

Original Research Articles

Effects of Dietary Protein and Lipid Levels on Growth, Feed Utilization, and Body Composition of Tire Track Eel (*Mastacembelus favus*) Fingerlings

Phan P. Loan^{1a}, Nguyen Thanh Phuong²¹ An Giang University, Vietnam National University Ho Chi Minh City, Viet Nam, ² Can Tho University, Viet NamKeywords: protein, lipid, *Mastacembelus favus*, growth, feed utilization<https://doi.org/10.46989/001c.128999>

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This study aimed to determine the optimal protein and lipid levels for tire track eel (*Mastacembelus favus*) fingerlings. Nine test diets comprised three protein levels (40, 45, and 50%) and three lipid levels (6, 9, and 12%). The experiment was conducted in triplicate for 56 days. The initial sizes of fish varied from 3 to 5 g. There were significant interaction effects between protein and lipid levels on final body weight, weight gain, and feed conversion ratio. The dietary protein levels affected weight gain and daily weight gain, while the dietary lipid levels affected length gain. The protein-sparing effect of lipids did not occur in the fingerling stage. Daily feed intake was affected by dietary protein levels but not lipid levels. Feed conversion ratios were lower with increased lipid levels; the lowest value was found at the protein level of 45%. The protein efficiency rates of fish were improved with increased dietary protein levels but were reduced with increased dietary lipids. Increasing dietary lipid levels of the fish resulted in higher body lipid deposition. Survival rates were high and were not affected by the experimental diets. The optimal levels of dietary protein and lipid for growth performance and feed utilization of the *Mastacembelus favus* fingerlings were 40% and 9%, respectively.

INTRODUCTION

Protein is the most important fish dietary nutrient, as it provides essential and non-essential amino acids for growth and energy production.¹ However, a given diet with excess protein and low energy leads to ammonia production through amino acid catabolization, a metabolic process that can be harmful to fish.² Moreover, protein is more expensive than other macro-nutrients in formulated feeds. Therefore, excess dietary protein leads to higher feed costs that can reduce the profits from fish farming. It is well known that the dietary protein requirement of fish depends on dietary energy.³ A diet containing an optimal combination of protein and non-protein energy-supplying nutrients is required for both the growth of cultured fish and the economic efficiency of fish farming. An effect referred to as the protein-sparing activity of carbohydrates and lipids, is commonly recognized.⁴⁻¹¹ Lipids can more effectively conserve protein for development than carbohydrates due to their higher energy content, and lipids are readily metabolized by fish.¹² In addition to being rich in energy, the inclusion of lipid sources, particularly fish oils, in diets also provides essential fatty acids for the normal performance

and health of fish. Excess dietary lipids may cause body fat deposition and deterioration of muscle quality in cultured fish.^{13,14} Hence, to promote optimal growth without adverse effects such as excessive nutrient loss or low meat quality, the fish diet should comprise an optimal ratio of protein and lipids. So far, a balanced protein-to-lipid ratio in diets in order to increase growth, improve flesh quality, and reduce environmental waste has been studied for many cultured fish species.¹⁵⁻¹⁹

Mastacembelus eels (striped spiny eel *M. pancalus*, tire-track spiny eel or zig-zag eel *M. armatus*, tire track eel or flower spiny eel *M. favus*) are considered highly valued fish in Asia where they are naturally distributed (Bangladesh, India, Pakistan, Nepal, Thailand, Lao PDR, Cambodia, Vietnam and Peninsular Malaysia). They have a good taste, delicious flesh quality (special flavor, characteristic texture, and high protein, oil, and vitamin C content), high market value, lucrative size, and important production potentials for food and aquarium fish.²⁰⁻²⁴ The importance of fish farming is underscored by the decline of wild fish stocks, driven by habitat degradation and overexploitation.²¹ In Vietnam, *M. favus* (known as chach lau or chach bong) is abundant in the Mekong River Delta (MRD) and was pre-

a Corresponding author, Email: ploan@agu.edu.vn

viously misidentified as *Mastacembelus armatus*, a species found only in countries such as India and Bangladesh.²⁵ *M. favus* is a carnivorous fish.²⁶ Artificial seed production of *M. favus*²⁴ and the separate dietary protein and lipid requirements of this species have been studied in the Mekong River Delta of Vietnam.²⁷⁻²⁹ *M. favus* is being farmed in the MRD and fed low-value fish and/or homemade feed.³⁰ Nguyen *et al.*³² also studied replacement levels of black soldier fly (*Hermetia illucens*) larvae meal for fishmeal in the diets of tire track eel fingerlings. To our knowledge, there are no previous studies assessing dietary protein-to-lipid ratios on the growth performance of *M. favus* at any development stages. Thus, to promote the farming of this species in the country, it is necessary to produce formulated feeds with an optimal protein-to-lipid ratio for cost-effective production.

