.067	0.150±0.004^{cd}	0.188±0.003^{b}	2.7±0.22^{ab}	15.19±1.99^{ab}	1.74±0.003^{b}	14.26±2.10^{a}	2.5±0.18^{b}	64±1.0^{ab}
						Two-way ANOVA (CL=6%)				
	GE		*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.4	*P*<0.09	*P*<0.001
	CP		*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.4	*P*<0.001	*P*<0.008
	GE×CP		*P*<0.001	*P*<0.001	*P*<0.03	*P*<0.001	*P*<0.001	*P*<0.4	*P*<0.001	*P*<0.001
10	20	0.067	0.156±0.004^{cd}	0.249±0.004^{c}	4.2±0.30^{c}	17.08±2.00^{c}	1.74±0.002^{b}	17.63±2.04^{d}	2.8±0.32^{b}	65±2.0^{b}
	25	0.066	0.145±0.005^{c}	0.207±0.003^{ab}	3.6±0.26^{bc}	15.94±2.13^{b}	1.70±0.002^{ab}	15.88±1.57^{b}	2.6±0.33^{b}	63±1.3^{c}
	30	0.069	0.153±0.004^{c}	0.224±0.004^{b}	3.7±0.30^{bc}	12.93±1.97^{a}	1.72±0.003^{b}	16.66±1.23^{c}	2.4±0.28^{ab}	62±1.6^{a}
	35	0.067	0.132±0.006^{b}	0.211±0.004^{b}	3.2±0.28^{b}	12.81±1.89^{a}	1.68±0.002^{a}	15.89±2.33^{b}	2.3±0.26^{ab}	64±1.1^{ab}
12.5	20	0.067	0.121±0.001^{ab}	0.198±0.005^{ab}	3.4±0.25^{b}	21.10±2.19^{d}	1.68±0.003^{a}	14.34±1.52^{a}	2.0±0.19^{a}	64±1.1^{ab}
	25	0.067	0.131±0.003^{b}	0.202±0.003^{ab}	2.6±0.21^{a}	22.08±2.33^{d}	1.68±0.002^{a}	15.15±1.24^{ab}	2.7±0.17^{b}	64±2.1^{ab}
	30	0.069	0.157±0.005^{cd}	0.245±0.005^{c}	2.8±0.27^{ab}	17.08±2.10^{c}	1.74±0.003^{b}	16.90±2.13^{c}	2.6±0.32^{b}	64±1.9^{ab}
	35	0.066	0.178±0.004^{d}	0.348±0.002^{d}	2.5±0.26^{a}	16.40±1.86^{bc}	1.79±0.003^{c}	19.56±2.32^{e}	3.9±0.41^{d}	66±1.6^{b}
15	20	0.067	0.113±0.003^{a}	0.186±0.002^{a}	3.4±0.31^{b}	21.10±2.30^{d}	1.65±0.002^{a}	14.46±1.24^{a}	1.9±0.15^{a}	62±2.4^{a}
	25	0.068	0.122±0.002^{ab}	0.197±0.003^{ab}	3.2±0.29^{b}	17.94±2.23^{c}	1.67±0.003^{a}	14.67±2.15^{a}	2.3±0.08^{ab}	62±1.7^{a}
	30	0.065	0.136±0.003^{b}	0.256±0.004^{c}	2.9±0.20^{ab}	16.49±2.11^{bc}	1.75±0.002^{b}	16.66±1.58^{c}	3.0±0.17^{c}	65±1.8^{b}
	35	0.066	0.160±0.005^{cd}	0.244±0.003^{c}	2.6±0.27^{a}	15.77±1.98^{b}	1.75±0.003^{b}	16.25±2.04^{bc}	2.6±0.24^{b}	65±2.1^{b}
						Two-way ANOVA (CL=3%)				
	GE		*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.6	*P*<0.001	*P*<0.03	*P*<0.004
	CP		*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.03	*P*<0.001	*P*<0.001	*P*<0.001
	GE×CP		*P*<0.001	*P*<0.001	*P*<0.03	*P*<0.001	*P*<0.04	*P*<0.001	*P*<0.001	*P*<0.001

