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The suitable dietary protein and lipid levels for juvenile hybrid sunfish (Lepomis cyanellus ♀× Lepomis macrochirus ♂)

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The suitable dietary protein and lipid levels for juvenile hybrid sunfish (*Lepomis cyanellus* ♀× *Lepomis macrochirus* ♂)

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Running head: Dietary protein and lipid requirements for hybrid sunfish

Abstract

An eight-week feeding trial was conducted to examine the suitable dietary protein and lipid levels for juvenile hybrid sunfish (*Lepomis cyanellus* ♀× *Lepomis macrochirus* ♂). A 5×2 factorial layout containing five protein levels (300, 340, 380, 420 and 460 g kg^{-1}) and two lipid levels (30 and 80 g kg^{-1}) were designed. Fish (initial body weight 1.21 ± 0.06 g) were fed the test diets twice daily. The final body weight (FBW) and percent weight gain (PWG) in fish increased with dietary protein level from 300 to 420 g kg^{-1}, and afterward decreased with dietary protein level from 420 to 460 g kg^{-1}. Feed intake and feed conversion ratio (FCR) significantly declined with increase dietary protein level. Fish fed at 380 and 420 g kg^{-1} dietary protein level exhibited highest nitrogen retention efficiency (NRE) and phosphorus retention efficiency (PRE), however, the lowest nitrogen waste and phosphorus waste. For different dietary lipid levels, the higher FBW, PWG, NRE and PRE, but the lower feed intake, FCR, nitrogen and phosphorus waste, were found in fish fed diet fish fed at 80 g kg^{-1} dietary lipid level than those in fish fed at 30 g kg^{-1} dietary lipid level. At the end of feeding trail, the contents of body moisture and lipid significantly influenced by dietary lipid level. According to these results, a diet containing 420 g kg^{-1} protein and 80 g kg^{-1} lipid is recommended for efficient growth of juvenile hybrid sunfish.

KEY WORDS: dietary lipid level, dietary protein level, hybrid sunfish, growth, feed utilization, waste output

1 INTRODUCTION

Protein and lipid in feed are essential nutrients for the normal growth and metabolism of fish. Dietary proteins provide amino acids for protein synthesis in fish (Glencross, Smith, Curnow, Smith, & Williams, 2001), and dietary lipids act as a source of energy fuel and essential fatty acids (Alam, Watanabe, Carroll, & Rezek, 2009). The ratio of protein to energy is one of the most important factors affecting feed utilization efficiency in fish (Rahimnejad, Bang, Park, Sade, Choi, & Lee, 2015), and protein-sparing action by elevating dietary lipid level has been reported in some fish species such as common dentex *Dentex dentex* (Skalli, Hidalgo, Abellán, Arizcun, & Cardenete, 2004), blunt snout bream *Megalobrama amblycephala* (Li, Liu, Jiang, Zhu, & Ge, 2010) and largemouth bass *Micropterus salmoides* (Huang, Wu, Lin, Chen, Karrow, Ren, & Wang, 2017). Generally, feeding at optimal dietary protein and lipid levels makes fish grow fast. Superfluous dietary protein increases feed cost and nitrogen waste output (Huang et al., 2017), and excess dietary lipid causes undesirable symptoms in fish such as fatty liver and poor fillet quality (Chatzifotis, Panagiotidou, Papaioannou, Pavlidis, Nengas, & Mylonas, 2010). Therefore, determination of dietary requirements for protein and lipid in fish helps in formulating cost-effective feed with high quality (Huang et al., 2017).

Hybrid sunfish is a freshwater omnivore fish species that generated by hybridization of female green sunfish *Lepomis cyanellus* and male bluegill *Lepomis macrochirus* (Twibell, Wilson, Sanders, & Brown, 2003). Hybrid sunfish possesses some desirable characteristics like high flesh quality and good flavor, acceptance of prepared diets (Lane & Morris, 2002), and capacity for rapid growth under a wide range of water temperature (Wang, Hayward, & Noltie, 2000), which make it as a good candidate for commercial rearing. Although some studies tried to evaluate the experimental and practice diets (Twibell et al., 2003) and effects of dietary protein levels on growth, feed conversion and nitrogen utilization of juvenile hybrid sunfish (Tidwell, Webster, & Clark, 1992), the optimum dietary protein and lipid levels for this species is still unknown. In the present study, we examined the effects of different dietary protein and lipid levels on feed intake, growth, feed utilization, body components and wastes output of hybrid sunfish. Our objective was to determine the suitable dietary protein and lipid levels for hybrid sunfish farming.

2 MATERIALS AND METHODS

2.1 Feed ingredients and experimental diets

Red fish meal (steam dried, manufactured in New Zealand), poultry by-product meal (pet-feed grade, manufactured in the USA), menhaden fish oil (manufactured in the USA), soybean meal, rapeseed meal, wheat flour, wheat bran and wheat gluten meal were supplied by Kesheng Feed Company (Shaoxing, China). All the feed ingredients were ground to pass through a 0.425 mm mesh before assay, and the proximate composition of the feed ingredients is presented in Table 1.