MATERIALS AND METHODS

STUDY LOCATION AND DURATION

The experiment was conducted in the Experimental farm of An Giang University, Long Xuyen City, An Giang Province in the Mekong River Delta of Vietnam from February to May 2021.

EXPERIMENTAL FISH AND HOLDING CONDITIONS

Fingerlings of *M. favus* with a body mass of 2-3 g each were bought from a local hatchery and transported to the wet lab of An Giang University (a distance of 5 km). The fish were acclimatized in three fiberglass tanks (2,000 L each) and fed commercial pellets for two weeks. The tanks were continuously aerated, and about 30% of the tank water was exchanged every two days to maintain optimal environmental conditions (dissolved oxygen over 5 mg/L, a pH of 7.5-8.0, and a temperature of 27-29°C).

Healthy fish with a body weight of 3-5 g were randomly distributed into 27 fiberglass experimental tanks (500 L each) at a stocking density of 50 fish per tank. The average sizes of fish by treatments were from 2.87 ± 0.92 to 3.37 ± 0.28 g/fish. Water flowed through the experimental tanks at a rate of 0.5-0.7 L/min. The tanks were continuously aerated during the experimental period. Four PVC pipes 9.0 cm in diameter and 30 cm in length were placed at the bottom of the tanks to create shelters for the fish. The water quality parameters were measured at 06:00 and 15:00 hours daily using portable DO and pH meters (Hanna Company, Woonsocket, RI, USA).

EXPERIMENTAL DESIGN AND DIETS

Due to a lack of available information on the dietary protein and lipid requirements of *M. favus*. The protein and lipid levels in this study were referred from the research of Hung *et al.*³ on Asian red-tailed catfish (*H. wyckioides*), an omnivore, with an adjustment of higher protein contents. The experiment was conducted using a 3 × 3 factorial design with three dietary protein levels (40, 45, and 50%) and three

lipid levels (6, 9, and 12%). Each treatment was performed in triplicate simultaneously. The diets were formulated using fish meal, soybean meal, fish oil, cassava flour, bone meal, and premix (Tables 1 and 2). All ingredients were thoroughly mixed and then pelleted by using an electronic meat grinder with a diameter of 1 mm. Diets were dried in an oven at 60°C until the moisture content was less than 10%, and the meal was broken into pellets with a length of 1-1.2 mm. All diets were stored at 5°C in sealed plastic bags until use. The experiment was carried out in accordance with the national guidelines on the protection of animals and experimental animal welfare in Vietnam.³³

FEEDING MANAGEMENT

The fish were manually fed the test diets twice a day from 08:00 to 09:00 and from 15:00 to 16:00 using a feeding rate of about 10% body weight. This species is a bottom feeder; therefore, plastic tubing was used to position the pellets on the bottom of the tanks (close to the shelters). The feed intake of the fish was closely monitored. Uneaten feed was collected, dried, and weighed to estimate actual feed intake. The feeding rate was adjusted depending on the feed amount consumed on the previous day. In addition, the feed intake of the fish was monitored.

SAMPLE COLLECTION AND ANALYSIS

Thirty fish from each tank were individually measured for length and weight at the start (day 1) and end (day 56) of the experiment. A ruler and a digital balance with a minimum precision of 1 mm and 0.1 g, respectively, were used for the measurements.

Three fish at stocking and three fish from each experimental tank (nine fish per treatment) at the harvest were collected for carcass composition analysis. Feed ingredients, experimental diets, and fish samples were preserved at -20°C until analysis. The moisture, ash, protein, fat, and carbohydrate (NFE) contents of feed and fish were determined using standard methods.³⁴ Lysine and methionine were calculated based on their values in the ingredients.¹³ Gross energy (GE) was calculated using values of 5.65 Kcal/g for crude protein, 4.25 Kcal/g for carbohydrates, and 9.45 Kcal/g for crude fat.¹²

DATA ANALYSIS

The initial mean weight (W_i) and final mean weight (W_f) of individual fish were determined before and after the experiment. The survival rate (SR), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) were computed as follows:

Survival rate (SR, %) = (number of fish at harvest)/(number of fish at stock) × 100

Length gain (LG, cm) = $L_f - L_i$

Weight gain (WG, g) = $W_f - W_i$

Daily weight gain (DWG, g/day) = $(W_f - W_i)/t$

Table 1. Ingredients included in the experimental diets (dry matter basis, %)

