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## **Effects of Dietary Protein and Lipid Levels on Growth Performance, Feed Utilization, Plasma Biochemical Parameters, and Antioxidant Capacity of Asian Red-Tailed Catfish (*Hemibagrus wyckioides*)**

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**Key words:** Asian red-tailed catfish *Hemibagrus wyckioides*; growth performance; feed utilization; antioxidant capacity; protein to lipid ratio

### **Abstract**

A feeding trial was conducted to compare the effects of different dietary protein (37%, 43%, 49%) and lipid (6%, 10%, 14%) levels with protein to energy (P/E) ratios ranging from 17.76 to 24.77 g protein/MJ on growth performance, feed utilization, plasma biochemical parameters, and antioxidant capacity of Asian red-tailed catfish (*Hemibagrus wyckioides*). Each diet was randomly assigned to triplicate groups of 25 fish (initial average weight 14.88 g) per aquarium in a rearing system maintained at  $28 \pm 1^\circ\text{C}$  for 9 weeks. The highest values of weight gain (WG) and specific growth rate (SGR) were observed in fish fed a diet with 43% protein and 10% lipid. However, fish fed a 37% protein and 14% lipid diet had similar WG and SGR as those fed the diet with 43% protein and 10% lipid but showed the highest values of protein efficiency ratio (PER) and protein retention, the lowest values of plasma aspartate aminotransferase, and alanine aminotransferase activities. Fish fed diets with 14% lipid exhibited the lowest values of feed conversion ratio and plasma malondialdehyde (MDA) content, and the highest values of WG, SGR, PER and energy retention. Further, fish fed a diet with 37% protein and 14% lipid had relatively high plasma peroxidase and glutathione peroxidase activities, and low plasma MDA content among the dietary treatments. These results indicate that the increase of dietary lipid level has a protein-sparing effect, and a diet containing 37% protein and 14% lipid with P/E of 17.77 g protein/MJ is suitable for growth and health of Asian red-tailed catfish.

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## Introduction

Protein is the most crucial and expensive nutrient affecting fish growth performance and feed cost, and an adequate level is necessary to ensure healthy growth of fish (Shapawi et al., 2014; Rahimnejad et al., 2015). However, excess dietary protein intake should be avoided due to increased feed cost and nitrogen emission into the environment (NRC, 2011). Thus, it is essential to improve protein utilization for body protein synthesis rather than for energy purposes. It is well known that lipid can be utilized as a dietary energy source and thereby spare protein for growth, and can limit ammonia production (Lee et al., 2002). Meanwhile, the excess of dietary lipid can also result in side effects including body lipid deposition and fatty liver disease (Gao et al., 2009). Thus, it is important to optimize dietary protein to energy (P/E) ratio for high-efficiency and environmentally friendly compound feed. Numerous studies have been conducted to evaluate the optimal dietary P/E ratio for some cultured fish (Wang et al., 2017; Zhang et al., 2018).

Asian red-tailed catfish (*Hemibagrus wyckioides*) is an economically important farmed freshwater fish widely distributed in the Lancang-Mekong River Basin including Laos, Myanmar, Thailand, Vietnam, Cambodia, and southwest China (Prasertwattana et al., 2005). This species is the largest bagrid catfish with wide adaptation, high disease resistance, and hypoxia tolerance, and may reach 80 kg in the wild (Ng and Rainboth, 1999; Amornsakun, 2000). Further, unlike other catfish there are no cannibalistic phenomena in the rearing of this species (Jiwyam and Nithikulworawong, 2014). This makes it an excellent species for intensive aquaculture (Ng and Rainboth, 1999). At present, Asian red-tailed catfish is popular in pond and cage culture in the Lancang-Mekong River Basin (Prasertwattana et al., 2005). However, limited information on the nutritional requirements of this species is available (Deng et al., 2011; Hung et al., 2015 -These are second-hand citations). Using 2×3 factorial design the dietary protein to lipid ratio for Asian red-tailed catfish was determined, whereas the dietary P/E ratio was not clearly determined (Hung et al. 2015). The objective of the present study was to evaluate the effects of dietary P/E ratio on growth performance, feed utilization, plasma biochemical parameters, and antioxidant capacity of Asian red-tailed catfish.

## Materials and Methods

### *Experimental diets.*

Fish meal, fish protein concentrate, and soy protein concentrate were used as dietary protein sources, fish oil, and soybean lecithin as lipid sources, and dextrin and wheat flour as carbohydrate sources. A 3×3 factorial design with three replicates per dietary treatment was used in this study. Nine experimental diets (P37L6, P37L10, P37L14, P43L6, P43L10, P43L14, P49L6, P49L10 and P49L14) were formulated to contain three crude protein levels (37%, 43%, 49%), and each protein level with three crude lipid levels (6%, 10%, 14%) to produce P/E ratios ranging from 17.76 to 24.77 g protein/MJ. Feed ingredients were ground into fine powder through a 320-µm mesh. After thoroughly mixing the dry ingredients, fish oil and soybean lecithin together with distilled water were added to produce dough. The dough was then extruded using a pellet feed maker (KS-180; Jiangsu Jingu Rice Mill Co., Ltd., Jiangsu, China) through a 2-mm die. The moist pellets were dried in a forced air oven at room temperature for about 12 h and stored at -20°C until use.

### *Experimental animals and conditions.*

Juvenile Asian red-tailed catfish were obtained from a private hatchery (Zhangming Fisheries Co., Ltd., Hekou, Yunnan, China). The fish were acclimated to the experimental tank conditions and fed a commercial feed (42% crude protein, 12% crude lipid; supplied by Beijing Bio-Tech Co., Ltd., Beijing, China) for 2 weeks. At the end of the acclimation period, a total of 675 fish (initial average weight 14.88 g) were randomly distributed into 27 flow-through fiberglass tanks (1.0 × 0.7 × 0.8 m<sup>3</sup>) with 25 juveniles per tank. Each tank was then randomly assigned to one of three replicates of the nine dietary treatments. Fish were hand-fed to apparent satiation twice daily (07:30 and 17:30) for 9 weeks. Water was recirculated through a 4000-L biological and mechanical filtration system containing vertical quartz sand filter and activated carbon purifier to remove solid and nitrogenous wastes, and water temperature was maintained at 28 ± 1°C. All rearing tanks were provided with continuous aeration and maintained under natural photoperiod.

**Table 1.** Formulation and proximate composition of experimental diets for Asian red-tailed catfish,

*Hemibagrus wyckioides* (% dry matter).

| Dietary protein level (%)                        | 37%   |       |       | 43%   |       |       | 49%   |       |       |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|  | 6%    | 10%   | 14%   | 6%    | 10%   | 14%   | 6%    | 10%   | 14%   |
| Dietary lipid level (%)                          |       |       |       |       |       |       |       |       |       |
| Fish meal <sup>1</sup>                           | 26.00 | 26.00 | 26.00 | 30.00 | 30.00 | 30.00 | 34.00 | 34.00 | 34.00 |
| Fish protein concentrate <sup>2</sup>            | 8.00  | 8.00  | 8.00  | 10.00 | 10.00 | 10.00 | 12.00 | 12.00 | 12.00 |
| Soy protein concentrate <sup>3</sup>             | 17.00 | 18.00 | 19.00 | 20.00 | 21.00 | 22.00 | 24.00 | 25.00 | 26.00 |
| Wheat flour                                      | 33.17 | 28.17 | 23.17 | 24.77 | 19.77 | 14.77 | 15.37 | 10.37 | 5.37  |
| Dextrin <sup>4</sup>                             | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Fish oil   | 1.20  | 5.20  | 9.20  | 0.60  | 4.60  | 8.60  | 0.00  | 4.00  | 8.00  |
| Soybean lecithin (40%) <sup>1</sup>              | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  |
| Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  |
| Choline chloride (50%) <sup>1</sup>              | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  | 0.30  |
| Vitamin C <sup>5</sup>                           | 0.10  | 0.10  | 0.10  | 0.10  | 0.10  | 0.10  | 0.10  | 0.10  | 0.10  |
| Ethoxyquin (30%) <sup>1</sup>                    | 0.03  | 0.03  | 0.03  | 0.03  | 0.03  | 0.03  | 0.03  | 0.03  | 0.03  |
| Mineral premix <sup>6</sup>                      | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  |
| Vitamin premix <sup>7</sup>                      | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  | 1.00  |
| <i>Proximate composition</i>                     |       |       |       |       |       |       |       |       |       |
| Dry matter (DM, %)                               | 91.18 | 92.49 | 92.45 | 90.70 | 91.48 | 92.04 | 89.78 | 91.70 | 93.51 |
| Crude protein (% DM)                             | 37.42 | 37.24 | 37.75 | 43.09 | 43.41 | 43.00 | 49.40 | 49.46 | 49.33 |
| Crude lipid (% DM)                               | 6.60  | 10.67 | 14.42 | 6.49  | 10.39 | 14.85 | 6.53  | 10.44 | 14.55 |
| Ash (% DM)                                       | 8.59  | 8.60  | 8.51  | 9.36  | 9.36  | 9.38  | 10.20 | 10.22 | 10.17 |
| Gross energy (kJ/g)                              | 19.47 | 20.37 | 21.26 | 19.68 | 20.57 | 21.54 | 19.94 | 20.82 | 21.74 |
| P/E (g protein/MJ)                               | 19.22 | 18.28 | 17.76 | 21.90 | 21.10 | 19.96 | 24.77 | 23.75 | 22.69 |