Different superscript letters in the same row indicate significant differences among treatments ($P<0.05$). IBW: initial body weight; 28 d BW: body weight at 28th day; FBW: final body weight; FCR (food conversion ratio)=total dry food intake/weight gain; PRE (protein retention efficiency, %)=100(final–initial body protein)/protein consumed]; CF (condition factor)=100(body weight/body length3); VSI (visceralsomatic index,%)=[100(weight of viscera index/body weight)]; HSI (hepatosomatic index,%)=[100(weight of liver/body weight)]; DSI (digestive tract index)=[100(length of gut/body length)].

Table 3 Three-way analysis of variance results for growth performance of rare minnows fed with varying crude protein (CP), gross energy (GE), and crude lipid (CL) (mean±S.E.)

	28 d BW	FBW	FCR	PRE	CF	VSI	HSI	DSI
CP	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.09	*P*<0.5	*P*<0.02	*P*<0.03
GE	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.07	*P*<0.3	*P*<0.005	*P*<0.001
CL	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.9	*P*<0.4	*P*<0.4	*P*<0.2
CP×GE	*P*<0.001	*P*<0.001	*P*<0.03	*P*<0.001	*P*<0.001	*P*<0.4	*P*<0.001	*P*<0.001
CP×CL	*P*<0.001	*P*<0.001	*P*<0.1	*P*<0.001	*P*<0.001	*P*<0.4	*P*<0.001	*P*<0.001
GE×CL	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.001	*P*<0.04	*P*<0.4	*P*<0.5	*P*<0.3
CP×GE×CL	*P*<0.001	*P*<0.001	*P*<0.1	*P*<0.001	*P*<0.05	*P*<0.4	*P*<0.001	*P*<0.001

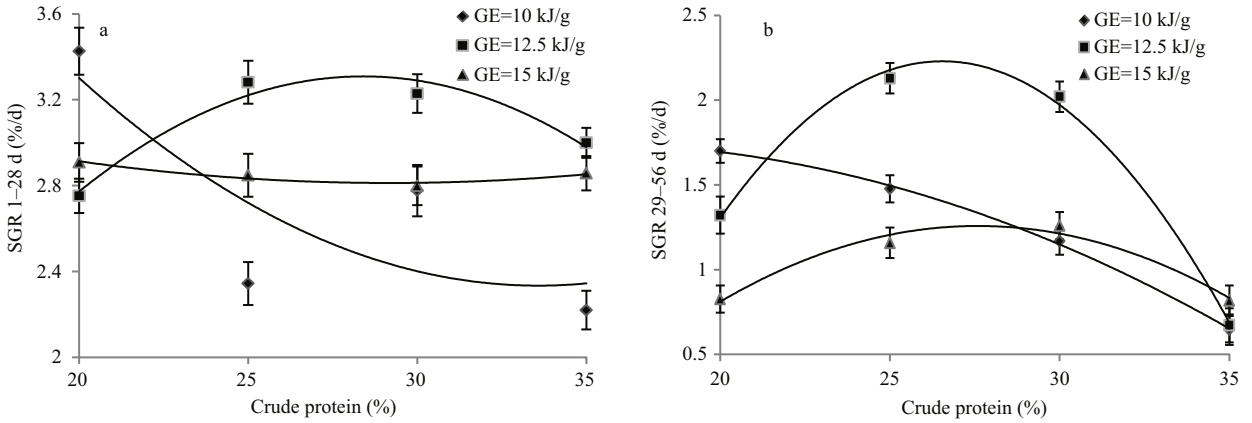

Fig.1 Specific growth rates (SGR 1–28 d=100(ln28 d BW-lnIBW)/28; SGR 29–56 d=100(lnFBW–ln28 d BW)/28) of fish during the first (a) and second (b) 4 weeks of the trial when fed diets with 6% crude lipid and varying protein and gross energy