Ingredients	Diet No. (Protein-Lipid)								
	Diet 1 (40-6)	Diet 2 (40-9)	Diet 3 (40-12)	Diet 4 (45-6)	Diet 5 (45-9)	Diet 6 (45-12)	Diet 7 (50-6)	Diet 8 (50-9)	Diet 9 (50-12)
Soybean oil	1.30	3.00	4.45	1.15	2.55	4.40	1.05	2.10	4.10
Fish oil	1.30	2.70	4.45	1.25	2.95	4.30	1.25	3.30	4.40
Soybean meal	36.5	37.0	37.0	38.0	36.0	37.0	44.0	42.0	45.0
Fish meal	19.0	17.5	16.0	20.0	22.0	22.2	23.5	24.7	29.0
Bone meal	14.0	16.0	18.0	23.0	22.4	21.3	22.1	22.5	14.8
Cassava flour	27.2	23.1	19.4	15.9	13.4	10.1	7.4	4.7	2.0
Premix* (vitamin- mineral)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CMC**	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Notes: *Vitamin and mineral premix content per kg: vitamin A: 4,000,000 UI; vitamin D3: 800,000 UI; vitamin E: 8,500 UI; vitamin K3: 750 UI; vitamin B1: 375 UI; vitamin C: 8,750 UI; vitamin B2: 1,600 mg; vitamin B6: 750 mg; folic acid: 200 mg; vitamin B12: 3,000 µg; biotin: 20,000 µg; methionine: 2,500 mg; and Mn, Zn, Mg, K and Na 10 mg. ** Carboxymethyl cellulose (CMC) from the Netherlands

Table 2. Proximate composition of the experimental diets (dry matter basis, %)

Nutrients	Diet No. (protein-lipid)								
	Diet 1 (40-6)	Diet 2 (40-9)	Diet 3 (40-12)	Diet 4 (45-6)	Diet 5 (45-9)	Diet 6 (45-12)	Diet 7 (50-6)	Diet 8 (50-9)	Diet 9 (50-12)
Dry matter	93.4	91.8	92.9	91.5	91.6	91.7	91.55	91.6	91.7
Crude protein	40.2	40.2	40.2	45.1	45.1	45.0	49.9	49.9	50.1
Crude lipid	6.03	9.01	12.0	6.03	9.01	12.1	6.01	9.04	12.04
Methionine*	0.51	0.50	0.49	0.55	0.43	0.44	0.50	0.49	0.52
Lysine*	3.54	3.52	3.48	3.81	2.41	2.53	2.69	2.76	3.01
GE Kcal/ kg)**	3,802	3,813	3,835	3,814	3,819	3,842	3,913	3,946	3,979
P : E (mg/ Kcal)	106	105	105	118	118	117	128	127	126

Specific growth rate (SGR, %/day) = $[(\ln W_f - \ln W_i) \times 100]/t$

Feed conversion ratio (FCR) = dry diet fed/wet weight gain)

Protein efficient ratio (PER) = $(W_f - W_i)/\text{protein intake}$

Daily feed intake (DFI, g/fish/day) = Consumed feed in tank (g)/(number of fish \times t)

Where W_i is the initial weight of the fish (g); W_f is the final weight of the fish (g); L_f is the final length of fish; L_i is the initial length of fish, and t is the experimental time (day).

STATISTICAL ANALYSIS

Treatment means were compared by a two-way ANOVA followed by Duncan's multiple range test using Minitab software version 16.0. The significance level was set at $P < 0.05$. Data are presented as mean \pm standard deviation. For the protein and lipid requirements, after considering the inter-

action of the two factors, the difference in each diet was examined to select the best treatment.

RESULTS

GROWTH AND SURVIVAL OF FISH

The growth performance of the fish fed nine test diets is presented in Tables 3 and 4. After 56 days, the average final body length (FBL) and length gain (LG) ranged from 12.9 to 14.4 cm and 2.32 to 4.27 cm, respectively (Table 3). There was no significant difference in the FBL or LG of the fish among the treatments. For the nutrient group analysis, the FBL (13.9 cm) and LG (3.49 cm) of the 45% protein diet group were the highest, and those (13.2 cm and 2.72 cm, respectively) of the 40% protein diet group were the lowest. Similarly, the averages of FBL (13.9 cm) and LG (3.54 cm) of the 9% lipid diet group were the highest, and those (13.1 cm and 2.59 cm, respectively) of the 6% lipid diet group were the lowest. The LG of the fish was affected by the dietary lipid content ($P < 0.05$) but not by the dietary protein con-

Table 3. Growth in length of *M. fавus* after 56 days of being reared with different test diets