Table 1

A 5×2 factorial layout containing five protein levels (300, 340, 380, 420 and 460 g kg^{-1}) and two lipid levels (30 and 80 g kg^{-1}) were designed, which correspondingly generated ten test diets. The diets contained low lipid level (30 g kg^{-1}) and gradient protein levels (300, 340, 380, 420 and 460 g kg^{-1}) were abbreviated as L30, L34, L38, L42 and L46, respectively, and the other five diets contained high lipid level (80 g kg^{-1}) and gradient lipid levels were abbreviated as H30, H34, H38, H42 and H46, respectively. Red fish meal and soybean meal were used as the major protein source, and menhaden fish oil was used as the major lipid source.

The feed ingredients were weighed in accordance with the designed feed formula and mixed by hand gradually. After being mixed, blend of feed ingredients were further mixed with quantitative water (4:1 w/v) for 15 min. Pellets (0.5mm in diameter, 1.0 mm in length) were extruded using a single screw laboratory-scale extruder. The test diets were dried at 25 °C in an air-conditioned room, and were grinded into smaller pellets, and then stored in plastic bags at -20 °C until use. Formulation, proximate composition and gross energy of the test diets are presented in Table 2.

Table 2

2.2 Fish and feeding trial

The feeding trial was conducted in Hangzhou Academy of Agricultural Sciences (Hangzhou, China). Healthy hybrid sunfish juveniles hatched by Hangzhou Academy of Agricultural Sciences were acclimated in two tanks (3 m in diameter, 1.5 m in height) for two month. During the acclimation, the fish were fed with a commercial feed twice daily.

Prior to the feeding trial, the acclimatized fish were deprived of feed for 24 h, thirty groups each of 30 fish were group-weighed and randomly distributed into 30 fiberglass tanks (100 L), with each test diet three replicates. The initial body weight of fish is 1.21 ± 0.06 g (mean ± SD, n = 30). Three groups each of 50 fish were sampled for the analysis of initial body composition, and the sampled fish were stored at -20 °C until analysis.

The feeding trial lasted eight weeks, during which fish were fed to apparent satiation with the test diets at 08:30 h and 16:30 h daily. Water in the experimental tanks was continually aerated and recirculated at 1.5 L/min, and 30% of water was renewed by aerated tap water every day. Water temperature was measured daily and averaged 28.5 ± 1.2 °C. Dissolved oxygen was measured weekly, and always greater than 6.5 mg/L.

The concentration of NH_3-N in the experimental tank was undetectable.

At the end of the feeding trial, deprived of feed for 24 h, the fish were captured from each tank and bulk weighed. All of the fish from each tank were sampled for analysis of final body composition. After measurement of body weight, body length and liver weight, the sampled fish were stored at -20 °C for chemical analysis.

2.3 Chemical analysis

Prior to chemical analysis, the sampled fish were thawed, weighed, autoclaved at 121 °C for 20 min, homogenized and dried at 105 °C. The contents of moisture, crude protein, crude lipid, ash and phosphorus of the feed ingredients, test diets and fish were analyzed following the methods described in AOAC (1995).

2.4 Calculations and statistics

Feed intake, percent weight gain (PWG), feed conversion ratio (FCR), retention efficiencies of nitrogen (NRE) and phosphorus (PRE), condition factor, hepatosomatic index, nitrogen waste and phosphorus waste are calculated as below:

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

$$\text{PWG (\%)} = 100 \times (W_t / N_t - W_0 / N_0) / (W_0 / N_0)$$

$$\text{FCR} = I / (W_t - W_0 + W_d)$$

$$\text{NRE (\%)} = 100 \times (W_t / N_t \times C_{Nt} - W_0 / N_0 \times C_{N0}) / [2 \times I / (N_t + N_0) \times C_{Nf}]$$

$$\text{PRE (\%)} = 100 \times (W_t / N_t \times C_{Pt} - W_0 / N_0 \times C_{P0}) / [2 \times I / (N_t + N_0) \times C_{Pf}]$$

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

$$\text{Hepatosomatic index (\%)} = 100 \times W_l / W_s$$

$$\text{Nitrogen waste [g N/ (kg fish gain)]} = 1000 \times (I \times C_{Nf}) \times (1 - \text{NRE}) / (W_t - W_0)$$

$$\text{Phosphorus waste [g P/ (kg fish gain)]} = 1000 \times (I \times C_{Pf}) \times (1 - \text{PRE}) / (W_t - W_0)$$

where I (g) is total amount of the dry feed; W_0 (g) is total initial body weight and W_t (g) is total final body weight; W_d (g) is total body weight of dead fish; t (d) is the duration of the trial; N_t is number of fish at the end of the trial and N_0 is at the start; C_{Nt} (%) is nitrogen content of fish body at the end of the trial and C_{N0} (%) is at the start; C_{Nf} (%) is nitrogen content of test diets; C_{Pt} (%) is phosphorus content of fish body at the end of the trial and C_{P0} (%) is at the start; C_{Pf} (%) is phosphorus content of test diets; W_s (g), L_s (cm) and W_l (g) are body weight, total length and liver weight of the sampled fish, respectively.

The manipulation effects of protein level, lipid level and their interaction on final body weight, PWG, feed intake, FCR, NRE, PRE, condition factor, hepatosomatic index, whole body components (moisture, crude protein, crude lipid, ash and phosphorus), wastes of nitrogen and phosphorus of fish fed the test diets were tested by two-way ANOVA. Duncan's test was used to examine the differences in the above parameters between different dietary protein levels or between different dietary lipid levels if the interaction effect was not significant. To determine the diet with best production performance, one-way ANOVA and Duncan's test was conducted to examine the differences in above parameters among all the test diets. The significant level was set at $P < 0.05$. All the statistical analyses were performed with IBM SPSS.