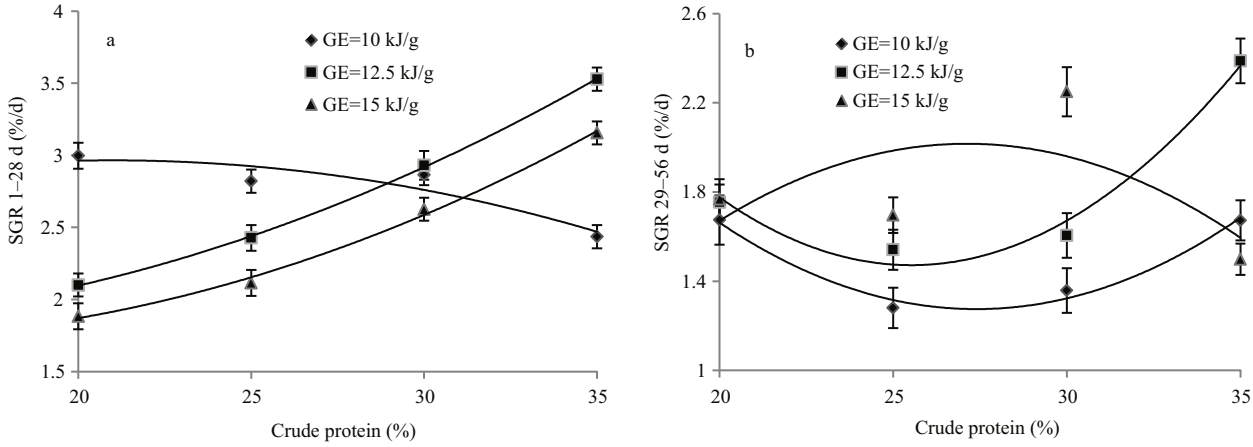

Fig.2 Specific growth rates (SGRs) of fish during the first (a) and second (b) 4 weeks of the trial when fed diets with 3% crude lipid and varying protein and gross energy

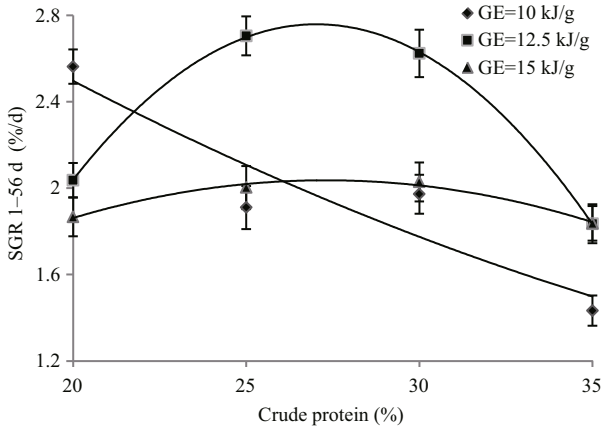

Fig.3 Specific growth rates (SGR 1–56 d=100(lnFBW–lnIBW)/56) during the entire feeding trial in fish fed diets with 6% crude lipid and varying protein and gross energy

Growth rates were divergent in different periods of the trial. Out of the groups fed 6% lipid diets, the maximum body weight was obtained in fish fed D(20, 6, 10) and SGR decreased rapidly with increasing protein at a low gross energy of 10 kJ/g in the first 4 weeks. There was no significant difference in SGRs when the energy was 15.0 kJ/g (P<0.05; Fig.1a). However, in the latter 4 weeks of the trial, groups fed D(25, 6, 12.5) and D(30, 6, 12.5) diets grew faster than others (Fig.1b). There were some differences when crude lipid was 3%. In the first 4 weeks, SGR increased with increasing protein when gross energy was either 12.5 kJ/g or 15.0 kJ/g, but decreased moderately with increasing protein at the low energy level of 10 kJ/g (Fig.2a). For the latter 4 weeks, the D(35, 3, 12.5) diet was obviously good for growth (Fig.2b).

