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The response of juvenile largemouth bass (*Micropterus salmoides*) to varying protein and carbohydrate levels in extruded feed

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Abstract
The present feeding trial was conducted to evaluate the growth and physiological-biochemical indexes response of largemouth bass (*Micropterus salmoides*) juvenile to varying protein and carbohydrate levels in extruded feed. Six experimental diets containing one of two carbohydrate (CHO) levels (10% or 13%) and three crude protein (CP) levels (42%, 45%, or 48%) were fed to three replicate groups of *M. salmoides* (18.21 ± 0.03 g) for 56 days in indoor concrete ponds. The results show that fish fed 42% CP level diets had significantly lower WG and SGR than those of fish fed 48% CP level diets(P<0.05), while there was no statistical difference between 45% and 48% CP levels diets (P>0.05). No significant differences in growth were observed by increment of dietary CHO level from 10% to 13% (P>0.05). Dietary 43% CP level increased feed conversion ratio (FCR) of fish when compared with 48% CP level treatment (P<0.05), however, fish fed 45% CP with two CHO diets showed very comparatively values in FCR and protein efficiency ratio (PER) with 48% CP diets (P>0.05). Hepatosomatic index of the experimental fish fed 13% CHO level was significantly higher than fish fed 10% CHO level (P<0.05), while this parameter was not affected by dietary CP levels (P>0.05). Dorsal muscle compositions of *M. salmoides* were unaffected by dietary treatments with exception of the lower protein content in fish fed 42% CP level diets (P<0.05). The highest GPT activity was observed in liver of *M. salmoides* fed 48%CP13%CHO diet, while GPT level was enhanced by either dietary CP or CHO level (P<0.05). High CP or CHO level in diets increased hepatic HK and PK activities, however, PEPCK activities were depressed by high CHO level diets. Pepsin activities in stomach of *M. salmoides* fed 42% CP level diets were significantly lower, while amylase activities of fish fed 13% CHO level diets were significantly higher (P<0.05). By overall consideration, extruded feed containing 45% CP with 10% or 13% CHO levels are applicable for *M. salmoides* juveniles under the current trial conditions.

Keywords: *Micropterus salmoides*, protein level, carbohydrate level, extruded feed, effects

1. Introduction
Largemouth bass, *Micropterus salmoides*, is a carnivorous fish commercially important for freshwater aquaculture native to North America [1]. *M. salmoides* is well accepted by farmers due to its many advantages that include its suitability to a wide variety of culture environments, fast growth, ease of handling, and short culture cycle [2]. It was firstly introduced into Guangdong in mainland China in 1983, and this species is now distributed throughout the country and has become a major freshwater product in Chinese aquaculture. The total culture production of China increased quickly and in 2018 was up to 432 thousand tons [3]. Because of the benefits to the physical attributes of pellets including nutrient digestibility, palatability, pellet durability, water stability, easy management, and minimal water pollution of extruded feed [4, 5], in main area such as Guangdong, Zhejiang, Jiangsu Provinces in China, extruded feed has became the dominant feed style in *M. salmoides* culture. However, the price of commercial extruded feed for *M. salmoides* general maintains 1.2~1.3 thousands RMB yuan per ton in China, of which occupies over 60% total cost in culture.
As it is known to all, protein is one of the most necessary and costly nutrients of most fish diets [6]. One of the main objectives of nutrition research is to minimize the amount of protein in diets and cover energy requirements using carbohydrates or lipids for this fish species [7]. However, the extremely limited glucose utilization of this fish seriously restricts the use of aquafeed since excessive dietary carbohydrate would lead to abnormal hepatic glycogen,

which may further impact the growth and health of this fish [8, 9]. Although a number of previous studies have assessed the protein or carbohydrate requirement for *M. salmoides* [10, 11, 12, 13, 14], most of these studies were performed using pellet feed. Therefore, the present study was designed to investigate the effects of growth performance, body composition and enzymes activities of juvenile *M. salmoides* to varying protein and carbohydrate levels in extruded feed. The results generated from this study may lead to more effective means of formulating low-cost and efficient extruded feed for this fish species.