3 RESULTS

3.1 Survival, growth, feed intake and feed utilization efficiency

Survival rate ranged from 95% to 100% in the feeding trial. The FBW, PWG and feed intake were affected by dietary protein level and lipid level, while FCR, NRE and PRE were affected by dietary protein level, lipid level and their interaction (two-way ANOVA, $P < 0.05$, Table 3). The FBW and PWG in fish increased with dietary protein level increased from 300 to 420 g kg^{-1}, and significantly higher FBW and PWG were found in fish fed at 80 g kg^{-1} dietary lipid level than in fish fed at 30 g kg^{-1} dietary lipid level (Duncan's test, $P < 0.05$). The relationship between PWG and dietary crude protein level (PL) was described as $PWG = -2.2425 \times (PL)^2 + 192.36 \times PL - 3115.3$ ($P < 0.05$, $r^2 = 0.8272$) at 30 g kg^{-1} dietary lipid level (Figure 1a), and $PWG = -3.765 \times (PL)^2 + 315.64 \times PL - 5302.4$ ($P < 0.05$, $r^2 = 0.8141$) at 80 g kg^{-1} dietary lipid level (Figure 1b). Feed intake and FCR declined with dietary protein level increased from 300 to 420 g kg^{-1} (Duncan's test, $P < 0.05$), and significantly lower feed intake and FCR were found in fish fed at 80 g kg^{-1} dietary lipid level than in fish fed at 30 g kg^{-1} dietary lipid level (Duncan's test, $P < 0.05$). The highest NRE were found in fish fed at 380 and 420 g kg^{-1} dietary protein levels (Duncan's test, $P < 0.05$), while the highest PRE were found in fish fed at 380 g kg^{-1} dietary protein levels (Duncan's test, $P < 0.05$). Significantly higher PRE and NRE were found in fish fed at 80 g kg^{-1} dietary lipid level than in fish fed at 30 g kg^{-1} dietary lipid level (Duncan's test, $P < 0.05$).

Table 3 and Figure 1

The FCR were significantly lower in fish fed diets H38, H42 and H46 than in fish fed other test diets (one-way ANOVA, $P < 0.05$). The NRE and PRE were significantly higher in fish fed diets H38 and H42 than in fish fed other test diets (Duncan's test, $P < 0.05$)

3.2 Morphology and body composition

At the end of the feeding trial, condition factor was affected by dietary lipid level and the interaction between protein level and lipid level (two-way ANOVA, $P < 0.05$, Table 4). The hepatosomatic index and body protein content were affected by dietary protein level, while body moisture content was affected by dietary lipid level (two-way ANOVA, $P < 0.05$). The body lipid content was affected by dietary protein level and lipid level (two-way ANOVA, $P < 0.05$), while body contents of ash and phosphorus were unaffected by dietary protein level, lipid level and their interaction (two-way

ANOVA, $P > 0.05$). No significant differences were found in condition factor, whole body contents of moisture, ash and phosphorus among fish fed diets containing different dietary protein levels, while no significant differences were found in hepatosomatic index, whole body contents of protein, ash and phosphorus among fish fed diets containing different dietary lipid levels (Duncan's test, $P > 0.05$). Hepatosomatic index was significantly higher in fish fed at 300 g kg^{-1} dietary protein level than in fish fed at other test protein levels (Duncan's test, $P < 0.05$). Whole body protein content was significantly higher in fish fed at 460 g kg^{-1} dietary protein level than in fish fed at 300 g kg^{-1} dietary protein level (Duncan's test, $P < 0.05$), while no significant difference was found in whole body protein content among fish fed at 340, 380 and 420 g kg^{-1} dietary protein levels (Duncan's test, $P > 0.05$). Whole body lipid content was significantly higher in fish fed at 300 g kg^{-1} dietary protein level than in fish fed at 420 and 460 g kg^{-1} dietary protein level (Duncan's test, $P < 0.05$). Condition factor and whole body lipid content was lower in fish fed at 3% dietary lipid level than in fish fed at 80 g kg^{-1} dietary lipid level, while whole body moisture content was higher in fish fed at 30 g kg^{-1} dietary lipid level than in fish fed at 80 g kg^{-1} dietary lipid level (Duncan's test, $P < 0.05$).

Table 4

Higher condition factor was found in fish fed diet H46 than in fish fed diets L38, H30 and H42 (one-way ANOVA, $P < 0.05$), while no significant difference was found in condition factor among fish fed diets L30, L34, L42, L46, H34 and H38 (Duncan's test, $P > 0.05$).

3.3 Wastes output

The nitrogen waste and phosphorus waste were affected by dietary lipid level and protein level (two-way ANOVA, $P < 0.05$, Table 5). Nitrogen waste was lower in fish fed at 380 g kg^{-1} dietary protein level than in fish fed at 300, 340 or 460 g kg^{-1} dietary protein level, and phosphorus waste was lower in fish fed at 380, 420 and 460 g kg^{-1} dietary protein level than in fish fed at 300 and 340 g kg^{-1} dietary protein level (Duncan's test, $P < 0.05$). Higher nitrogen and phosphorus wastes were found in fish fed at 30 g kg^{-1} dietary lipid level than in fish fed at 80 g kg^{-1} dietary lipid level (Duncan's test, $P < 0.05$).