3.2 Suitable E/P

Figure 3 shows that, at 6% dietary lipid, SGR reached its maximum and then decreased with increased dietary protein at 12.5 kJ/g and 15 kJ/g gross energy, but decreased with increased dietary

protein at 10 kJ/g gross energy. The groups fed diets with 12.5 kJ/g gross energy grew relatively well compared with those on lower (10.0 kJ/g) or higher (15.0 kJ/g) gross energy. The optimal content of crude protein was 27.0% based on the regression relationship. When gross energy was 10 kJ/g, SGR decreased with a decrease in E/P from 50.0 kJ/g to 28.6 kJ/g, and relative optimal crude protein was 20%. There was no significant difference in SGR for different protein contents (20%−35%) or E/P (75−42.9 kJ/g) when gross energy increased to 15 kJ/g, but the relative optimal concentration of crude protein was 30%, especially in the latter period of the trial. According to the SGR in various groups, we speculated that the optimal E/P for juvenile rare minnows was 41.7−50 kJ/g when energy was 10−15 kJ/g and crude lipid 6%. The level of protein should increase when gross energy increases, but by no more than 30% for this particular species.

There were some differences when crude lipid was 3%. As Fig.4 shows, SGR increased with increased dietary protein at 12.5 kJ/g and 15 kJ/g gross energy, but the SGR did not increase with increased dietary protein at 10 kJ/g gross energy. There was no significant difference in SGR at a low gross energy of 10 kJ/g. Maximum SGR also occurred at 12.5 kJ/g, but the protein requirement increased even more to 35%. SGR increased markedly with the decrease in E/P from 62.5 kJ/g to 35.7 kJ/g at a gross energy of 12.5 kJ/g; and from 75 kJ/g to 42.9 kJ/g at a gross energy of 15.0 kJ/g.

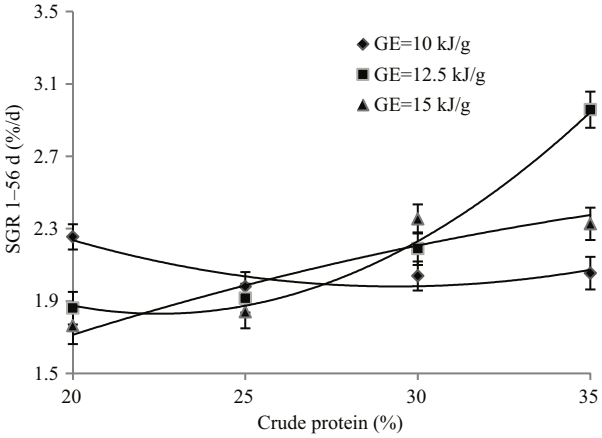

Fig.4 Specific growth rates (SGRs) during the entire feeding trial in fish fed diets with 3% crude lipid and varying protein and gross energy

4 DISCUSSION

4.1 Suitable E/P for growth

The effect of E/P on rare minnow growth was obvious, as the growth rate decreased with increased dietary protein at low gross energy of 10 kJ/g. Fish tend to consume more food at low gross energy. This response brings about an increased protein intake, which would lead to intensive specific dynamic action (SDA) because of complex catabolic and synthesis activities. That increased dietary protein leads to increases in SDA has been reported in many fish species (Medland and Beamish, 1985; Peres and Oliva-Teles, 1999; Fu et al., 2005). Other studies have shown that dietary protein affects SDA more than lipids and carbohydrates (Jobling and Davies, 1980; Tandler and Beamish, 1981; Zanotto et al., 1997). Some research has indicated a positive relationship between SDA and growth, showing that intensive SDA stimulates fast growth (Jobling, 1983; Brown and Cameron, 1991; Chakraborty et al., 1995; Carter and Hauler, 2000). In the present study, growth decreased with increased dietary protein. This result supports the idea that there is a competitive relationship between SDA and growth, which has been reported in metabolic studies for species like catfish and rainbow trout (Legrow and Beamish, 1986; Cui and Liu, 1990; Ai and Xie, 2006). However, increased dietary protein did not seem to affect SGR at the high gross energy of 15.0 kJ/g. E/P plays an important role in improving the utilization of both protein and energy as well as enhancing feed efficiency (Winfree and Stickney, 1981; Lee et al., 2002; Mathis et al., 2003; Wang et al., 2006). Excess protein may be used for energy rather than for growth, whereas the dietary lipid or carbohydrate did not provide energy. Excessive energy could restrict food intake, which would subsequently reduce protein consumption if dietary protein was low (NRC, 1993). Long-term dietary consumption of foods high in calories, protein, and fat could lead to decreases in growth hormones (Yang et al., 1987). In this study, excessive energy also brought about lipid deposition, especially in viscera. Fish usually respond to being fed low-energy diets by increasing feed consumption, apparently to maintain nutrient and energy intake (Boujard and Médale, 1994). For rare minnows, suitable E/Ps are 41.7−50 kJ/g at 6% lipid and 35.7 kJ/g at 3% lipid. In the present study a diet of 20%−35% protein and 10−12.5 kJ/g achieved a reasonable E/P for juvenile rare minnows.