2. Materials and Methods

2.1 Diets composition

A 3 × 2 factorial layout was established. Six test diets were formulated at 3 crude protein levels (48%, 45% and 42%) and 2 carbohydrate levels (13% and 10%,dry-matter basis), designed as diets P48C13 (D1), P48C10 (D2), P45C13 (D3), P45C10 (D4), P42C13 (D5) and P42C10 (D6), respectively. Fish meal and soybean meal were used as protein source, fish oil and soybean oil were used as lipid source, and wheat flour was used as carbohydrate source, respectively. Feed ingredients were purchased from Hangzhou Wangcheng Biotech Limited Company (Hangzhou, China), and were ground to pass through a 0.5 mm mesh. The extruded feed was produced by Zhejiang Liangxing Feed Limited Company (TongXiang, Jiaxing, China).

Each diet was extruded into 2 mm diameter pellets under the following extrusion condition as: the barrel temperature was adjusted to 100 and 105 °C, moisture content was 28% and 35%, and screw speed was 40–45 rpm using a Twin-screwed extruder (HR118, HENGRUN MACHINE, Zhanjiang, China). Extruded fish feed was dried at 40 °C for spraying oil and ground into powder to pass through a 50 mm mesh sieve. All diets were stored at room temperature (25–30°C) in the summer and kept out of the sun and rainy, as the same storage conditions used at fish farms. The diet formulation and proximate composition are shown in Table 1.

Table 1: Formulations and analyzed composition of experimental diets (dry matter basis)

Ingredients (%)	D1 (P48C13)	D2(P48C10)	D3(P45C13)	D4(P45C10)	D5(P42C13)	D6(P42C10)
Fish meal	52	52.5	47.5	48	43	43.5
Soybean meal (dehulled)	12.5	12.5	12.8	12.8	13.1	13.1
Soybean protein concentrate	3	3	3	3	3	3
Fermented soybean meal	3	3	3	3	3	3
Beer yeast	1.5	1.5	1.5	1.5	1.5	1.5
Wheat flour	17	13	17	13	17	13
Wheat gluten	2.2	2.5	2.2	2.5	2.2	2.5
Fish oil	1.8	1.8	2	2	2.2	2.2
Soybean oil	1.8	1.8	2	2	2.2	2.2
Phospholipid	0.8	0.8	0.8	0.8	0.8	0.8
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Calcium biphosphate	1.5	1.5	1.9	1.9	2.3	2.3
Vitamin premix	1	1	1	1	1	1
Mineralpremix	1	1	1	1	1	1
Zeolite powder	0.4	2.1	1.1	3.8	2.5	5
Microcrystalline cellulose	0	1.5	2.0	2.5	3.5	4.2
Crystalline lysine	0	0	0.3	0.3	0.5	0.5
Methionine hydroxy analog-Ca	0	0	0.3	0.3	0.5	0.5
Butyrin	0	0	0.1	0.1	0.2	0.2
Total	100	100	100	100	100	100
Proximate composition (% dry matter)						
Crude protein	48.02	48.04	45.18	45.20	42.35	42.37
Crude lipid	9.32	9.28	9.34	9.30	9.36	9.32
Crude ash	11.71	12.11	11.94	12.22	11.83	12.28
Carbohydrates	13.08	10.13	13.08	10.13	13.08	10.13
Gross energy (KJ/g)	12.27	11.92	11.88	11.53	11.49	11.14
P/E(mg/KJ)	39.14	40.30	38.03	39.20	36.85	38.02
Calcium	2.23	2.25	2.11	2.13	2.00	2.02
Phosphorus	1.87	1.87	1.85	1.85	1.84	1.83
LYS	3.40	3.41	3.47	3.49	3.45	3.46
MET	1.16	1.16	1.18	1.18	1.17	1.17

Notes:

Vitamin premix (IU or mg/kg dry diet): vitamin A, 16000 IU; vitamin D3, 8000 IU; vitamin K3, 14.72; thiamin, 17.80; riboflavin, 48.00; cyanocobalamine, 0.24; pyridoxine, 29.52; niacinamide,79.20; tocopherol acetate, 160.00; ascorbic acid (35%), 800.00; folic acid, 6.40; biotin, 0.64; L-carnitine,100.00; calcium-D-pantothenate, 73.6.