Table 5

4 DISCUSSIONS

Tidwell et al. (1992) investigated the effects three isocaloric (4.0-4.2 Kcal g^{-1}) diets containing varying dietary protein levels (260, 310 and 370 g kg^{-1}) on growth and feed utilization of hybrid sunfish, and they found that higher PWG was observed in fish fed a diet containing 370 g kg^{-1} protein than in fish fed diets containing 260 or 310 g kg^{-1} protein. In the present study, the FBW and PWG in fish increased with dietary protein level from 300 to 420 g kg^{-1}, whereas further elevation of dietary protein to 460 g kg^{-1} led to a decline of growth performance. The regression equation indicated that the dietary protein level for maximum PWG was 429 g kg^{-1} at 30 g kg^{-1} dietary lipid level, and was 418 g kg^{-1} at 80 g kg^{-1} dietary lipid level, respectively. These results indicate that 420 g kg^{-1} dietary protein could support the best growth of hybrid sunfish juveniles. This protein level is similar to the protein requirement (415.1- 423.7 g kg^{-1}) determined for the seed-parent of bluegill sunfish *Lepomis macrochirus* (Yang, Wang, Han, Yang, Li, & Jiang, 2016). Significantly higher FBW and PWG were found in fish fed at 80 g kg^{-1} dietary lipid level than in fish fed at 30 g kg^{-1} dietary lipid level, suggesting that 80 g kg^{-1} dietary lipid level is more suitable for the fast growth of hybrid sunfish. This lipid level is as same as that (80 g kg^{-1}) determined for juvenile bluegill sunfish *Lepomis* *macrochirus* (Hoagland, Davis, Tuan, & Mcgraw, 2003). Therefore, the optimal dietary protein and lipid contents for fast growth of hybrid sunfish are 420 g kg^{-1} and 80 g kg^{-1}, respectively.

In the present study, feed intake of hybrid sunfish declined with the increase of dietary protein and lipid levels. Similar results were found in Asian catfish *Pangasius hypophthalmus* (Liu, Wang, & Ji, 2011), *Pseudobagrus ussuriensis* (Wang, Ma, Shi, Liu, Guo, Yang, & Chen, 2012), giant croaker *Nibea japonica* (Chai, Ji, Han, Dai, & Wang, 2013) and hybrid grouper (Rahimnejad et al., 2015). In the present study, the FCR declined while the NRE and PRE enhanced with dietary protein level increased from 300 to 380 g kg^{-1}, lower FCR but higher NRE and PRE were found in fish fed at 80 g kg^{-1} dietary lipid level compared to fish fed at 30 g kg^{-1} dietary lipid level, suggesting that the based on feed utilization efficiency, the optimal dietary protein and lipid levels for hybrid sunfish were respectively 380 g kg^{-1} and 80 g kg^{-1}. Besides, no statistically differences were found in FCR, NRE and PRE between fish fed dietary protein at 380 and 420 g kg^{-1}. Thereby, the best feed utilization efficiency in hybrid sunfish was not impaired when fed at 420 g kg^{-1} protein and 80 g kg^{-1} lipid. According to the above mentioned results, the fast growth of hybrid sunfish is ascribed to high feed utilization rather than high feed intake.

Lipid deposition in fish body is closely associated with dietary lipid level (Yi, Zhang, Xu, Li, Zhang, & Mai, 2014), increasing dietary lipid level generally result in an increment in whole body lipid while the shrinkage in whole body moisture. For instance, previous studies reported that the body lipid content increased with the dietary lipid level rose in various fish species, e.g. grouper *Epinephelus malabaricus* (Lin & Shiau, 2003), cobia *Rachycentron canadum* (Wang, Liu, Tian, Mai, Du, Wang, & Yang, 2005), Atlantic halibut *Hippoglossus hippoglossus* (Martins, Valente, & Lall, 2007), white seabass *Atractoscion nobilis* (López, Durazo, Viana, Drawbridge, & Bureau, 2009; Jirsa, Deng, Davis, Wang, Hung, & Drawbridge, 2013), starry flounder *Platichthys stellatus* (Ding, Zhang, Wang, Ma, Meng, Duan, Sun, & Sun, 2010) and shortfin corvina *Cynoscion parvipinnis* (González-Félix, Maldonado-Othón, & Perez-Velazquez, 2016). In the present study, elevating dietary lipid level from 30 to 80 g kg^{-1}, whole body lipid content increased and whole body moisture content declined, while no significant difference was found in hepatosomatic index, suggesting enhancing dietary lipid level will increase lipid deposition in body but not in liver of hybrid sunfish.