Diet No. (protein-lipid)	Initial body length (IBL, cm)	Final body length (FBL, cm)	Length gain (LG, cm)	Survival rate (SR, %)
Diet 1 (40-6)	10.2±1.18	13.1±1.22	2.89±0.35 ^b	78.7±2.31
Diet 2 (40-9)	10.6±0.37	13.1±0.08	2.53±0.26 ^b	75.3±5.03
Diet 3 (40-12)	10.6±0.51	13.3±2.98	2.74±0.32 ^b	71.3±1.15
Diet 4 (45-6)	10.8±0.45	13.3±0.72	2.57±0.29 ^b	80.0±10.0
Diet 5 (45-9)	10.2±2.63	14.4±0.44	4.27±0.23 ^a	82.7±3.06
Diet 6 (45-12)	10.4±0.11	14.0±0.22	3.63±0.36 ^b	72.7±3.06
Diet 7 (50-6)	10.7±0.81	13.0±0.47	2.32±1.28 ^b	74.0±2.00
Diet 8 (50-9)	10.2±0.40	14.0±0.13	3.82±0.53 ^b	71.3±8.08
Diet 9 (50-12)	10.3±0.18	13.5±0.36	3.20±0.25 ^b	80.0±2.00
40% protein	10.5±0.20	13.2±0.13	2.72±0.18	75.1±3.67
45% protein	10.4±0.29	13.9±0.56	3.49±0.86	78.5±5.18
50% protein	10.4±0.24	13.5±0.52	3.11±0.75	75.1±4.44
6% lipid	10.6±0.27	13.1±0.17	2.59±0.29 ^b	77.6±3.15
9% lipid	10.3±0.22	13.9±0.68	3.54±0.90 ^a	76.4±5.75
12% lipid	10.4±0.14	13.6±0.35	3.19±0.45 ^{ab}	74.7±4.67
ANOVA analysis of two factors				
Protein (P)	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Lipid (L)	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$
P × L	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Notes: Values are presented as mean ± standard deviation; values in the same column with the same superscript are not significantly different ($P > 0.05$).

tent ($P > 0.05$). There was no interaction effect between dietary protein and lipid nutrients on the growth parameters of the fish ($P > 0.05$). The diet of 45% protein and 9% lipid obtained the best growth of the fish (Table 3).

There were significant interaction effects between dietary protein and lipid nutrients on final body weight (FBW) and weight gain (WG) ($P < 0.05$), but not on daily weight gain (DWG) or specific growth rate (SGR) ($P > 0.05$) (Table 4). The FBW ranged from 5.48 to 8.44 g/fish, WG from 2.36 to 5.38 g, DWG from 0.16 to 0.43 g/day, and SGR from 3.70 to 6.80 %/day (Table 4). There was a significant difference ($P < 0.05$) between the means of FBW, WG, and DWG, but not among those of SGR of the fish ($P > 0.05$). Based on nutrient group analysis, the average FBW (7.05 g), WG (4.08 g), DWG (0.31 g/day), and SGR (5.75 %/day) of the 9% lipid diets were the highest and those (5.97 g, 2.8 g, 0.19 g/day and 4.36 %/day, respectively) of the 6% lipid group were the lowest. Similarly, at the end of the experiment, the average BW (6.86 g), WG (3.74 g), and DWG (0.3 g/day) of the 45% protein diets and the SGR (5.58 %/day) of 50% protein diets were the highest, and those (6.19 g, 3.18 g, 0.19 g/day, and 4.5 %/day, respectively) of the 40% protein diets were the lowest. The WG and DWG were affected by the dietary protein but not by the dietary lipid ($P > 0.05$). The diet of 45% protein and 9% lipid showed the best weight gain (Table 4).

The survival rates (SR) ranged from 71.3 to 82.7%. There was no significant difference among the average SRs of fish fed different test diets ($P > 0.05$). However, the SR of fish fed 45% protein diets was the highest (78.5%) compared to fish in the 40% and 50% protein diet groups (75.1%). The increase in lipid content of the diets from 6% to 9% and

12% resulted in a decline in the SRs of the fish (77.6, 76.4, and 74.7%, respectively). There was no interaction effect between dietary protein and lipid nutrients on the SR of the fish ($P > 0.05$) (Table 3).

FEED UTILIZATION EFFICIENCY

The average daily feed intake (DFI) ranged from 0.21 to 0.30 g/fish/day. The feed conversion ratio (FCR) and protein efficiency ratio (PER) were 1.91 to 4.07 and 0.81 to 1.70. These parameters were not separately affected by dietary protein or lipid levels except for DFI by dietary protein ($P < 0.05$). There was a significant interaction of the two dietary nutrients on the FCR but not on the DFI or PER. The DFI value of the diet of 45% protein and 6% lipid resulted in the lowest efficiency, and the diet of 50% protein and 9% lipid had the highest efficiency. The FCR of the diet of 40% protein and 9% lipid was the highest, and that of the diet of 45% protein and 9% lipid was the lowest (Table 5).