Mineral premix (mg/kg dry diet): Zn($ZnSO_4$), 34.4; Cu ($CuSO_4$), 2.0; Mn($MnSO_4$), 6.2; Fe($FeSO_4$), 21.1; I($Ca(IO_3)_2$), 1.63; Co($CoCl_2$), 0.24; Mg($MgSO_4 \cdot H_2O$), 52.7; Se(Na_2SeO_3), 0.18.

Gross energy was calculated using physiological fuel values of 4.0, 4.0 and 9.0 kcal/g (16.7, 16.7 and 37.7 kJ/g) for carbohydrate, protein and lipid, respectively. All experimental diets had the same gross energy of kcal per 100 g dry matter.

2.2 Feeding trial

A 56-day feeding trial was conducted at the Hangzhou Xi-ba field station which belongs to the Zhejiang Fisheries Technical Extension Center (Hangzhou, China). *M. salmoides* juveniles were obtained from the commercial fish hatchery of Huzhou (Zhejiang, China). Prior to the experiment, fishes were reared in several concrete ponds (3.2m length × 2.6m width × 1m depth, each) in a greenhouse for 2 weeks to acclimate to the experiment conditions. After the conditioning period, fishes of similar sizes (average weight: 18.21 ± 0.03

g) were randomly distributed into 18 same size concrete ponds of 200 fishes per pond. Fishes in each pond were randomly assigned one of six experimental diets. Each diet was tested in three replicates. *M. salmoides* juveniles were fed to apparent satiation two times daily (08:00 am and 17:00 pm) for 8 weeks. In each feeding, some pellets of the test diets were dropped in the fixed point of the experimental pond edge until no fish were observed to eat the dropped diets. No feed residue was left in the pond after feeding to ensure that the fish can eat up the diet.

The insides of the ponds were routinely cleaned, and about 30% water in the ponds was replaced with sand-filtered fresh water twice a week. The water quality parameters including temperature and pH were measured routinely and their values were 28.5 ± 0.7 °C and 7.3 ± 0.1 respectively. Dissolved oxygen was maintained above 5.0 mg L^{-1} using air stones for continuous aeration; total ammonia nitrogen and nitrite were kept less than 0.2 and 0.01 mg L^{-1}, respectively.

2.3 Sample Collection

At the end of the feeding trials, *M. salmoides* were starved overnight prior to harvest. Then, the experimental fishes were anesthetized in diluted MS-222 (tricaine methane sulfonate, Sigma, St. Louis, MO, USA) at the concentration of 100 mg L^{-1}. Total numbers and weight of fishes in each pond were measured. A sample of 10 fish at the beginning and 10 fish per pond at the end of the feeding trial were collected and stored at -80 °C for body composition analysis. The remaining fishes were all sampled for the analysis of biometric parameters. Also, individuals liver and stomach were quickly removed and stored at -20 °C for subsequent analysis.

Moisture, ash, crude protein and crude lipid were determined following methods of the Association of Official Analytical Chemists [15] Moisture concentration was determined by drying minced samples for 6 h in a forced-air oven maintained at 105 °C. Ash content was analysed by incinerating samples at 600 °C for 24 h in a muffle furnace. Crude protein was estimated as Kjeldahl-nitrogen using factor 6.25, and crude lipid was determined by Soxhlet extraction with petroleum ether for 6 h. The enzymes activities, including glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), Hexokinase (HK), pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK) in liver, as well as the digestive enzymes in stomach of experimental fishes were all measured within 3 days, using the Diagnostic Reagent Kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China) according to the manufacturer's instructions.