Environmental impacts should not be ignored when determining the optimal dietary protein and lipid levels for fish. In the present study, nitrogen waste and phosphorus waste decreased with dietary lipid level raised from 300 to 380 g kg^{-1}, and no statistically difference was detected in both nitrogen waste and phosphorus waste between fish fed at 380 and 420 g kg^{-1} dietary protein level although the values of wastes output tended to increase with dietary protein level exceeding 380 g kg^{-1}. Furthermore, compared to fish fed at 30 g kg^{-1} dietary lipid level, significantly lower wastes of nitrogen and phosphorus were found in fish fed at 80 g kg^{-1} dietary lipid level. These results indicate that feeding at optimal dietary protein and lipid levels could effectively decline wastes output in juvenile hybrid sunfish farming. To the best of our knowledge, the effects of different dietary protein and lipid levels on wastes output in fish were merely evaluated in a few fish species including giant croaker *Nibea japonica* (Chai et al., 2013), golden pompano *Trachinotus ovatus* (Wang, Han, Wang, & Ma, 2013) and largemouth bass *Micropterus salmoides* (Huang et al., 2017). In the present study, the wastes output for hybrid sunfish fed at optimal combination of dietary protein and lipid were 42.54 g kg^{-1} N and 8.26 g kg^{-1} P, which is lower than that evaluated in giant croaker (~50 g kg^{-1} N, Chai et al., 2013), golden pompano (64 g kg^{-1} N, Wang et al., 2013) and largemouth bass (49.8 g kg^{-1} N, 8.7 g kg^{-1} P, Huang et al., 2017). According to these results, it seems that hybrid sunfish farming may generate less nitrogen and phosphorus wastes than that produced from the farming of giant croaker, golden pompano and largemouth bass.

In conclusion, the FBW, PWG, NRE and PRE of hybrid sunfish increased, while feed intake and FCR decreased, with dietary protein increased from 300 to 420 g kg^{-1} and dietary lipid increased from 30 to 80 g kg^{-1}. Therefore, the optimal combination of dietary protein and lipid for hybrid sunfish should be 420 g kg^{-1} and 80 g kg^{-1}, respectively. In fish fed diets containing 380-420 g kg^{-1} protein and 80 g kg^{-1} lipid, the wastes output of nitrogen and phosphorus were obvious lower than that generated of fish fed diets containing other combination of dietary protein and lipid. Feeding at optimal combination of dietary protein and lipid is an efficient strategy to reduce waste discharge in hybrid sunfish farming.

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DATA A VAILABILITY STATEMENT

The data that support the findings of this study are appearing in the submitted article.

CONFLICT OF INTEREST

The authors have no conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL STATEMENT

All the fish were treated following the guideline of Administration of Laboratory Animals published by the State Science and Technology Commission of China.

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1 **Table 1** Proximate composition (g kg^{-1}) of the feed ingredients

Ingredient	Dry matter	Crude protein	Crude lipid	Ash
Red fish meal	926	656	87	154
Poultry by-product meal	965	657	127	136
Soybean meal	894	447	14	61
Wheat bran	875	177	41	33
Wheat gluten meal	92.2	805	4	5
Rapeseed meal	898	401	15	65
Wheat flour	864	139	20	7

2 Crude protein, crude lipid and ash are expressed on a dry matter basis (n = 2).

Table 2 Formulation (g kg^{-1}), proximate composition (g kg^{-1}) and gross energy (MJ kg^{-1}) of the test diets

Ingredient	L30[1]	L34[1]	L38[1]	L42[1]	L46[1]	H30[1]	H34[1]	H38[1]	H42[1]	H46[1]
Red fish meal	80	130	180	230	280	80	130	180	230	280
Soybean meal	220	220	220	220	220	220	220	220	220	220
Wheat flour	267	237	195	120	90	267	237	195	126	40
Wheat bran	180	180	180	180	180	180	180	180	180	180
Poultry by-product meal	50	50	50	50	50	50	50	50	50	50
Rapeseed meal	30	30	30	50	60	30	30	30	50	60
Wheat gluten meal	10	20	35	50	64	10	20	35	50	72
Bentonite powder	96	71	52	47	17	55	29	11	0	59
$CaHPO_4$	15	15	15	15	15	15	15	15	15	15
Choline chloride	2	2	2	2	2	2	2	2	2	2
Starch, gel.	2	2	2	2	2	2	2	2	2	2
Mineral premix[2]	20	20	20	20	20	20	20	20	20	20
Menhaden fish oil	28	23	19	14	10	69	65	60	55	10
Dry matter[3]	926	929	939	937	933	939	934	939	938	949
Crude protein[3]	298	333	373	409	451	306	337	374	414	453
Crude lipid[3]	35	31	33	30	34	77	86	78	83	84
Ash[3]	151	142	132	134	106	118	103	97	92	101
Phosphorus[3]	10	12	12	13	14	16	11	13	14	15
Gross energy	15.6	16.1	16.5	16.7	17.0	17.2	17.7	18.1	18.4	18.8

[1] L30: Diet designed with 30 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; L34: Diet designed with 30 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; L38: Diet designed with 30 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; L42: Diet designed with 30 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; L46: Diet designed with 30 g kg^{-1} crude

lipid and 460 g kg^{-1} crude protein; H30: Diet designed with 80 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; H34: Diet designed with 80 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; H38: Diet designed with 80 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; H42: Diet designed with 80 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; H46: Diet designed with 80 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein.