4.2 Effect of lipid on E/P

Growth increased when dietary protein increased

from 20% to 35% at 3% lipid and a higher gross energy of 12.5 kJ/g and 15 kJ/g, but was restricted at a low gross energy of 10 kJ/g (Fig.2). Optimal dietary protein was 27% at 6% lipid, and a low-lipid diet of 3% could potentially be used in daily feeding if paired with high protein levels of 35% or more. Both protein and gross energy levels were lower than other carnivorous fishes such as groupers, which suggested that when the energy and protein requirements were 14.3−15.8 kJ/g and 44%−50%, respectively, the optimal E/P was 32.5−35.8 kJ/g (Shiau and Lan, 1996). For black carp, the optimal protein level is 35%−40%, energy is 13.4−15.3 kJ/g, and E/P is 38.0 kJ/g (Dai et al., 1988). Optimal E/P fluctuated with variations in nutrition levels. In the present study, increasing lipid also increased optimal E/P, which suggests that increasing lipids from 3% to 6% could significantly decrease the requirement of dietary protein without adverse effects on growth for rare minnows. Dietary lipids can provide energy while decreasing dietary protein requirements (Reinitz et al., 1978; Shiau and Huang, 1990). A protein-sparing effect has been reported in several fish species, and so an appropriate increase of either lipids or carbohydrates would improve the efficiency of protein utilization (Wilson, 1989; Cho and Kaushik, 1990; Lee et al., 2002; Kim and Lee, 2005). Certainly, nutrition content should meet the requirement for amino and fatty acids. The excessive increase of lipids did not enhance growth, and even caused adverse effects, especially in the latter half of the trial period. Overabundance of lipids food perishability and adversely affects fish (Company, 1999). The fish were fed to apparent satiation in this study, which implies that fish had eaten to satisfy their energy requirements (Lee and Putnam, 1973; Lee et al., 2002). Rare minnows could also adjust food intake to satisfy nutritional requirements. The fish grew well on D(20, 6.10), and tended to eat more when diets were low in protein and energy. Lower protein in diets leads to higher protein utilization efficiency (Samantaray and Mohanty, 1997). Optimal energy was estimated to be 12.5 kJ/g in the present study and high energy had negative effects on growth for *G. rarus*, not only because of nutrient imbalance but also because of the reduction in appetite and lower nutrient intake (Page and Andrews, 1973; Bromley, 1980; Raven et al., 2006).

In conclusion, this study reveals the effect of E/P on growth. Diets with 10−12.5 kJ/g gross energy and 20%−35% protein could represent optimal E/P for maximum growth; however, optimal E/P is affected by dietary lipid. There is an apparent protein-sparing effect by increasing lipid in diets with suitable energy levels, and the utilization of protein is more efficient in diets with 6% lipid than those with 3% lipid. Dietary gross energy and lipid content could affect the use of protein in rare minnows. The requirements for dietary protein, lipid, and gross energy were also affected by environmental factors such as temperature, dissolved oxygen, and feeding regime. The nutrition standardization for this model fish demands artificial diets with balanced nutrients and suitable feeding strategies.

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