<sup>1</sup> Kunming Tianyuan Feed Co., Ltd., Yunnan, China.

<sup>2</sup> Fisheries Research Institute of Shanghai, Shanghai, China.

<sup>3</sup> Dongying Wonderful Vegetable Protein Science and Technology Co., Ltd., Shandong, China.

<sup>4</sup> Qufu Tianli Medical Supplements Co., Ltd., Shandong, China.

<sup>5</sup> L-Ascorbate-2-polyphosphate (35%).

<sup>6</sup> g/kg mixture: MgSO<sub>4</sub>·7H<sub>2</sub>O, 180; KI, 1; FeSO<sub>4</sub>·H<sub>2</sub>O, 260; ZnSO<sub>4</sub>·H<sub>2</sub>O, 180; CuSO<sub>4</sub>·5H<sub>2</sub>O, 25; Na<sub>2</sub>Se<sub>2</sub>O<sub>3</sub>, 0.01; MnSO<sub>4</sub>·H<sub>2</sub>O, 180; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.75.

<sup>7</sup> g/kg mixture: retinyl acetate (2800000 IU/g), 2; cholecalciferol, 0.03; DL- $\alpha$ -tocopheryl acetate, 30; menadione, 3; thiamine hydrochloride, 8; riboflavin, 11; pyridoxine hydrochloride, 8; vitamin B<sub>12</sub>, 0.02; ascorbic acid, 50; folic acid, 1; biotin, 0.1; niacin, 30; calcium D-pantothenate, 32; inositol, 25.

*Sample collection and analysis.*

At the end of the feeding trial, fish were fasted for 24 h before harvest. All experimental fish were anesthetized with eugenol (1:12000) and weighed to calculate growth rate and feed utilization. A total of fifteen fish at initiation of feeding trial and five fish per tank at termination were randomly collected and stored at -20°C for proximate composition analyses. Another six fish per tank were sampled for analysis of biochemical parameters in plasma. Plasma samples were collected from the caudal vein with a heparinized syringe and transferred into a heparinized tube. Plasma was recovered after centrifugation (6000 g, 10 min) and immediately stored at -80°C until analysis. All samples were pooled by tank of analysis.

Analysis of dry matter (105°C, 24 h), crude protein (Kjeldahl nitrogen  $\times$  6.25), crude lipid (ether extraction by Soxhlet method), and ash (550°C, 18 h) in feed ingredients, experimental diets, and whole-body samples were performed following standard laboratory procedures (AOAC, 1995). Gross energy content in experimental diets and whole-body samples were measured using a bomb calorimeter (Parr 1351; Parr Instrument Co., Moline, IL, USA). Plasma total protein, blood urea nitrogen (BUN), calcium, phosphorus, malondialdehyde (MDA), nitric oxide (NO), total cholesterol (TC), triglyceride (TAG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents, and  $\gamma$ -glutamyl transferase ( $\gamma$ -GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AKP), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPx) and total antioxidant capacity (TAC) activities were determined by colorimetric enzymatic methods using commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). One unit of  $\gamma$ -GT is defined as the amount of enzyme that produced 1 mmol of p-nitroaniline per min. One unit of AST is defined as the amount of enzyme that will generate 1 mmol of glutamate per min. One unit of ALT is defined as the amount of enzyme that generates 1 mmol of pyruvate per min. One unit of AKP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg phenol in 15 min. One unit of

CAT activity was defined as the amount of enzyme that catalyzed the decomposition of 1 mmol of H<sub>2</sub>O<sub>2</sub> per min. One unit of POD activity was defined as the amount of enzyme required to produce 1 µg substrate-enzyme per milliliter plasma per min. One unit of GPx activity was defined as the amount of enzyme that reduced the glutathione concentration in the reaction system at 1 µmol/L per min. One unit of TAC was defined as a 0.01 increment of absorbance of the reaction system caused by plasma per milliliter for 1 min.

#### Statistical analysis.

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

## Results

### Growth performance and feed utilization.

Survival of fish ranged from 91.7% to 100% and was not significantly different between the differing dietary treatments (Table 2). Dietary protein and lipid levels either individually or in combination had significant effects on feed intake (FI), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). The highest values of WG (159%) and SGR (1.51%/d) were observed in fish fed the P43L10 diet, whereas the lowest values (124%, 1.28%/d) were recorded in fish fed the P37L6 diet. The best FCR and PER were observed in fish fed the P43L6 and P37L14 diets, respectively. With respect to dietary protein and lipid levels, WG and SGR were higher in fish fed diets with 43% protein compared to fish fed diets with 37% or 49% protein, and higher in fish fed diets with 10% or 14% lipid compared to fish fed diets with 6% lipid. FCR was lower in fish fed diets with 43% protein compared to fish fed diets with 37% or 49% protein, and lower in fish fed diets with 14% lipids compared to fish fed diets with 6% lipid. Conversely, PER was higher in fish fed diets with 37% protein compared to fish fed diets with 43% or 49% protein, and higher in fish fed diets with 10% or 14% lipids compared to fish fed diets with 6% lipid.

**Table 2.** Growth performance of Asian red-tailed catfish *Hemibagrus wyckioides* fed diets with different protein and lipid levels for 9 weeks.