[2]Vitamin and mineral premix provides per kg of feed: retinyl acetate, 3000 IU; cholecalciferol, 2400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid), 120 mg; cyanocobalamine, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine.HCl, 6 mg; riboflavin, 7.2 mg; thiamin.HCl, 1.2 mg; sodium chloride (39% Na, 61% Cl), 3077 mg; ferrous sulfate (20% Fe), 65mg; manganese sulfate (36% Mn), 89 mg; zinc sulfate (40% Zn), 150 mg; copper sulfate (25% Cu), 28 mg; potassium iodide (24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1000 mg.

[3] Crude protein, crude lipid, ash and phosphorus are expressed as they are in air and given as means of two measurements (n = 2).

Table 3 Final body weight (FBW, g), percent weight gain (PWG, %), feed intake (g d^{-1}), feed conversion ratio (FCR), retention efficiencies of nitrogen (NRE, %) and phosphorus (PRE, %), and survival (%) of hybrid sunfish juveniles

Diet	FBW	PWG	Feed intake	FCR	NRE	PRE	Survival
L30	9.68 ± 0.22	645.80 ± 25.38	4.98 ± 0.24	1.83 ± 0.10^{E}	29.32 ± 1.96^{A}	31.64 ± 4.43^{B}	100
L34	10.78 ± 0.25	789.23 ± 11.33	4.37 ± 0.22	1.53 ± 0.07^{D}	32.89 ± 0.93^{B}	35.85 ± 1.28^{BC}	95
L38	12.26 ± 0.11	882.56 ± 42.86	3.99 ± 0.10	1.37 ± 0.02^{C}	32.68 ± 1.15^{B}	37.48 ± 1.86^{C}	95
L42	14.42 ± 0.67	1087.86 ± 52.39	3.77 ± 0.09	1.25 ± 0.04^{B}	33.06 ± 0.93^{BC}	38.47 ± 0.88^{C}	95
L46	13.32 ± 0.10	967.50 ± 88.28	3.43 ± 0.04	1.16 ± 0.01^{B}	33.13 ± 0.36^{BC}	39.73 ± 0.69^{C}	97
H30	11.46 ± 0.28	881.09 ± 52.89	4.51 ± 0.07	1.55 ± 0.04^{D}	33.62 ± 0.93^{BC}	25.81 ± 0.68^{A}	97
H34	12.72 ± 0.10	943.82 ± 19.30	4.26 ± 0.08	1.44 ± 0.03^{CD}	33.64 ± 1.02^{BC}	41.10 ± 1.58^{C}	97
H38	17.60 ± 0.04	1309.77 ± 50.78	3.16 ± 0.06	1.02 ± 0.01^{A}	41.31 ± 0.09^{E}	47.30 ± 1.94^{D}	97
H42	16.93 ± 0.32	1317.51 ± 105.49	3.24 ± 0.06	1.04 ± 0.01^{A}	38.45 ± 1.39^{D}	41.80 ± 4.26^{C}	95
H46	17.22 ± 0.77	1257.38 ± 68.04	3.16 ± 0.02	1.03 ± 0.01^{A}	35.81 ± 0.67^{C}	39.22 ± 0.55^{C}	100
Protein level							
300 g kg^{-1}	10.55 ± 1.02^{a}	763.45 ± 134.11^{a}	4.74 ± 0.30^{d}	1.69 ± 0.17^{d}	31.47 ± 2.73^{a}	28.73 ± 4.27^{a}	98
340 g kg^{-1}	11.75 ± 1.08^{b}	866.52 ± 85.85^{b}	4.32 ± 0.16^{c}	1.49 ± 0.07^{c}	33.26 ± 0.96^{b}	38.48 ± 3.15^{b}	96
380 g kg^{-1}	14.93 ± 2.92^{c}	1096.16 ± 237.74^{c}	3.58 ± 0.46^{b}	1.20 ± 0.19^{b}	36.99 ± 4.78^{d}	42.39 ± 5.64^{c}	96

420 g kg^{-1}	15.67 ± 1.46^{d}	1202.69 ± 146.19^{d}	3.50 ± 0.30^{b}	1.15 ± 0.12^{ab}	35.75 ± 3.14^{cd}	40.13 ± 3.30^{bc}	95
460 g kg^{-1}	15.27 ± 2.19^{cd}	1112.44 ± 173.72^{c}	3.29 ± 0.15^{a}	1.09 ± 0.07^{a}	34.47 ± 1.54^{bc}	39.47 ± 0.63^{bc}	98
Lipid level							
30 g kg^{-1}	12.08 ± 1.80^{x}	874.59 ± 162.07^{x}	4.11 ± 0.57^{y}	1.43 ± 0.25^{y}	32.22 ± 1.81^{x}	36.63 ± 3.48^{x}	96
80 g kg^{-1}	15.19 ± 2.68^{y}	1141.92 ± 203.87^{y}	3.67 ± 0.61^{x}	1.22 ± 0.24^{x}	36.56 ± 3.16^{y}	39.22 ± 0.55^{y}	97
Two-way ANOVA							
Protein level	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	
Lipid level	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	
Protein × lipid	NS	NS	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$	

[1] L30: Diet designed with 30 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; L34: Diet designed with 30 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; L38: Diet designed with 30 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; L42: Diet designed with 30 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; L46: Diet designed with 30 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein; H30: Diet designed with 80 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; H34: Diet designed with 80 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; H38: Diet designed with 80 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; H42: Diet designed with 80 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; H46: Diet designed with 80 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein.