For the nutrient group analysis, the average DFI of the 50% protein diets was the highest (0.28 g/fish/day) compared to others (0.24 g/fish/day), and the average DFI of the 9% lipid diets was the highest (0.26 g/fish/day) followed by the 12% and 6% lipid diets (0.25 and 0.24 g/fish/day, respectively). The average FCR of the 40% protein diets was the highest (3.24), followed by the 50% and 45% protein levels (2.99 and 2.75, respectively). The increase in lipid level of the diets from 6% to 12% resulted in the decline of the FCR (3.49, 2.80, and 2.70, respectively). The average PER of the 50% protein diets was the highest (1.49), followed by the 40% and 45% protein diets (1.26 and 1.24, respec-

Table 4. Changes in weight of *M. favus* after 56 days of being reared with nine different diets

Diet No. (protein-lipid)	Initial body weight (IBW, g)	Final body weight (FBW, g)	Weight gain (WG, g)	Daily weight gain (DWG, g/day)	Specific growth rate (SGR, %/day)
Diet 1 (40-6)	2.87 ± 0.92	5.74 ± 1.85 ^b	2.87 ± 1.00 ^{ab}	0.20 ± 0.07 ^{ab}	4.95 ± 0.14
Diet 2 (40-9)	3.10 ± 0.34	5.48 ± 0.05 ^b	2.37 ± 0.38 ^b	0.17 ± 0.03 ^b	4.08 ± 0.20
Diet 3 (40-12)	3.06 ± 0.57	7.34 ± 1.27 ^{ab}	4.31 ± 0.76 ^{ab}	0.19 ± 0.05 ^{ab}	4.48 ± 0.13
Diet 4 (45-6)	3.37 ± 0.28	5.73 ± 0.54 ^b	2.36 ± 0.83 ^b	0.16 ± 0.06 ^b	3.70 ± 0.32
Diet 5 (45-9)	3.02 ± 0.37	8.44 ± 0.87 ^a	5.38 ± 0.49 ^a	0.43 ± 0.04 ^a	6.37 ± 0.04
Diet 6 (45-12)	2.97 ± 0.20	6.42 ± 0.34 ^{ab}	3.44 ± 0.23 ^{ab}	0.31 ± 0.02 ^{ab}	6.49 ± 0.08
Diet 7 (50-6)	3.26 ± 0.72	6.43 ± 1.98 ^{ab}	3.26 ± 2.46 ^{ab}	0.21 ± 0.18 ^{ab}	4.43 ± 0.80
Diet 8 (50-9)	2.81 ± 0.37	7.24 ± 0.40 ^{ab}	4.43 ± 0.76 ^{ab}	0.32 ± 0.05 ^{ab}	6.80 ± 0.32
Diet 9 (50-12)	2.97 ± 0.14	6.38 ± 0.28 ^{ab}	3.42 ± 0.22 ^{ab}	0.25 ± 0.02 ^{ab}	5.50 ± 0.06
40% protein	3.01 ± 0.12	6.19 ± 1.01	3.18 ± 0.99	0.19 ± 0.02 ^b	4.50 ± 0.44
45% protein	3.12 ± 0.22	6.86 ± 1.41	3.74 ± 1.55	0.30 ± 0.14 ^a	5.52 ± 1.58
50% protein	3.01 ± 0.23	6.68 ± 0.48	3.67 ± 0.67	0.26 ± 0.06 ^a	5.58 ± 1.19
6% lipid	3.17 ± 0.26	5.97 ± 0.40 ^b	2.80 ± 0.41 ^b	0.19 ± 0.03	4.36 ± 0.63
9% lipid	2.98 ± 0.15	7.05 ± 1.49 ^a	4.07 ± 1.55 ^a	0.31 ± 0.13	5.75 ± 1.46
12% lipid	3.00 ± 0.05	6.71 ± 0.54 ^{ab}	3.73 ± 0.49 ^a	0.25 ± 0.06	5.49 ± 1.01
ANOVA analysis of two factors					
Protein (P)	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> > 0.05
Lipid (L)	<i>P</i> > 0.05	<i>P</i> = 0.05	<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05
<i>P</i> × <i>L</i>	<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> = 0.05	<i>P</i> > 0.05

Notes: Values are presented as mean ± standard deviation; values in the same column with the same superscript are not significantly different (*p* > 0.05).

tively). The increase in the lipid level in the diets also resulted in a decline in the average PER (1.57, 1.22, and 1.20, respectively). There was no interaction effect between dietary protein and lipid on the DFI and PER (*P* > 0.05), but this effect was documented in the FCR (*P* < 0.05) (Table 5).

CHEMICAL COMPOSITION OF FISH

The proximate composition of the whole body of the fish fed the nine test diets is presented in Table 6. There were significant differences between means in the moisture and lipid contents (*P* < 0.05) but not between those of the organic matter and protein contents. The proximate composition was separately affected by lipid levels, except for the organic matter content, and was unaffected by the protein levels, except for the protein content (*P* > 0.05). There were significant interactive effects of the two dietary nutrients on the moisture and lipid contents, but not on organic matter or protein (*P* > 0.05).

For the nutrient group analysis, the average protein content of the 45% protein diets was the highest (25.4%) (*P* < 0.05). The increase in the lipid level in the diets resulted in a decline in the average protein content and an increase in the average lipid content. There were no significant inter-

action effects between dietary protein and lipid nutrients on the organic matter or protein contents, but there was a significant interaction in the moisture and lipid contents (*P* < 0.05) (Table 6).