| Diet              | Final body Weight (g)      | Feed intake <sup>1</sup> (g/fish) | Weight gain <sup>2</sup>  | SGR <sup>3</sup> (%/day)  | FCR <sup>4</sup>          | PER <sup>5</sup>         | Survival (%) |
|-------------------|----------------------------|-----------------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------|
| P37L6             | 33.48 ± 0.18 <sup>a</sup>  | 32.73 ± 0.06 <sup>abc</sup>       | 1.24 ± 0.01 <sup>a</sup>  | 1.28 ± 0.01 <sup>a</sup>  | 1.77 ± 0.01 <sup>c</sup>  | 1.51 ± 0.01 <sup>a</sup> | 100.0 ± 0.0  |
| P37L10            | 36.86 ± 1.07 <sup>b</sup>  | 33.13 ± 0.49 <sup>bc</sup>        | 1.49 ± 0.07 <sup>b</sup>  | 1.45 ± 0.05 <sup>c</sup>  | 1.51 ± 0.05 <sup>ab</sup> | 1.79 ± 0.06 <sup>b</sup> | 95.8 ± 4.2   |
| P37L14            | 37.83 ± 0.48 <sup>b</sup>  | 33.51 ± 0.45 <sup>c</sup>         | 1.54 ± 0.03 <sup>b</sup>  | 1.48 ± 0.02 <sup>c</sup>  | 1.46 ± 0.02 <sup>a</sup>  | 1.81 ± 0.03 <sup>b</sup> | 96.9 ± 3.1   |
| P43L6             | 37.44 ± 0.90 <sup>b</sup>  | 30.91 ± 0.38 <sup>a</sup>         | 1.52 ± 0.06 <sup>b</sup>  | 1.46 ± 0.04 <sup>c</sup>  | 1.37 ± 0.04 <sup>a</sup>  | 1.48 ± 0.05 <sup>a</sup> | 95.8 ± 2.1   |
| P43L10            | 38.63 ± 0.35 <sup>b</sup>  | 34.12 ± 0.31 <sup>c</sup>         | 1.59 ± 0.03 <sup>b</sup>  | 1.51 ± 0.02 <sup>c</sup>  | 1.44 ± 0.03 <sup>a</sup>  | 1.41 ± 0.03 <sup>a</sup> | 91.7 ± 2.1   |
| P43L14            | 38.17 ± 0.50 <sup>b</sup>  | 33.22 ± 0.14 <sup>bc</sup>        | 1.58 ± 0.04 <sup>b</sup>  | 1.50 ± 0.02 <sup>c</sup>  | 1.42 ± 0.04 <sup>a</sup>  | 1.43 ± 0.04 <sup>a</sup> | 97.9 ± 2.1   |
| P49L6             | 33.76 ± 0.05 <sup>a</sup>  | 30.95 ± 0.38 <sup>a</sup>         | 1.27 ± 0.01 <sup>a</sup>  | 1.30 ± 0.01 <sup>ab</sup> | 1.64 ± 0.03 <sup>bc</sup> | 1.42 ± 0.03 <sup>a</sup> | 96.9 ± 3.1   |
| P49L10            | 36.17 ± 0.18 <sup>ab</sup> | 31.49 ± 0.33 <sup>ab</sup>        | 1.43 ± 0.01 <sup>ab</sup> | 1.41 ± 0.01 <sup>bc</sup> | 1.48 ± 0.02 <sup>a</sup>  | 1.55 ± 0.02 <sup>a</sup> | 93.8 ± 6.3   |
| P49L14            | 36.71 ± 0.40 <sup>b</sup>  | 32.59 ± 0.66 <sup>abc</sup>       | 1.47 ± 0.03 <sup>b</sup>  | 1.43 ± 0.02 <sup>c</sup>  | 1.49 ± 0.01 <sup>ab</sup> | 1.56 ± 0.01 <sup>a</sup> | 91.7 ± 8.3   |
| Protein level (%) |                            |                                   |                           |                           |                           |                          |              |
| 37                | 36.05 ± 0.74 <sup>u</sup>  | 33.13 ± 0.22 <sup>v</sup>         | 1.42 ± 0.05 <sup>u</sup>  | 1.40 ± 0.03 <sup>u</sup>  | 1.58 ± 0.05 <sup>v</sup>  | 1.70 ± 0.05 <sup>v</sup> | 97.7 ± 1.6   |
| 43                | 38.08 ± 0.36 <sup>v</sup>  | 32.75 ± 0.50 <sup>w</sup>         | 1.56 ± 0.03 <sup>v</sup>  | 1.49 ± 0.02 <sup>v</sup>  | 1.41 ± 0.02 <sup>u</sup>  | 1.44 ± 0.02 <sup>u</sup> | 95.1 ± 1.4   |
| 49                | 35.55 ± 0.47 <sup>u</sup>  | 31.68 ± 0.34 <sup>u</sup>         | 1.39 ± 0.03 <sup>u</sup>  | 1.38 ± 0.02 <sup>u</sup>  | 1.54 ± 0.03 <sup>v</sup>  | 1.51 ± 0.03 <sup>u</sup> | 93.8 ± 3.6   |
| Lipid level (%)   |                            |                                   |                           |                           |                           |                          |              |
| 6                 | 34.89 ± 0.69 <sup>x</sup>  | 31.53 ± 0.34 <sup>x</sup>         | 1.34 ± 0.05 <sup>x</sup>  | 1.35 ± 0.03 <sup>x</sup>  | 1.59 ± 0.06 <sup>y</sup>  | 1.47 ± 0.02 <sup>x</sup> | 97.7 ± 1.1   |
| 10                | 37.22 ± 0.49 <sup>y</sup>  | 32.91 ± 0.43 <sup>y</sup>         | 1.50 ± 0.03 <sup>y</sup>  | 1.46 ± 0.03 <sup>y</sup>  | 1.48 ± 0.02 <sup>xy</sup> | 1.58 ± 0.04 <sup>y</sup> | 93.8 ± 2.0   |
| 14                | 37.57 ± 0.32 <sup>y</sup>  | 33.11 ± 0.27 <sup>y</sup>         | 1.53 ± 0.02 <sup>y</sup>  | 1.47 ± 0.02 <sup>y</sup>  | 1.46 ± 0.02 <sup>x</sup>  | 1.60 ± 0.04 <sup>y</sup> | 95.3 ± 3.1   |
| ANOVA (P value)   |                            |                                   |                           |                           |                           |                          |              |
| Protein           | <0.001                     | <0.001                            | <0.001                    | <0.001                    | <0.001                    | <0.001                   | 0.528        |
| Lipid             | <0.001                     | <0.001                            | <0.001                    | <0.001                    | <0.001                    | 0.001                    | 0.561        |
| Protein × lipid   | 0.046                      | 0.003                             | 0.049                     | 0.043                     | 0.001                     | 0.001                    | 0.828        |

\*Values in a column with different superscripts significantly differ.

<sup>1</sup> Feed intake:  $\Sigma[(\text{daily feed consumption per tank(g)})/(\text{number of fish})]$ .

<sup>2</sup> Weight gain:  $(\text{final body weight (g)} - \text{initial body weight (g)})/\text{initial body weight (g)}$ .

<sup>3</sup> SGR: specific growth rate =  $((\ln \text{ final body weight} - \ln \text{ initial body weight})/\text{feeding days}) \times 100$ .

<sup>4</sup> FCR: feed conversion ratio =  $\text{dry feed fed (g)}/\text{weight gain (g)}$ .

<sup>5</sup> PER: protein efficiency ratio =  $\text{weight gain (g)}/\text{protein fed (g)}$ .

### Protein and energy retention.

Dietary treatments significantly affected protein intake, energy intake, protein gain,

energy gain, protein retention, and energy retention (Table 3). However, energy intake was not affected by dietary protein level; protein intake and protein retention were not affected by dietary lipid level. The highest values of protein intake (10.01 g/kg ABW/day), energy intake (0.43 MJ/kg ABW/day), protein gain (2.41 g/kg ABW/day), energy gain (0.14 MJ/kg ABW/day), protein retention (31.59%) and energy retention (32.74%) were observed in fish fed the P49L10, P49L14, P37L14, P37L14, P37L14 and P43L10 diets, respectively. Conversely, the lowest values of protein intake (7.59 g/kg ABW/day), energy intake (0.38 MJ/kg ABW/day), protein gain (2.10 g/kg ABW/day), energy gain (0.10 MJ/kg ABW/day), protein retention (23.68%) and energy retention (24.69%) were recorded in fish fed the P37L10, P49L6, P37L6, P37L6, P49L14 and P37L6 diets, respectively. With respect to dietary protein and lipid levels, protein intake, energy gain, and energy retention of fish increased with increasing dietary protein level. Protein gain was higher in fish fed diets with 49% protein compared to fish fed diets with 37% or 43% protein. However, protein retention was higher in fish fed diets with 37% protein compared to fish fed diets with 43% or 49% protein. Energy intake, protein gain, energy gain and energy retention of fish increased with increasing dietary lipid level.