[2] Data are expressed as mean ± SD (n = 3). Data with different superscripts in the same column are significantly different at $P < 0.05$. Uppercases

(ABCDE) in the same column indicate the statistical results when there was significant interaction between protein level and lipid level (P < 0.05), and lowercases (abcd and xy) in the same column indicate the statistical results when there was no significant interaction between protein level and lipid level (P > 0.05).

37 **Table 4** Condition factor (g cm^{-3}), hepatosomatic index (%), and body composition (g kg^{-1}) of hybrid sunfish juveniles.

Diet	Condition factor	Hepatosomatic index	Moisture	Crude protein	Crude lipid	Ash	Phosphorus
L30	2.70 ± 0.16^{AB}	2.81 ± 0.57	733 ± 4.4	157 ± 2.7	62 ± 3.6	37 ± 0.8	6 ± 0.6
L34	2.86 ± 0.32^{AB}	2.42 ± 0.40	726 ± 5.8	165 ± 4.2	57 ± 3.4	36 ± 1.6	6 ± 0.1
L38	2.65 ± 0.14^{A}	1.97 ± 0.25	728 ± 6.2	165 ± 4.5	58 ± 2.3	35 ± 0.8	6 ± 0.3
L42	2.80 ± 0.29^{AB}	2.00 ± 0.29	735 ± 0.4	167 ± 0.3	58 ± 2.4	36 ± 0.8	6 ± 0.1
L46	2.79 ± 0.44^{AB}	1.90 ± 0.45	729 ± 2.8	170 ± 0.1	58 ± 0.8	39 ± 3.5	6 ± 0.0
H30	2.67 ± 0.18^{A}	2.63 ± 0.28	712 ± 2.0	158 ± 0.5	88 ± 0.1	37 ± 0.4	6 ± 0.0
H34	2.84 ± 0.23^{AB}	2.18 ± 0.37	714 ± 7.5	161 ± 2.9	81 ± 3.0	37 ± 1.0	7 ± 0.2
H38	2.88 ± 0.18^{AB}	2.67 ± 0.46	717 ± 5.0	157 ± 3.2	81 ± 5.6	36 ± 0.7	6 ± 0.2
H42	2.66 ± 0.20^{A}	2.03 ± 0.24	721 ± 6.9	165 ± 10.0	72 ± 0.6	36 ± 1.2	6 ± 0.9
H46	3.18 ± 0.34^{B}	1.97 ± 0.22	720 ± 5.4	165 ± 1.7	75 ± 4.9	36 ± 0.1	6 ± 0.0
Protein level							
300 g kg^{-1}	2.68 ± 0.16	2.72 ± 0.44^{b}	722 ± 12.5	157 ± 1.6^{a}	75 ± 15.1^{b}	37 ± 0.6	6 ± 0.4
340 g kg^{-1}	2.85 ± 0.27	2.30 ± 0.39^{a}	720 ± 8.7	164 ± 3.6^{ab}	69 ± 14.0^{ab}	36 ± 1.2	7 ± 0.2
380 g kg^{-1}	2.76 ± 0.20	2.32 ± 0.51^{a}	722 ± 8.0	161 ± 5.7^{ab}	70 ± 14.0^{ab}	36 ± 1.0	6 ± 0.2
420 g kg^{-1}	2.73 ± 0.24	2.02 ± 0.25^{a}	728 ± 9.0	166 ± 5.9^{ab}	65 ± 8.4^{a}	36 ± 0.9	6 ± 0.6
460 g kg^{-1}	2.98 ± 0.43	1.94 ± 0.34^{a}	725 ± 6.0	167 ± 3.4^{b}	66 ± 10.0^{a}	37 ± 2.8	6 ± 0.2

Lipid level							
30 g kg^{-1}	2.76 ± 0.28^{x}	2.22 ± 0.52	730 ± 4.8^{y}	165 ± 5.1	59 ± 2.7^{x}	36 ± 2.0	6 ± 0.3
80 g kg^{-1}	2.85 ± 0.29^{y}	2.30 ± 0.43	717 ± 5.6^{x}	161 ± 5.1	80 ± 6.3^{y}	36 ± 0.8	6 ± 0.4
Two-way ANOVA							
Protein level	NS	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$	NS	NS
Lipid level	$P < 0.05$	NS	$P < 0.05$	NS	$P < 0.05$	NS	NS
Protein × lipid	$P < 0.05$	NS	NS	NS	NS	NS	NS

[1] L30: Diet designed with 30 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; L34: Diet designed with 30 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; L38: Diet designed with 30 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; L42: Diet designed with 30 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; L46: Diet designed with 30 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein; H30: Diet designed with 80 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; H34: Diet designed with 80 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; H38: Diet designed with 80 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; H42: Diet designed with 80 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; H46: Diet designed with 80 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein.

[2] Data are expressed as mean ± SD (n = 3). Data with different superscripts in the same column are significantly different at $P < 0.05$. Uppercases (AB) in the same column indicate the statistical results when there was significant interaction between protein level and lipid level ($P < 0.05$),

and lowercases (ab and xy) in the same column indicate the statistical results when there was no significant interaction between protein level and lipid level (*P* > 0.05).