DISCUSSION

The water quality parameters of the experimental tanks by treatments were 5.23 ± 0.24 to 5.62 ± 0.28 mg/L for dissolved oxygen, 27.2 ± 0.79 to 28.5 ± 1.97 °C for temperature, and 8.36 ± 1.12 to 8.47 ± 0.14 for pH. These parameters were suitable for the normal development of the fish.³⁵⁻³⁷

Nguyen *et al.*²⁸ found that a dietary content of 44% protein stimulated the best growth and the lowest FCR of *M. favus* fingerlings; they proposed that the value of 45.2% protein in the feed resulted in optimal growth of 2.7 g fish. Nguyen *et al.*³¹ also found that a dietary content of 7.5% lipid resulted in the maximum growth of *M. favus* fingerlings. Therefore, in the experiment involving the combination of three protein levels and three dietary lipid levels, it was reasonable to detect the protein-sparing effect of lipids in *M. favus* fingerlings. In this study, there was no interaction between dietary protein and lipid nutrients on the

Table 5. Feed utilization efficiency of *M. favus* after 56 days reared with nine different diets

Diet No. (protein-lipid)	Daily feed intake (DFI, g/fish/day)	Feed conversion ratio (FCR)	Protein efficiency ratio (PER)
Diet 1 (40-6)	0.23 ± 0.01 ^{ab}	3.41 ± 1.63 ^{ab}	1.36 ± 0.71
Diet 2 (40-9)	0.25 ± 0.02 ^{ab}	4.07 ± 0.71 ^a	1.61 ± 0.25
Diet 3 (40-12)	0.23 ± 0.02 ^{ab}	2.24 ± 0.29 ^{ab}	0.81 ± 0.11
Diet 4 (45-6)	0.21 ± 0.04 ^b	3.65 ± 1.28 ^{ab}	1.65 ± 0.60
Diet 5 (45-9)	0.25 ± 0.01 ^{ab}	1.91 ± 0.13 ^b	0.86 ± 0.05
Diet 6 (45-12)	0.26 ± 0.03 ^{ab}	2.70 ± 0.45 ^{ab}	1.22 ± 0.21
Diet 7 (50-6)	0.27 ± 0.02 ^{ab}	3.41 ± 5.15 ^{ab}	1.70 ± 2.55
Diet 8 (50-9)	0.30 ± 0.04 ^a	2.41 ± 0.34 ^{ab}	1.20 ± 0.18
Diet 9 (50-12)	0.27 ± 0.02 ^{ab}	3.15 ± 0.43 ^{ab}	1.57 ± 0.22
40% protein	0.24 ± 0.01 ^b	3.24 ± 0.93	1.26 ± 0.41
45% protein	0.24 ± 0.03 ^b	2.75 ± 0.87	1.24 ± 0.40
50% protein	0.28 ± 0.02 ^a	2.99 ± 0.52	1.49 ± 0.26
6% lipid	0.24 ± 0.03	3.49 ± 0.14 ^a	1.57 ± 0.18
9% lipid	0.26 ± 0.03	2.80 ± 0.13 ^b	1.22 ± 0.38
12% lipid	0.25 ± 0.02	2.70 ± 0.46 ^b	1.20 ± 0.38
ANOVA analysis of two factors			
Protein (P)	<i>P</i> < 0.01	<i>P</i> > 0.05	<i>P</i> > 0.05
Lipid (L)	<i>P</i> > 0.05	<i>P</i> = 0.05	<i>P</i> > 0.05
<i>P</i> × <i>L</i>	<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> > 0.05

Notes: Values are presented as mean ± standard deviation; values in the same column with the same superscript are not significantly different (*p* > 0.05).