**Table 3.** Protein and energy utilization of Asian red-tailed catfish *Hemibagrus wyckioides* fed diets with different protein and lipid levels.

| Diet                    | Protein intake<br>(g/kg ABW/day) | Energy intake<br>(MJ/kg ABW/day) | Protein gain<br>(g/kg ABW/day) | Energy gain<br>(kJ/kg ABW/day) | Protein retention <sup>3</sup><br>(%) | Energy retention <sup>4</sup><br>(%) |
|-------------------------|----------------------------------|----------------------------------|--------------------------------|--------------------------------|---------------------------------------|--------------------------------------|
| P37L6                   | 8.03 ± 0.03 <sup>ab</sup>        | 0.42 ± 0.00 <sup>bc</sup>        | 2.10 ± 0.02 <sup>a</sup>       | 0.10 ± 0.00 <sup>a</sup>       | 26.19 ± 0.29 <sup>ab</sup>            | 24.69 ± 0.28 <sup>a</sup>            |
| P37L10                  | 7.59 ± 0.05 <sup>a</sup>         | 0.42 ± 0.00 <sup>bc</sup>        | 2.21 ± 0.06 <sup>abc</sup>     | 0.12 ± 0.01 <sup>bc</sup>      | 29.19 ± 0.62 <sup>bc</sup>            | 29.10 ± 0.89 <sup>b</sup>            |
| P37L14                  | 7.62 ± 0.07 <sup>a</sup>         | 0.43 ± 0.00 <sup>c</sup>         | 2.41 ± 0.04 <sup>d</sup>       | 0.14 ± 0.01 <sup>d</sup>       | 31.59 ± 0.61 <sup>c</sup>             | 32.37 ± 0.55 <sup>b</sup>            |
| P43L6                   | 8.71 ± 0.11 <sup>c</sup>         | 0.40 ± 0.01 <sup>b</sup>         | 2.14 ± 0.01 <sup>ab</sup>      | 0.12 ± 0.00 <sup>ab</sup>      | 24.64 ± 0.39 <sup>a</sup>             | 29.20 ± 0.17 <sup>b</sup>            |
| P43L10                  | 8.49 ± 0.08 <sup>bc</sup>        | 0.40 ± 0.00 <sup>b</sup>         | 2.22 ± 0.01 <sup>abc</sup>     | 0.13 ± 0.00 <sup>cd</sup>      | 26.15 ± 0.29 <sup>ab</sup>            | 32.74 ± 0.92 <sup>b</sup>            |
| P43L14                  | 8.62 ± 0.12 <sup>c</sup>         | 0.43 ± 0.01 <sup>c</sup>         | 2.25 ± 0.01 <sup>abcd</sup>    | 0.13 ± 0.00 <sup>cd</sup>      | 26.13 ± 0.27 <sup>ab</sup>            | 30.37 ± 0.69 <sup>b</sup>            |
| P49L6                   | 9.26 ± 0.12 <sup>d</sup>         | 0.38 ± 0.00 <sup>a</sup>         | 2.41 ± 0.05 <sup>d</sup>       | 0.12 ± 0.00 <sup>bc</sup>      | 26.00 ± 0.79 <sup>a</sup>             | 32.59 ± 0.64 <sup>b</sup>            |
| P49L10                  | 10.01 ± 0.11 <sup>e</sup>        | 0.42 ± 0.01 <sup>bc</sup>        | 2.39 ± 0.06 <sup>cd</sup>      | 0.13 ± 0.00 <sup>bcd</sup>     | 23.91 ± 0.84 <sup>a</sup>             | 30.75 ± 0.42 <sup>b</sup>            |
| P49L14                  | 9.76 ± 0.13 <sup>e</sup>         | 0.43 ± 0.01 <sup>c</sup>         | 2.31 ± 0.03 <sup>bcd</sup>     | 0.14 ± 0.00 <sup>d</sup>       | 23.68 ± 0.66 <sup>a</sup>             | 32.41 ± 0.92 <sup>b</sup>            |
| Protein level (%)       |                                  |                                  |                                |                                |                                       |                                      |
| 37                      | 7.74 ± 0.08 <sup>u</sup>         | 0.42 ± 0.00                      | 2.24 ± 0.05 <sup>u</sup>       | 0.12 ± 0.01 <sup>u</sup>       | 28.99 ± 0.86 <sup>v</sup>             | 28.72 ± 0.59 <sup>u</sup>            |
| 43                      | 8.61 ± 0.06 <sup>v</sup>         | 0.41 ± 0.01                      | 2.21 ± 0.02 <sup>u</sup>       | 0.13 ± 0.00 <sup>uv</sup>      | 25.64 ± 0.30 <sup>u</sup>             | 30.77 ± 0.62 <sup>uv</sup>           |
| 49                      | 9.68 ± 0.13 <sup>w</sup>         | 0.41 ± 0.01                      | 2.37 ± 0.03 <sup>v</sup>       | 0.13 ± 0.00 <sup>v</sup>       | 24.53 ± 0.53 <sup>u</sup>             | 31.92 ± 0.45 <sup>v</sup>            |
| Lipid level (%)         |                                  |                                  |                                |                                |                                       |                                      |
| 6                       | 8.67 ± 0.18                      | 0.40 ± 0.01 <sup>x</sup>         | 2.22 ± 0.05 <sup>x</sup>       | 0.11 ± 0.00 <sup>x</sup>       | 25.61 ± 0.36                          | 28.83 ± 0.62 <sup>x</sup>            |
| 10                      | 8.70 ± 0.36                      | 0.41 ± 0.00 <sup>xy</sup>        | 2.27 ± 0.04 <sup>xy</sup>      | 0.13 ± 0.00 <sup>y</sup>       | 26.42 ± 0.86                          | 30.87 ± 0.65 <sup>xy</sup>           |
| 14                      | 8.67 ± 0.31                      | 0.43 ± 0.00 <sup>y</sup>         | 2.32 ± 0.03 <sup>y</sup>       | 0.14 ± 0.00 <sup>z</sup>       | 27.13 ± 0.97                          | 31.72 ± 0.59 <sup>y</sup>            |
| ANOVA ( <i>P</i> value) |                                  |                                  |                                |                                |                                       |                                      |
| Protein                 | <0.001                           | 0.332                            | <0.001                         | 0.020                          | <0.001                                | 0.034                                |
| Lipid                   | 0.910                            | <0.001                           | 0.009                          | <0.001                         | 0.328                                 | 0.046                                |
| Protein × lipid         | <0.001                           | <0.001                           | 0.001                          | 0.026                          | <0.001                                | <0.001                               |

\*Values in a column with different superscripts significantly differ.

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

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

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

#### Plasma biochemical parameters.

Dietary treatments significantly affected plasma calcium and phosphorus contents, and  $\gamma$ -GT, AST, ALT and AKP activities, but did not affect plasma total protein and BUN contents (Table 4). However, plasma AKP activity was not affected by dietary lipid level. The highest values of plasma calcium and phosphorus contents, and  $\gamma$ -GT, AST, ALT and AKP activities were observed in fish fed the P49L6, P49L14, P37L6, P49L6, P49L6 and P49L14 diets, whereas the lowest values were recorded in fish fed the P43L10, P37L10, P43L10, P37L14, P37L14 and P43L10 diets, respectively. With respect to dietary protein and lipid levels, fish fed diets with 49% protein had the highest plasma total protein, calcium, and phosphorus contents, and ALT and AKP activities. However, fish fed diets with 37% protein had the highest plasma  $\gamma$ -GT activity and the lowest plasma AST activity. Plasma calcium content, and  $\gamma$ -GT, AST and ALT activities were higher in fish fed diets with 6% lipid compared to fish fed diets with 10% or 14% lipid. Fish fed diets with 14% lipid had the highest plasma phosphorus content.

**Table 4.** Plasma total protein, blood urea nitrogen (BUN), calcium and phosphorus contents, and  $\gamma$ -glutamyl transferase ( $\gamma$ -GT), aspartate aminotransferase (AST), Alanine amino-transferase (ALT) and alkaline phosphatase (AKP) activities of Asian red-tailed catfish *Hemibagrus wyckioides* fed diets with different protein and lipid levels.