Table 5 Nitrogen waste [g N (kg fish gain)$^{-1}$] and phosphorus waste [g P (kg fish gain)$^{-1}$] of hybrid sunfish juveniles

Diet	Nitrogen waste	Phosphorus waste
L30	61.68 ± 4.95	12.76 ± 1.49
L34	54.97 ± 3.40	11.43 ± 0.78
L38	55.16 ± 1.79	10.55 ± 0.47
L42	54.79 ± 2.43	10.23 ± 0.46
L46	55.91 ± 0.96	9.70 ± 0.22
H30	50.36 ± 2.00	18.05 ± 0.63
H34	51.73 ± 1.76	9.53 ± 0.43
H38	35.86 ± 0.42	6.95 ± 0.35
H42	42.54 ± 0.62	8.26 ± 0.53
H46	47.72 ± 1.04	9.23 ± 0.19
Protein level		
300 g kg^{-1}	56.02 ± 7.06^{d}	15.40 ± 3.07^{c}
340 g kg^{-1}	53.35 ± 3.00^{cd}	10.48 ± 1.18^{b}
380 g kg^{-1}	45.51 ± 10.63^{a}	8.75 ± 2.01^{a}
420 g kg^{-1}	48.66 ± 6.90^{ab}	9.25 ± 1.16^{a}
460 g kg^{-1}	51.81 ± 4.57^{bc}	9.47 ± 0.32^{a}
Lipid level		
30 g kg^{-1}	56.50 ± 3.73^{y}	10.93 ± 1.31^{y}
80 g kg^{-1}	45.64 ± 6.12^{x}	10.40 ± 4.08^{x}
Two-way ANOVA		
Protein level	$P < 0.05$	$P < 0.05$
Lipid level	$P < 0.05$	$P < 0.05$
Protein × lipid	NS	NS

[1] L30: Diet designed with 30 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; L34: Diet designed with 30 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; L38: Diet designed

with 30 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; L42: Diet designed with 30 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; L46: Diet designed with 30 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein; H30: Diet designed with 80 g kg^{-1} crude lipid and 300 g kg^{-1} crude protein; H34: Diet designed with 80 g kg^{-1} crude lipid and 340 g kg^{-1} crude protein; H38: Diet designed with 80 g kg^{-1} crude lipid and 380 g kg^{-1} crude protein; H42: Diet designed with 80 g kg^{-1} crude lipid and 420 g kg^{-1} crude protein; H46: Diet designed with 80 g kg^{-1} crude lipid and 460 g kg^{-1} crude protein.

[2]Data are expressed as mean ± SD (n = 3). Data with different superscripts in the same column are significantly different at $P < 0.05$. Lowercases (abcd and xy) in the same column indicate the statistical results when there was no significant interaction between protein level and lipid level ($P > 0.05$).

Figure Legend

Figure 1 The relationship between percent weight gain (PWG) and dietary crude protein level (PL) of hybrid sunfish fed at dietary lipid level 3% (a) and 8% (b). The regression equation in figure (a) was described as $PWG = –2.2425 \times (PL)^2 + 192.36 \times PL - 3115.3$ ($P < 0.05$, $r^2 = 0.8272$), and the regression equation in figure (b) was described as $PWG = –3.765 \times (PL)^2 + 315.64 \times PL - 5302.4$ ($P < 0.05$, $r^2 = 0.8141$).

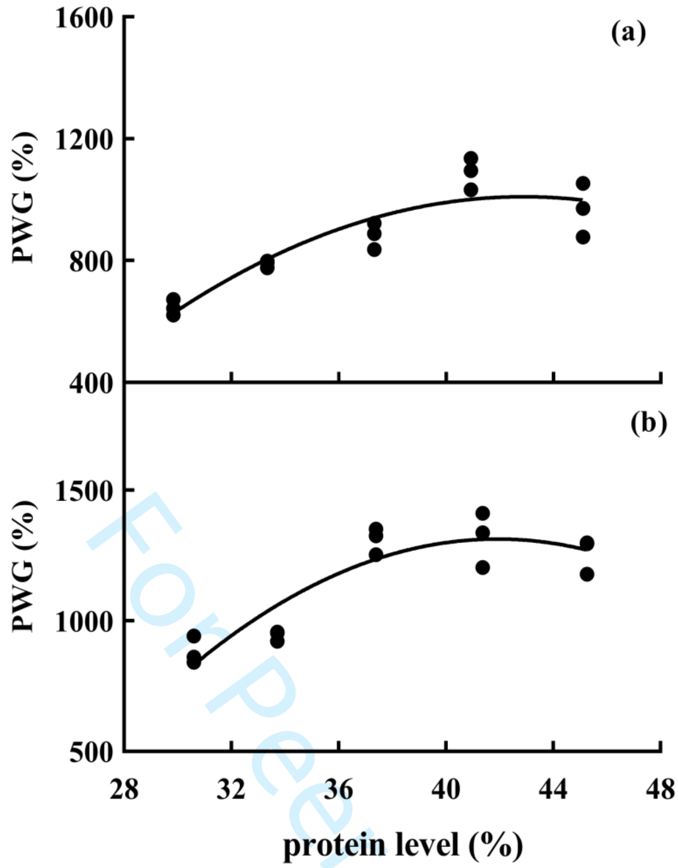
(a)
1600
1200
800
400
PWG (%)
(b)
1500
1000
500
PWG (%)
28
32
36
40
44
48
protein level (%)

Fig. 1