2.4 Statistical analysis

All the data are presented as mean ± SD. Mean values for all monitored parameters were analyzed by one- and two-way ANOVAs to determine whether there were significant differences due to the dietary levels of protein, carbohydrate or the interaction. When ANOVA identified differences among groups, the difference in means was made with Tukey's test. Statistical significance was determined at $P < 0.05$. All statistical analyses were carried out by using SPSS version 16.0 (SPSS, Michigan Avenue, Chicago, Illinois, USA).

3. Results

Growth performance and feed utilization of *M. salmoides* juveniles fed different dietary protein and carbohydrate levels are shown in Table 2. During the feeding trial, survival rate was above 90% and no significant differences were observed among treatments ($P>0.05$). Fish fed 42% protein level diets had significantly lower WG and SGR than fish fed 48% protein level diets ($P<0.05$). There had no significant differences in body weight gain values of fish fed 45% and 48% protein levels diets. No significant improvement in growth was observed by increment of dietary carbohydrate level from 10% to 13% ($P>0.05$). The best growth performance was found in fish fed the diet containing 48% protein and 10% carbohydrate with P/E ratio of 11.92 mg protein kJ^{-1}, and fish fed the diet containing 42% protein level and 10% carbohydrate level had the poorest growth. Daily feed intake was not affected by protein or carbohydrate levels. Feed conversion ratio (FCR) of fish fed 43% protein level diets were significantly higher than fish fed 48% protein level diets ($P<0.05$), but values of this parameter in fish fed 45% protein level showed no significant differences with other two groups ($P>0.05$).The values of PER remained stable and were not remarkably affected by dietary treatments ($P>0.05$). Morphometrical parameters, HSI and CF showed different variation. HSI of the experimental fish fed 13% carbohydrate level diets was significantly higher than fish fed 10% carbohydrate level diets ($P<0.05$), while this parameter was not affected by dietary protein levels ($P>0.05$). The CF data of the experimental fish was not related to dietary treatments ($P>0.05$) (Table 2).

In general, dorsal muscle composition of *M. salmoides* was mostly not related to dietary treatments with the exception of protein contents (Table 3). Significant changes were found in dorsal muscle protein content by variation of dietary protein, fish fed 42% protein level diets showed much lower than the values obtained in the other groups ($P<0.05$), however, muscle protein contents were not significantly affected by dietary carbohydrate level or their interactions ($P>0.05$).

The results of liver enzymes activities of *M. salmoides* juveniles fed diets with varying protein and carbohydrate levels were presented in Table 4. Significant enhancement in liver GPT level was observed with increasing dietary protein from 42% to 48% and the relatively high value was found in fish fed the diet containing 48% protein and 13% carbohydrate level. GOP level increased as dietary protein or carbohydrate level was increased ($P<0.05$). However, there was no significant interaction between dietary protein and carbohydrate in hepatic GPT or GOP activities of *M. salmoides*. The enzymes which relate to the glycometabolism were all observed to be influenced by the dietary treatments in the present study (Table 4). The hepatic HK level of the fish fed 42% protein and 10% carbohydrate level diet was significantly lower than fish fed other diets ($P<0.05$), but no significant interactions between dietary protein and carbohydrate in this enzyme were noticeable ($P>0.05$). The PK activities displayed very similar variation with HK, however, the value in the fish fed 45% protein level diets have no significant difference when compared to fish fed 42% protein diets ($P>0.05$). The PEPCK level of liver of *M. salmoides* were not significantly influenced by different dietary protein levels but decreased with increment of dietary carbohydrate levels, no significant interactions in PEPCK activities were found between dietary protein and carbohydrate ($P>0.05$).