| Diet              | Total protein (g/L)        | BUN (mmol/L) | Calcium (mmol/L)          | Phosphorus (mmol/L)       | $\gamma$ -GT (mmol/L)      | AST (IU/L)                 | ALT (IU/L)                | AKP (mmol/L)              |
|-------------------|----------------------------|--------------|---------------------------|---------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
| P37L6             | 31.13 ± 1.72               | 1.52 ± 0.12  | 3.73 ± 0.21 <sup>bc</sup> | 1.47 ± 0.13 <sup>a</sup>  | 65.70 ± 4.36 <sup>c</sup>  | 55.00 ± 7.61 <sup>a</sup>  | 20.26 ± 1.38 <sup>a</sup> | 5.82 ± 0.81 <sup>ab</sup> |
| P37L10            | 31.13 ± 3.54               | 1.40 ± 0.02  | 3.32 ± 0.24 <sup>ab</sup> | 1.45 ± 0.02 <sup>a</sup>  | 33.72 ± 1.34 <sup>ab</sup> | 35.88 ± 3.48 <sup>a</sup>  | 17.55 ± 0.96 <sup>a</sup> | 5.82 ± 1.04 <sup>ab</sup> |
| P37L14            | 30.80 ± 2.68               | 1.52 ± 0.08  | 3.25 ± 0.15 <sup>ab</sup> | 1.69 ± 0.02 <sup>ab</sup> | 42.18 ± 1.68 <sup>b</sup>  | 35.79 ± 4.18 <sup>a</sup>  | 13.97 ± 1.04 <sup>a</sup> | 5.22 ± 0.68 <sup>ab</sup> |
| P43L6             | 30.14 ± 4.30               | 1.56 ± 0.21  | 3.92 ± 0.15 <sup>bc</sup> | 1.60 ± 0.02 <sup>ab</sup> | 44.19 ± 0.67 <sup>b</sup>  | 66.92 ± 5.62 <sup>bc</sup> | 14.59 ± 0.73 <sup>b</sup> | 5.62 ± 0.63 <sup>ab</sup> |
| P43L10            | 32.04 ± 1.67               | 1.63 ± 0.02  | 2.57 ± 0.04 <sup>a</sup>  | 1.57 ± 0.05 <sup>ab</sup> | 26.16 ± 1.68 <sup>a</sup>  | 43.20 ± 2.27 <sup>ab</sup> | 16.92 ± 1.72 <sup>a</sup> | 5.02 ± 0.58 <sup>a</sup>  |
| P43L14            | 27.90 ± 1.29               | 1.60 ± 0.07  | 3.73 ± 0.21 <sup>bc</sup> | 1.83 ± 0.12 <sup>ab</sup> | 33.14 ± 1.68 <sup>ab</sup> | 47.94 ± 2.78 <sup>ab</sup> | 16.56 ± 1.14 <sup>a</sup> | 6.22 ± 0.58 <sup>ab</sup> |
| P49L6             | 34.19 ± 0.43               | 1.60 ± 0.18  | 4.22 ± 0.11 <sup>c</sup>  | 1.83 ± 0.04 <sup>ab</sup> | 42.44 ± 1.33 <sup>b</sup>  | 82.71 ± 8.39 <sup>c</sup>  | 27.72 ± 2.11 <sup>b</sup> | 7.15 ± 0.46 <sup>ab</sup> |
| P49L10            | 31.63 ± 0.86               | 1.43 ± 0.08  | 3.81 ± 0.08 <sup>bc</sup> | 1.52 ± 0.07 <sup>ab</sup> | 36.63 ± 3.02 <sup>ab</sup> | 38.75 ± 2.53 <sup>a</sup>  | 16.26 ± 1.71 <sup>a</sup> | 7.03 ± 0.35 <sup>ab</sup> |
| P49L14            | 38.09 ± 0.57               | 1.40 ± 0.08  | 3.28 ± 0.08 <sup>ab</sup> | 1.85 ± 0.12 <sup>b</sup>  | 41.86 ± 3.35 <sup>b</sup>  | 40.17 ± 4.03 <sup>a</sup>  | 16.55 ± 0.76 <sup>a</sup> | 7.83 ± 0.35 <sup>b</sup>  |
| Protein level (%) |                            |              |                           |                           |                            |                            |                           |                           |
| 37                | 31.02 ± 1.38 <sup>uv</sup> | 1.48 ± 0.05  | 3.43 ± 0.13 <sup>u</sup>  | 1.54 ± 0.05 <sup>u</sup>  | 46.90 ± 5.02 <sup>w</sup>  | 42.22 ± 4.18 <sup>u</sup>  | 17.26 ± 1.12 <sup>u</sup> | 5.62 ± 0.39 <sup>u</sup>  |
| 43                | 30.03 ± 1.51 <sup>u</sup>  | 1.60 ± 0.06  | 3.41 ± 0.22 <sup>u</sup>  | 1.67 ± 0.06 <sup>uv</sup> | 34.50 ± 2.72 <sup>u</sup>  | 52.69 ± 4.10 <sup>v</sup>  | 16.02 ± 0.71 <sup>u</sup> | 5.62 ± 0.30 <sup>u</sup>  |
| 49                | 35.01 ± 1.00 <sup>v</sup>  | 1.48 ± 0.07  | 3.77 ± 0.14 <sup>v</sup>  | 1.73 ± 0.07 <sup>v</sup>  | 40.31 ± 1.60 <sup>v</sup>  | 53.87 ± 7.73 <sup>v</sup>  | 20.17 ± 1.05 <sup>v</sup> | 7.34 ± 0.19 <sup>v</sup>  |
| Lipid level (%)   |                            |              |                           |                           |                            |                            |                           |                           |
| 6                 | 31.82 ± 1.48               | 1.56 ± 0.09  | 3.96 ± 0.11 <sup>y</sup>  | 1.63 ± 0.06 <sup>xy</sup> | 50.78 ± 1.95 <sup>y</sup>  | 68.21 ± 2.42 <sup>y</sup>  | 20.85 ± 1.04 <sup>y</sup> | 6.20 ± 0.34               |
| 10                | 31.60 ± 1.29               | 1.49 ± 0.04  | 3.23 ± 0.19 <sup>x</sup>  | 1.51 ± 0.03 <sup>x</sup>  | 32.17 ± 1.89 <sup>x</sup>  | 39.28 ± 1.77 <sup>x</sup>  | 16.91 ± 0.77 <sup>x</sup> | 5.96 ± 0.46               |
| 14                | 32.26 ± 1.75               | 1.51 ± 0.05  | 3.42 ± 0.11 <sup>x</sup>  | 1.79 ± 0.06 <sup>y</sup>  | 38.76 ± 1.84 <sup>x</sup>  | 41.30 ± 2.56 <sup>x</sup>  | 15.70 ± 0.72 <sup>x</sup> | 6.42 ± 0.43               |
| ANOVA (P value)   |                            |              |                           |                           |                            |                            |                           |                           |
| Protein           | 0.048                      | 0.345        | 0.016                     | 0.019                     | <0.001                     | 0.020                      | 0.005                     | 0.001                     |
| Lipid             | 0.942                      | 0.724        | <0.001                    | 0.001                     | <0.001                     | <0.001                     | 0.001                     | 0.590                     |
| Protein × lipid   | 0.346                      | 0.728        | <0.001                    | 0.337                     | <0.001                     | 0.086                      | 0.001                     | 0.517                     |

\*Values in a column with different superscripts significantly differ.

#### Plasma lipoprotein.

Dietary treatments significantly affected plasma TAG, HDL-C and LDL-C levels and LDL-C/HDL-C ratio but did not affect plasma TC level (Table 5). The highest values of plasma TAG, HDL-C and LDL-C contents and LDL-C/HDL-C ratio were observed in fish fed the P49L10, P49L6, P37L10 and P37L14 diets, whereas the lowest values were recorded in fish fed the P43L10, P37L14, P43L6 and P49L6 diets, respectively. With respect to dietary protein and lipid levels, fish fed diets with 43% protein exhibited the lowest plasma TC, TAG and LDL-C contents. Plasma HDL-C content increased with increasing dietary protein level. Fish fed diets with 37% protein had the highest plasma LDL-C/HDL-C ratio. Plasma HDL-C level decreased with increasing dietary lipid level. Fish fed diets with 6% lipid exhibited the lowest plasma LDL-C level and LDL-C/HDL-C ratio.