Table 6. Proximate analysis of *M. favus* eels at the end of the feeding trial

Diet No. (protein-lipid)	Moisture (%)	Dry matter (%)	Protein (% dry matter basis)	Lipid (% dry matter basis)
Diet 1 (40-6)	68.9 ± 1.68 ^b	95.5 ± 0.39	22.9 ± 0.23	2.01 ± 0.08 ^b
Diet 2 (40-9)	77.8 ± 2.12 ^a	95.5 ± 0.83	20.2 ± 0.94	3.54 ± 0.15 ^{ab}
Diet 3 (40-12)	76.0 ± 2.19 ^a	95.3 ± 0.60	20.7 ± 0.93	4.04 ± 0.59 ^a
Diet 4 (45-6)	73.6 ± 0.30 ^{ab}	95.5 ± 0.13	25.7 ± 1.96	2.38 ± 0.20 ^{bc}
Diet 5 (45-9)	78.5 ± 1.59 ^a	95.7 ± 0.31	26.4 ± 0.69	3.76 ± 0.48 ^a
Diet 6 (45-12)	77.7 ± 1.02 ^a	95.4 ± 0.40	24.0 ± 2.33	4.14 ± 0.80 ^a
Diet 7 (50-6)	74.9 ± 3.12 ^a	95.2 ± 0.44	23.7 ± 1.59	3.45 ± 0.36 ^{ab}
Diet 8 (50-9)	74.6 ± 2.03 ^a	95.9 ± 0.35	23.0 ± 1.16	3.00 ± 0.26 ^{abc}
Diet 9 (50-12)	75.3 ± 2.35 ^a	95.7 ± 0.64	21.6 ± 1.75	4.17 ± 0.42 ^a
40% Protein	74.2 ± 4.46	95.4 ± 0.32	21.3 ± 1.76 ^b	3.20 ± 0.94
45% Protein	76.6 ± 2.81	95.6 ± 0.49	25.4 ± 1.33 ^a	3.43 ± 0.85
50% Protein	74.9 ± 1.71	95.6 ± 0.56	22.8 ± 1.80 ^b	3.54 ± 0.74
6% Lipid	72.5 ± 3.25 ^b	95.4 ± 0.56	24.1 ± 1.43 ^a	2.61 ± 0.72 ^c
9% Lipid	77.0 ± 2.04 ^a	95.7 ± 0.30	23.2 ± 3.10 ^{ab}	3.43 ± 0.59 ^b
12% Lipid	76.3 ± 2.44 ^a	95.5 ± 0.47	22.1 ± 1.99 ^b	4.12 ± 0.31 ^a
ANOVA analysis of two factors				
Protein (P)	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> > 0.05
Lipid (L)	<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05
<i>P</i> × <i>L</i>	<i>P</i> < 0.05	<i>P</i> > 0.05	<i>P</i> > 0.05	<i>P</i> < 0.05

growth performance of the fish (Tables 3 and 4). This result was similar to the findings of Hung *et al.*³ on red-tail catfish (*Hemibagrus wyckioides*), but different from that of Hien

*et al.*³⁸ on clown knife fish (*Chitala chitala*) fed diets with a combination of different protein and lipid levels. The protein-sparing effect of lipids in fish has been demonstrated

by many authors such as Orire & Sadiku⁸ in Nile tilapia (*Oreochromis niloticus*), Fan *et al.*¹⁰ in common carp (*Cyprinus carpio*), Hung *et al.*³ in *H. wyckioides*, and Welengane *et al.*³⁹ in hybrid fish tibatiga ($\text{♀}C. macropomum \times \text{♂}P. brachypomus$). However, Mohammadi *et al.*⁴⁰ found that growth indices were reduced by lipid contents increasing from 5 to 13% in combination with dietary protein levels of 15, 22, 29, and 36%, and concluded that the protein-sparing effect of lipids did not occur in *O. niloticus*. Hien *et al.*³⁸ found that the growth of the fish fed a dietary lipid level of 9% was the highest compared to the lipid levels of 6 and 12% at different dietary protein levels of 25, 30, 35 and 40%, and also concluded that the protein-sparing effect of lipids was not present in *C. chitala*. In this study, at low and high dietary protein levels (40 and 50%), the increase in lipid levels from 6 to 12% had no significant impact on length and weight gain. In contrast, at the dietary protein level of 45%, increasing lipid levels from 6 to 9% significantly improved the growth performance of fish, and a higher lipid level (12%) had no significant effect on fish growth (Tables 3 and 4). This implied that the protein-sparing effect of lipids occurred in *M. favus* fingerlings and the effect was obvious with dietary protein levels close to the optimum level (45%), as was found by Nguyen *et al.*²⁸ Orire & Sadiku⁸ found that the growth of *O. niloticus* fed Palm oil based diets containing three levels of protein (P) and three levels of lipid (L) ratios (15P:25L, 10P:30L and 5P: 35L%) declined when the lipid level was lowered to 5% and crude protein level increased to 35%. To detect the protein-sparing effect of lipids in *M. favus*, more studies need to be carried out with wider ranges of dietary protein and lipid content, and different fish sizes.

The correlations between changes in DFI and the dietary inclusion ratios of protein and lipids in fish fed diets with varying levels of these nutrients have shown inconsistent patterns. There were no correlations between DFI values and dietary protein or lipid levels in *H. wyckioides*.³ The DFI of snakehead fish (*Channa striata*) was not different from the combined protein and lipid diets.⁴¹ Total feed intake and DFI were reduced with the increase of dietary lipid levels at all protein inclusion ratios in *O. niloticus*³⁸ and tibatiga ($\text{♀}C. macropomum \times \text{♂}P. brachypomus$).³⁷ Welengane *et al.*³⁹ also found that increased protein levels lower the DFI of $\text{♀}C. macropomum \times \text{♂}P. brachypomus$ juveniles. In the present work, the average of DFI increased with increased dietary protein levels and was not correlated with lipid levels. This result was similar to the findings of Hung *et al.*,³ but different from the findings on $\text{♀}C. macropomum \times \text{♂}P. brachypomus$ ³⁹ and *O. niloticus*.⁴⁰ The correlation between DFI and dietary protein and lipid nutrients depends on the feeding habits of fish. There were no correlations in studies of carnivorous fish, e.g., *H. wyckioides*,³ *C. striata*,⁴¹ and *M. favus*, while such correlations were observed in omnivorous fish, e.g. *O. niloticus*⁴⁰ and $\text{♀}C. macropomum \times \text{♂}P. brachypomus$.³⁹