For the analysis of the activity of stomach digestive enzymes (Table 5), pepsin activities of *M. salmoides* fed 42% protein

level diets were significantly lower than that of fish fed other two protein levels diets but did not significantly differ from those of fish fed 45% or 48% protein level diet. Dietary carbohydrate levels had significant effects on stomach amylase activities and fish fed 13% carbohydrate level diets had significantly higher values than fish fed 10% carbohydrate level diets(P<0.05). Lipase activities were more consistent and no significant differences were found among the experimental diets (P>0.05). No significant interactions between dietary dietary protein and carbohydrate in stomach digestive enzymes of *M. salmoides* were noticeable (P>0.05) (Table 5).

Table 2: Growth performance and feed utilization of *M. salmoides* juveniles fed diets with varying protein and carbohydrate levels for 8 weeks

Diets	FBW(g)	Survival(%)	WG(%)	SGR (%/day)	DFI (g/day)	FCR	PER	HSI (%)	CF (g/cm^3)
D1（P48C13）	67.57	92.83	274.60	2.40	128.69	1.10	2.04	2.40	2.10
D2（P48C10）	70.20	90.00	289.14	2.47	126.58	1.08	2.09	2.20	2.13
D3（P45C13）	65.90	90.50	266.44	2.36	135.21	1.13	2.11	2.39	2.15
D4（P45C10）	66.30	92.00	268.63	2.37	132.23	1.15	2.08	2.13	2.12
D5（P42C13）	64.90	90.17	260.12	2.33	130.27	1.21	2.12	2.23	2.10
D6（P42C10）	63.30	91.83	251.69	2.29	133.46	1.24	2.07	2.17	2.09
Pooled SE	0.56	0.72	3.06	0.02	3.22	0.07	0.01	0.04	0.02
Means of main effects									
Protein									
P48	68.88^a	91.47	281.87^a	2.44^a	127.64	1.09^b	2.07	2.30	2.12
P45	66.10^{ab}	91.25	267.54^{ab}	2.37^{ab}	133.72	1.14^{ab}	2.09	2.27	2.13
P42	64.10^b	91.00	255.90^b	2.31^b	131.86	1.22^a	2.09	2.20	2.09
Carbohydrate									
C13	67.00	90.22	271.90	2.38	130.69	1.14	2.11	2.34a	2.13
C10	65.72	92.77	264.98	2.35	131.46	1.16	2.06	2.17b	2.10
ANOVA (*P*-value)									
Protein	0.015	0.972	0.015	0.018	0.312	0.01	0.548	0.553	0.681
Carbohydrate	0.277	0.189	0.280	0.324	0.014	0.412	0.076	0.034	0.521
Protein× Carbohydrate	0.549	0.919	0.546	0.612	0.087	0.972	0.971	0.552	0.962

Values are means of triplicate groups and presented as mean ± SD.
a Weight gain (WG) = [(final body weight - initial body weight) / initial body weight × 100].
b Specific growth rate (SGR) = [ln (final body weight) - ln (initial body weight)] × 100 / days reared.
c Daily feed intake (DFI) = feed intake × 100 / [(initial body weight + final body weight + dead fish weight) × days reared / 2].
d Feed efficiency(FCR) = (wet weight gain / feed intake) × 100.
e Protein efficiency ratio (PER) = wet weight gain / protein intake.
f Hepatosomatic index (HSI)=100×liver wet weight (g)/body wet weight (g).
g Condition factor (CF)= 100 × body wet weight (g) / body length (cm)^3.

Table 3: Dorsal muscle composition (wet weight basis %) of *M. salmoides* juveniles fed diets with varying protein and carbohydrate levels for 8 weeks

`	Moisture	Protein	Lipid	Ash
D1（P48C13）	76.73	20.43	1.17	1.36
D2（P48C10）	76.60	20.77	1.15	1.39
D3（P45C13）	76.57	20.77	1.18	1.41
D4（P45C10）	76.73	20.53	1.17	1.40
D5（P42C13）	73.37	19.63	1.21	1.46
D6（P42C10）	76.57	19.83	1.23	1.50
Pooled SE	0.15	0.12	0.01	0.02
Means of main effects				
Protein				
P48	76.67	20.60a	1.16	1.37
P45	76.65	20.65a	1.18	1.40
P42	76.47	19.73b	1.22	1.48
Carbohydrate				
C13	76.56	20.28	1.19	1.41
C10	76.63	20.77	1.18	1.43
ANOVA (P-value)				
Protein	0.837	0.012	0.106	0.064
Carbohydrate	0.801	0.675	1.000	0.537
Protein× Carbohydrate	0.885	0.596	0.692	0.835