**Table 5.** Lipid profiles in plasma of Asian red-tailed catfish *Hemibagrus wyckioides* fed diets with different protein and lipid levels.

| Diet              | TC (mmol/L)               | TAG (mmol/L)              | HDL-C (mmol/L)            | LDL-C (mmol/L)            | LDL-C/HDL-C               |
|-------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| P37L6             | 7.18 ± 0.48               | 6.82 ± 0.02 <sup>bc</sup> | 2.11 ± 0.33 <sup>ab</sup> | 3.71 ± 0.16 <sup>ab</sup> | 1.82 ± 0.22 <sup>ab</sup> |
| P37L10            | 8.09 ± 0.49               | 6.87 ± 0.10 <sup>bc</sup> | 2.18 ± 0.17 <sup>ab</sup> | 4.54 ± 0.29 <sup>b</sup>  | 2.09 ± 0.06 <sup>ab</sup> |
| P37L14            | 6.87 ± 0.14               | 7.17 ± 0.50 <sup>bc</sup> | 1.64 ± 0.25 <sup>a</sup>  | 3.79 ± 0.17 <sup>ab</sup> | 2.43 ± 0.39 <sup>b</sup>  |
| P43L6             | 6.99 ± 0.12               | 5.87 ± 0.16 <sup>ab</sup> | 2.78 ± 0.40 <sup>ab</sup> | 3.04 ± 0.42 <sup>a</sup>  | 1.19 ± 0.34 <sup>a</sup>  |
| P43L10            | 7.24 ± 0.12               | 4.01 ± 0.67 <sup>a</sup>  | 2.51 ± 0.25 <sup>ab</sup> | 3.92 ± 0.19 <sup>ab</sup> | 1.61 ± 0.24 <sup>ab</sup> |
| P43L14            | 6.46 ± 0.14               | 7.27 ± 0.72 <sup>bc</sup> | 1.85 ± 0.24 <sup>a</sup>  | 3.06 ± 0.12 <sup>a</sup>  | 1.75 ± 0.29 <sup>ab</sup> |
| P49L6             | 8.31 ± 0.53               | 6.59 ± 0.38 <sup>bc</sup> | 3.28 ± 0.27 <sup>b</sup>  | 3.71 ± 0.18 <sup>ab</sup> | 1.13 ± 0.07 <sup>a</sup>  |
| P49L10            | 7.51 ± 0.18               | 8.15 ± 0.35 <sup>c</sup>  | 2.01 ± 0.19 <sup>ab</sup> | 3.87 ± 0.06 <sup>ab</sup> | 1.96 ± 0.16 <sup>ab</sup> |
| P49L14            | 7.88 ± 0.84               | 5.26 ± 0.25 <sup>ab</sup> | 2.58 ± 0.29 <sup>ab</sup> | 4.25 ± 0.50 <sup>ab</sup> | 1.65 ± 0.15 <sup>ab</sup> |
| Protein level (%) |                           |                           |                           |                           |                           |
| 37                | 7.38 ± 0.27 <sup>uv</sup> | 6.95 ± 0.16 <sup>v</sup>  | 1.98 ± 0.15 <sup>u</sup>  | 4.01 ± 0.16 <sup>v</sup>  | 2.11 ± 0.16 <sup>v</sup>  |
| 43                | 6.95 ± 0.12 <sup>u</sup>  | 5.71 ± 0.55 <sup>u</sup>  | 2.45 ± 0.21 <sup>uv</sup> | 3.40 ± 0.22 <sup>u</sup>  | 1.49 ± 0.17 <sup>u</sup>  |
| 49                | 7.90 ± 0.31 <sup>v</sup>  | 6.67 ± 0.45 <sup>v</sup>  | 2.62 ± 0.22 <sup>v</sup>  | 3.94 ± 0.17 <sup>uv</sup> | 1.58 ± 0.13 <sup>u</sup>  |
| Lipid level (%)   |                           |                           |                           |                           |                           |
| 6                 | 7.50 ± 0.29               | 6.43 ± 0.19               | 2.72 ± 0.24 <sup>y</sup>  | 3.49 ± 0.18 <sup>x</sup>  | 1.38 ± 0.16 <sup>x</sup>  |
| 10                | 7.61 ± 0.20               | 6.34 ± 0.65               | 2.24 ± 0.13 <sup>xy</sup> | 4.11 ± 0.15 <sup>y</sup>  | 1.89 ± 0.11 <sup>y</sup>  |
| 14                | 7.14 ± 0.36               | 6.57 ± 0.42               | 2.05 ± 0.21 <sup>x</sup>  | 3.81 ± 0.23 <sup>xy</sup> | 1.97 ± 0.20 <sup>y</sup>  |
| ANOVA (P value)   |                           |                           |                           |                           |                           |
| Protein           | 0.040                     | 0.005                     | 0.032                     | 0.025                     | 0.009                     |
| Lipid             | 0.309                     | 0.803                     | 0.021                     | 0.034                     | 0.014                     |
| Protein × lipid   | 0.298                     | <0.001                    | 0.121                     | 0.251                     | 0.638                     |

\*Values in a column with different superscripts significantly differ.

#### Plasma antioxidant-related parameters.

Dietary treatments significantly affected plasma CAT, POD, GPx and TAC activities, and MDA and NO contents (Table 6). However, plasma POD and TAC activities were not affected by dietary protein level; plasma CAT activity was not affected by dietary lipid level. The highest values of plasma CAT, POD, GPx and TAC activities, and MDA and NO contents were observed in fish fed the P37L6, P49L10, P43L10, P43L6, P49L6 and P43L6 diets, whereas the lowest values were recorded in fish fed the P43L10, P49L6, P49L6,

P43L14, P43L14 and P37L10, respectively. With respect to dietary protein and lipid levels, fish fed diets with 37% protein had the highest plasma CAT activity and the lowest plasma MDA content. Plasma GPx activity and NO content were higher in fish fed diets with 43% protein compared to fish fed diets with 37% or 49% protein. Fish fed diets with 10% lipid exhibited the highest plasma POD and GPx activities and the lowest plasma NO content. Plasma TAC activity was higher in fish fed diets with 6% or 10% lipid compared to fish fed diets with 14% lipid. Plasma MDA content was higher in fish fed diets with 6% lipid compared to fish fed diets with 10% or 14% lipid.

**Table 6.** Antioxidant-related parameters in plasma of Asian red-tailed catfish *Hemibagrus wyckioides* fed diets with different protein and lipid levels.