In species showing the protein-sparing effect of lipids, such as Nile perch (*Lates niloticus*), increasing dietary lipid levels improved the FCR of the fish fed with iso-protein level diets.⁴² In *O. niloticus*, the increment of lipid to the

highest level of 15% resulted in improved growth performance for all of the lipid sources of groundnut, palm, and fish oil; the palm oil-based diets had the highest level of growth and feed utilization, thereby yielding the lowest FCR.⁴¹ With two dietary levels of protein (39 and 44%) and three dietary levels of lipid (6, 9, and 12%), Hung *et al.*³ found that increasing dietary protein significantly improved FCR, but increasing dietary lipid significantly reduced the FCR of *H. wyckioides*. However, in species showing no protein-sparing effect of lipids such as *C. chitala*, in an experiment with four dietary levels of protein (25, 30, 35, and 40%) and three dietary levels of lipid (6, 9, and 12%), the FCR was reduced and reached the lowest values at the levels of 35% protein and 9% lipid.³⁸ Moreover, the FCR of *C. striata* fed a combined protein and lipid diet showed no differences at all levels of the two nutrients.⁴¹ In this work, with three dietary levels of protein (40, 45, and 50%) and lipid (6, 9, and 12%), the FCR values of *M. favus* were reduced with the increase in lipid levels. A similar result was found in *H. wyckioides*,³ and FCR was the lowest at 45% protein in *C. chitala*.³⁸

In the present study, the PER of *M. favus* fingerlings increased with the dietary protein level and declined with an increase in dietary lipid level. The increase in lipid and decrease in protein levels increased the PER of red-tail catfish.³ The decrease in PER with the increase in dietary lipid levels was found in *O. niloticus*⁴⁰ and *C. chitala*.³⁸ Therefore, the difference in the correlation of PER, as well as of FCR, and dietary protein and lipid levels between this study and others could be due to different experimental conditions, such as species, size, age, nutrient quality, environmental parameters, or other unknown factors.

This work has demonstrated that the body protein contents of *M. favus* were higher than those of *H. wyckioides*,³ the hybrid of $\text{♀}C. macropomum \times \text{♂}P. brachypomus$,³⁹ the Nile tilapia *O. niloticus*⁴⁰ and clown knife fish,³⁸ but lower than those of *L. niloticus*.⁴² The body lipid contents of the fish were lower than those of others.^{3,36-38,42} An increase in the dietary protein did not alter the carcass of *M. favus* in terms of moisture, organic matter, or lipid content. This effect was similar to that of *H. wyckioides*.³ The tendency of decrease in body protein contents with increasing dietary lipid levels of the present study was different from *C. chitala*,³⁸ but the tendency of increase in body lipid contents was in agreement with patterns observed in *L. niloticus*,⁴² the hybrid of $\text{♀}C. macropomum \times \text{♂}P. brachypomus$,³⁹ and *O. niloticus*.⁴⁰ The impact of dietary protein and lipid levels on the proximate composition of fish bodies tends to vary across species. However, a common trend observed in fish is that higher dietary lipid levels often lead to increased body lipid deposition.

The survival rate of *M. favus* eels in the present work was not affected by the experimental diets. Similar results were demonstrated for *H. wyckioides*,³ *C. carpio*,¹⁰ and *C. chitala*.³⁸

CONCLUSIONS

Based on the growth performances and FCR, the dietary requirements of 3-5 g *M. favus* fingerlings are 45% protein and 9% lipid, corresponding to an energy level of 3,819 Kcal. kg⁻¹ and a P/E of 118 mg. Kcal⁻¹.

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AUTHORS’ CONTRIBUTION - CREDIT

Conceptualization: Phan P. Loan (Lead). Methodology: Phan P. Loan (Equal), Nguyen Thanh Phuong (Equal). Investigation: Phan P. Loan (Lead). Resources: Phan P. Loan (Equal), Nguyen Thanh Phuong (Equal). Writing – original draft: Phan P. Loan (Lead). Supervision: Nguyen Thanh Phuong (Lead). Writing – review & editing: Nguyen Thanh Phuong (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

Tire Track Eel (*Mastacembelus favus*) is not considered endangered or protected, so experiments with this species in Vietnam do not require special permission. The animal experiment received approval from the Scientific and Training Council of the Faculty of Agriculture and Natural Resources of An Giang University (Approval No. 29A-08092023)

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data that has been used is confidential.

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