| Diet                    | CAT (U/ml)                 | POD (U/ml)                  | GPx (U/ml)                 | TAC (U/ml)                 | MDA (nmol/L)              | NO (mmol/L)               |
|-------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
| P37L6                   | 14.45 ± 0.78 <sup>e</sup>  | 33.33 ± 1.64 <sup>abc</sup> | 0.39 ± 0.03 <sup>ab</sup>  | 8.88 ± 0.14 <sup>abc</sup> | 5.45 ± 0.26 <sup>a</sup>  | 0.81 ± 0.20 <sup>ab</sup> |
| P37L10                  | 12.20 ± 1.35 <sup>de</sup> | 37.00 ± 1.06 <sup>bc</sup>  | 0.43 ± 0.01 <sup>abc</sup> | 11.88 ± 0.62 <sup>cd</sup> | 7.05 ± 0.13 <sup>ab</sup> | 0.64 ± 0.11 <sup>a</sup>  |
| P37L14                  | 6.77 ± 0.78 <sup>abc</sup> | 38.17 ± 0.58 <sup>bc</sup>  | 0.47 ± 0.01 <sup>cde</sup> | 6.72 ± 0.69 <sup>ab</sup>  | 5.68 ± 0.13 <sup>a</sup>  | 1.02 ± 0.21 <sup>ab</sup> |
| P43L6                   | 7.90 ± 0.91 <sup>bcd</sup> | 28.33 ± 3.85 <sup>ab</sup>  | 0.42 ± 0.02 <sup>abc</sup> | 14.64 ± 1.94 <sup>d</sup>  | 12.50 ± 1.39 <sup>c</sup> | 1.89 ± 0.12 <sup>c</sup>  |
| P43L10                  | 2.71 ± 0.26 <sup>a</sup>   | 35.00 ± 2.41 <sup>abc</sup> | 0.55 ± 0.00 <sup>e</sup>   | 7.93 ± 0.28 <sup>abc</sup> | 9.09 ± 0.26 <sup>b</sup>  | 1.31 ± 0.03 <sup>bc</sup> |
| P43L14                  | 6.55 ± 0.39 <sup>abc</sup> | 29.67 ± 2.79 <sup>ab</sup>  | 0.46 ± 0.02 <sup>bcd</sup> | 5.04 ± 0.14 <sup>a</sup>   | 5.00 ± 0.26 <sup>a</sup>  | 1.85 ± 0.17 <sup>c</sup>  |
| P49L6                   | 3.61 ± 0.26 <sup>ab</sup>  | 23.50 ± 2.50 <sup>a</sup>   | 0.38 ± 0.01 <sup>a</sup>   | 9.84 ± 0.81 <sup>bc</sup>  | 17.73 ± 1.31 <sup>d</sup> | 0.89 ± 0.04 <sup>ab</sup> |
| P49L10                  | 6.55 ± 0.39 <sup>abc</sup> | 45.83 ± 3.08 <sup>c</sup>   | 0.52 ± 0.00 <sup>de</sup>  | 8.52 ± 0.76 <sup>abc</sup> | 6.59 ± 0.39 <sup>ab</sup> | 0.71 ± 0.06 <sup>ab</sup> |
| P49L14                  | 9.71 ± 1.17 <sup>cde</sup> | 28.17 ± 3.85 <sup>ab</sup>  | 0.44 ± 0.01 <sup>ab</sup>  | 7.56 ± 0.76 <sup>abc</sup> | 5.23 ± 0.13 <sup>a</sup>  | 0.98 ± 0.05 <sup>ab</sup> |
| Protein level (%)       |                            |                             |                            |                            |                           |                           |
| 37                      | 11.14 ± 1.36 <sup>v</sup>  | 36.17 ± 1.94                | 0.43 ± 0.02 <sup>u</sup>   | 9.16 ± 0.80                | 6.06 ± 0.27 <sup>u</sup>  | 0.82 ± 0.10 <sup>u</sup>  |
| 43                      | 5.72 ± 0.83 <sup>u</sup>   | 31.17 ± 1.82                | 0.48 ± 0.02 <sup>v</sup>   | 9.20 ± 1.53                | 8.86 ± 1.16 <sup>v</sup>  | 1.69 ± 0.11 <sup>v</sup>  |
| 49                      | 6.62 ± 0.95 <sup>u</sup>   | 32.50 ± 3.76                | 0.45 ± 0.02 <sup>u</sup>   | 8.64 ± 0.61                | 9.85 ± 2.02 <sup>v</sup>  | 0.86 ± 0.05 <sup>u</sup>  |
| Lipid level (%)         |                            |                             |                            |                            |                           |                           |
| 6                       | 8.66 ± 1.62                | 28.56 ± 2.00 <sup>x</sup>   | 0.40 ± 0.13 <sup>x</sup>   | 11.12 ± 1.05 <sup>y</sup>  | 11.89 ± 1.86 <sup>y</sup> | 1.20 ± 0.19 <sup>y</sup>  |
| 10                      | 7.15 ± 1.54                | 39.28 ± 2.03 <sup>y</sup>   | 0.50 ± 0.18 <sup>z</sup>   | 9.44 ± 0.79 <sup>y</sup>   | 7.58 ± 0.41 <sup>x</sup>  | 0.89 ± 0.11 <sup>x</sup>  |
| 14                      | 7.68 ± 0.66                | 32.00 ± 2.08 <sup>x</sup>   | 0.46 ± 0.10 <sup>y</sup>   | 6.44 ± 0.48 <sup>x</sup>   | 5.30 ± 0.14 <sup>x</sup>  | 1.28 ± 0.16 <sup>y</sup>  |
| ANOVA ( <i>P</i> value) |                            |                             |                            |                            |                           |                           |
| Protein                 | <0.001                     | 0.083                       | 0.002                      | 0.720                      | <0.001                    | <0.001                    |
| Lipid                   | 0.215                      | <0.001                      | <0.001                     | <0.001                     | <0.001                    | 0.003                     |
| Protein × lipid         | <0.001                     | 0.009                       | 0.016                      | <0.001                     | <0.001                    | 0.475                     |

\*Values in a column with different superscripts significantly differ.

#### Whole-body composition.

Dietary treatments significantly affected the whole-body moisture, crude protein, crude lipid, ash and energy contents (Table 7). However, the whole-body crude protein content was not affected by dietary protein level; the whole-body ash content was not affected by dietary lipid levels.

**Table 7.** Proximate composition of the whole-body of Asian red-tailed catfish *Hemibagrus wyckioides* fed diets with different protein and lipid levels.

| Diet                    | Moisture (%)               | Crude protein (%)           | Crude lipid (%)             | Ash (%)                   | Energy (kJ/g)             |
|-------------------------|----------------------------|-----------------------------|-----------------------------|---------------------------|---------------------------|
| Initial                 | 74.22                      | 12.16                       | 10.47                       | 2.02                      | 7.37                      |
| P37L6                   | 71.38 ± 0.30 <sup>d</sup>  | 15.01 ± 0.10 <sup>abc</sup> | 10.01 ± 0.06 <sup>a</sup>   | 2.81 ± 0.03 <sup>a</sup>  | 8.00 ± 0.09 <sup>a</sup>  |
| P37L10                  | 70.45 ± 0.12 <sup>c</sup>  | 14.66 ± 0.05 <sup>a</sup>   | 10.96 ± 0.04 <sup>abc</sup> | 2.87 ± 0.03 <sup>ab</sup> | 8.31 ± 0.05 <sup>ab</sup> |
| P37L14                  | 68.95 ± 0.23 <sup>a</sup>  | 15.36 ± 0.06 <sup>c</sup>   | 12.35 ± 0.34 <sup>de</sup>  | 2.85 ± 0.03 <sup>a</sup>  | 9.00 ± 0.17 <sup>d</sup>  |
| P43L6                   | 70.03 ± 0.05 <sup>bc</sup> | 15.09 ± 0.01 <sup>abc</sup> | 11.26 ± 0.16 <sup>bcd</sup> | 2.90 ± 0.05 <sup>ab</sup> | 8.53 ± 0.10 <sup>bc</sup> |
| P43L10                  | 68.89 ± 0.21 <sup>a</sup>  | 14.90 ± 0.03 <sup>ab</sup>  | 12.37 ± 0.41 <sup>de</sup>  | 2.86 ± 0.04 <sup>ab</sup> | 8.92 ± 0.21 <sup>cd</sup> |
| P43L14                  | 69.34 ± 0.09 <sup>ab</sup> | 14.90 ± 0.09 <sup>ab</sup>  | 12.09 ± 0.37 <sup>cde</sup> | 2.88 ± 0.06 <sup>ab</sup> | 8.81 ± 0.12 <sup>cd</sup> |
| P49L6                   | 70.56 ± 0.06 <sup>cd</sup> | 15.44 ± 0.03 <sup>c</sup>   | 10.50 ± 0.16 <sup>ab</sup>  | 2.92 ± 0.02 <sup>ab</sup> | 8.32 ± 0.11 <sup>ab</sup> |
| P49L10                  | 69.94 ± 0.20 <sup>bc</sup> | 15.13 ± 0.21 <sup>bc</sup>  | 11.12 ± 0.18 <sup>abc</sup> | 3.07 ± 0.01 <sup>b</sup>  | 8.51 ± 0.04 <sup>bc</sup> |
| P49L14                  | 68.87 ± 0.09 <sup>a</sup>  | 14.81 ± 0.03 <sup>ab</sup>  | 12.64 ± 0.13 <sup>e</sup>   | 2.84 ± 0.09 <sup>a</sup>  | 9.01 ± 0.05 <sup>d</sup>  |
| Protein level (%)       |                            |                             |                             |                           |                           |
| 37                      | 70.26 ± 0.37 <sup>z</sup>  | 15.01 ± 0.11                | 11.11 ± 0.35 <sup>u</sup>   | 2.84 ± 0.02 <sup>u</sup>  | 8.44 ± 0.20 <sup>u</sup>  |
| 43                      | 69.42 ± 0.18 <sup>x</sup>  | 14.96 ± 0.13                | 11.91 ± 0.23 <sup>v</sup>   | 2.88 ± 0.03 <sup>uv</sup> | 8.75 ± 0.12 <sup>v</sup>  |
| 49                      | 69.79 ± 0.26 <sup>y</sup>  | 15.13 ± 0.11                | 11.42 ± 0.32 <sup>uv</sup>  | 2.94 ± 0.04 <sup>v</sup>  | 8.61 ± 0.14 <sup>uv</sup> |
| Lipid level (%)         |                            |                             |                             |                           |                           |
| 6                       | 70.66 ± 0.22 <sup>z</sup>  | 15.18 ± 0.07 <sup>y</sup>   | 10.59 ± 0.19 <sup>x</sup>   | 2.88 ± 0.02               | 8.28 ± 0.11 <sup>x</sup>  |
| 10                      | 69.76 ± 0.25 <sup>y</sup>  | 14.90 ± 0.09 <sup>x</sup>   | 11.48 ± 0.26 <sup>y</sup>   | 2.93 ± 0.04               | 8.58 ± 0.13 <sup>y</sup>  |
| 14                      | 69.05 ± 0.11 <sup>x</sup>  | 15.02 ± 0.09 <sup>xy</sup>  | 12.36 ± 0.16 <sup>z</sup>   | 2.86 ± 0.03               | 8.94 ± 0.10 <sup>z</sup>  |
| ANOVA ( <i>P</i> value) |                            |                             |                             |                           |                           |
| Protein                 | <0.001                     | 0.093                       | 0.002                       | 0.037                     | 0.002                     |
| Lipid                   | <0.001                     | 0.004                       | <0.001                      | 0.123                     | <0.001                    |
| Protein × lipid         | <0.001                     | <0.001                      | 0.006                       | 0.066                     | 0.002                     |

\*Values in a column with different superscripts significantly differ.

The highest values of whole-body moisture, crude protein, crude lipid, ash, and energy contents were observed in fish fed the P37L6, P49L6, P49L14, P49L10 and P49L14 diets, whereas the lowest values were recorded in fish fed the P49L14, P37L10, P37L6, P37L6 and P37L6 diets, respectively. With respect to dietary protein and lipid levels, fish fed diets with 43% protein exhibited the lowest whole-body moisture content, but the highest whole-body lipid and energy contents. The whole-body ash content increased with increasing dietary protein level. The whole-body protein content was higher in fish fed diets with 6% lipid compared to fish fed diets with 10% lipid. The whole-body moisture content decreased with increasing dietary lipid level. Conversely, the whole-body lipid and energy contents increased with increasing dietary lipid levels.

### Discussion

In this study, the feed intake of Asian red-tailed catfish increased with increasing dietary lipid levels, suggesting the extra lipid may to some extent enhance the palatability of diets. However, an effect of dietary carbohydrate level cannot be excluded. The increase of dietary lipid level from 6 to 14% was accompanied by a decrease of 10% wheat flour in diets at all dietary protein levels. The expansion of starch (wheat flour) during feed extrusion may reduce the bulk density of the pellets. According to Colgan (1973), stomach fullness should be responsible for short-term feed intake regulation in fish. Thus, the increased feed intake of Asian red-tailed catfish fed high dietary lipid content may be partly related to the physical compaction of feed since the volume of feed that a fish can eat depends on the stomach capacity (Saravanan *et al.*, 2012).

The present study showed that dietary protein and lipid levels with various P/E ratio (17.76 to 24.77 g protein/MJ) markedly affected the growth rate and feed utilization of Asian red-tailed catfish. The highest values of WG and SGR were observed in fish fed the P43L10 diet, the dietary protein level was in line with the recommended protein requirements (43.9–44.1% of diets containing 10% lipid) for this species (Deng *et al.*, 2011; Hong Tham *et al.*, 2013), the dietary lipid level was in accordance with the determined lipid requirement (9.4–11.4% of diets containing 42% protein, unpublished data). However, fish fed a diet containing 37% protein and 14% lipid had similar WG and SGR as those fed the P43L10 diet, but showed the best PER, protein gain and protein retention among the dietary treatments. Further, fish fed diets with 49% protein showed relatively higher plasma total protein content, and AST and ALT activities as compared with fish fed diets with 37% and 43% protein, suggesting the dietary protein of 49% is an excess level for Asian red-tailed catfish. Additionally, the increase of dietary lipid level from 6 to 14% resulted in increased PER, protein and energy gains, protein and energy retentions, and depressed FCR and plasma ALT activity, suggesting excess lipid was metabolized for energy to reduce protein catabolism. Thus, a diet containing 37% protein and 14% lipid with P/E ratio of 17.76 g protein/MJ is suitable for Asian red-tailed catfish.

It is well known that inadequate dietary P/E ratio may result in lower growth rate as well as lower protein and energy retentions (Ai *et al.*, 2004). In this study, SGR, protein and energy retention of Asian red-tailed catfish linearly improved with the increase of dietary lipid content at 37% protein level, whereas those were not observed at 43% and 49% protein levels, which may be attributed to the imbalance of dietary P/E ratio. An earlier study on Asian red-tailed catfish showed that the highest growth rate was found in fish fed a diet containing 39% protein and 12% lipid with P/E ratio of 20.48 g protein/MJ (Hung *et al.*, 2015), but it is insufficient to demonstrate that this diet is the best suited requirement for this species since the dietary protein and lipid levels ranged from 39 to 44% and 6 to 12%, respectively. In this study, the dietary protein and lipid levels were formulated to range from 37 to 49% and 6 to 14%, respectively; the best dietary P/E ratio for growth of Asian red-tailed catfish was 17.76 g protein/MJ, which is within the range reported for other fish species. The optimal P/E ratio has been reported to be 15.1 g protein/MJ for African catfish *Clarias gariepinus* (Ahmad, 2008), 18.3 g protein/MJ for African catfish *Heterobranchus longifilis* (Babalola and Apata, 2006), 19.4 g protein/MJ for bagrid catfish *Pseudobagrus fulvidraco* (Kim and Lee, 2005). However, it should be emphasized that dietary P/E ratio should be used in combination with dietary nutrient content (Zhang *et al.*, 2017). In this study, the dietary P/E ratio of P37L6 and P43L14 groups was similar (19.22 versus 19.96 g protein/MJ), but they resulted in obvious differences in growth rate and feed utilization. By contrast, P37L14 and P43L10 diets had apparent differences in the P/E ratio (17.76 versus 21.10 g protein/MJ), but they had

comparable growth rate and feed utilization. This phenomenon had also been observed in some other fish species (Lee *et al.*, 2002; Zhang *et al.*, 2017; Zhang *et al.*, 2018). Thus, dietary P/E ratio must be used together with absolute amounts of dietary protein and lipid levels.

It has been well established for many fish species that protein utilization can be improved by increasing dietary lipid levels because it has a protein-sparing effect (NRC, 2011). In this study, the PER, protein gain, and protein retention increased with increasing dietary lipid level at the 37% dietary protein level, indicating that increasing dietary energy level by lipids will provide a more efficient utilization of dietary protein for growth of fish. A similar result showing the protein sparing effect of dietary lipid was observed in the catfish (Hung *et al.* 2015). Furthermore, the protein-sparing effects by dietary lipid were observed in other catfish species, such as bagrid catfish (Kim and Lee, 2005), African catfish *Clarias gariepinus* (Ahmad, 2008), Asian catfish *Pangasius hypophthalmus* (Liu *et al.*, 2011a), far eastern catfish *Silurus asotus* (Kim *et al.*, 2012) and South American catfish *Pseudoplatystoma sp.* (Arslan *et al.*, 2012). However, the protein-sparing effect of excess energy with 14% lipid in the diets with 43% and 49% protein was not observed in this study. Thus, it is important to provide an optimum level and ratio of dietary protein and lipid in order to reduce catabolism of protein for energy.

Previous studies have shown that high dietary energy inclusion resulted in high fat deposition in fish (Millikin, 1983). Moreover, higher levels of dietary lipid usually resulted in some pathological damage in fish (Rueda-Jasso *et al.*, 2004). Blood  $\gamma$ -GT, AST, and ALT activities are usually used as general indicators of liver function (Chien *et al.*, 2003). High blood  $\gamma$ -GT, AST, and ALT generally, but not definitively, indicate a weakening or damage of normal liver function. Malondialdehyde is the end-product of lipid peroxidation, which causes toxic stress in cells and is used as a biomarker to measure oxidative stress (Liu *et al.*, 2011b). In this study, higher dietary lipid levels also caused higher lipid accumulation in the catfish body. However, the depressed plasma  $\gamma$ -GT, AST and ALT activities and MDA content in fish fed diets with 14% lipid as compared with fish fed diets with 6% lipid, imply that dietary lipid levels up to 14% did not cause obvious damage to Asian red-tailed catfish. Thus, it is appropriate to feed a diet containing 37% protein and 14% lipid with P/E ratio of 17.76 g protein/MJ for health.

In conclusion, the results of the present study demonstrated that a diet containing 37% protein and 14% lipid with P/E ratio of 17.76 g protein/MJ is suitable for optimum growth and health of Asian red-tailed catfish.

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