Table 4: Liver enzymes activities (U/gprot) of *M. salmoides* juveniles fed diets with varying protein and carbohydrate levels for 8 weeks

Diets	GPT	GOT	HK	PK	PEPCK
D1（P48C13）	11.69	3.25	32.60	94.57	47.11
D2（P48C10）	11.14	2.94	22.70	80.53	73.18
D3（P45C13）	11.38	3.11	27.44	90.49	46.30
D4（P45C10）	9.08	2.68	20.83	72.82	77.59
D5（P42C13）	4.56	1.83	22.06	79.83	48.69
D6（P42C10）	4.95	1.54	12.96	64.14	72.86
Pooled SE	0.30	0.04	0.94	1.97	2.04
Means of main effects					
Protein					
P48	11.42a	3.09a	27.65a	87.55a	60.14
P45	10.23a	2.90a	24.13a	81.65ab	61.95
P42	4.76b	1.68b	17.15b	71.99b	60.77
Carbohydrate					
C13	9.21	2.73a	27.36a	10.63a	47.36b
C10	8.39	2.39b	18.83b	9.25b	74.54a
ANOVA (*P*-value)					
Protein	0.00	0.000	0.003	0.022	0.936
Carbohydrate	0.194	0.002	0.001	0.002	0.000
Protein×Carbohydrate	0.214	0.794	0.762	0.932	0.765

Table 5: The activity of stomach digestive enzymes of *M. salmoides* juveniles fed diets with varying protein and carbohydrate levels for 8 weeks.

Diets	Pepsin（U/mg prot）	Lipase（U/g prot）	Amylase (U/mg prot）
D1（P48C13）	56.35	3.22	0.42
D2（P48C10）	59.10	2.97	0.38
D3（P45C13）	51.55	2.99	0.43
D4（P45C10）	52.72	3.47	0.37
D5（P42C13）	44.46	3.76	0.40
D6（P42C10）	44.57	3.33	0.36
Pooled SE	1.05	0.19	0.01
Means of main effects			
Protein			
P48	57.73a	3.09	0.40
P45	52.14a	3.23	0.40
P42	44.51b	3.54	0.38
Carbohydrate			
C13	50.79	3.32	0.41a
C10	52.13	3.26	0.37b
ANOVA (*P*-value)			
Protein	0.001	0.624	0.629
Carbohydrate	0.535	0.869	0.041
Protein × Carbohydrate	0.879	0.602	0.914

4. Discussion

Carbohydrates are considered to supply energy at low cost and carbohydrate utilization have been studied in most cultured fish [16]. However, it has been generally accepted that fish do not have a specific requirement for dietary glucose [17], many fish species showed a long postprandial hyperglycemia after feeding high digestible carbohydrates, which is associated with a retarding growth performance and sometimes a "fatty liver" [18, 19]. The aim of the present study was to investigate the metabolic consequences of a different carbohydrate/protein level feeding and examine whether the *M. salmoides* was able to adapt to the diets. It is well known that, omnivorous or herbivorous warm-water fish tolerate high dietary carbohydrate levels, utilizing them as a source of energy more effectively than carnivorous species [16]. For *M. salmoides*, previous studies reported that the optimum dietary starch level ranged from 10% to 19% [9, 12, 20]. These results indicated that the inclusion of high amounts of dietary starch remains controversial, but high starch contents would result in poor growth and feed utilization of *M. salmoides*. The result observed in the present study showed that decreasing the protein content from 48% to 42% reduced the growth and the feed utilization efficiency of the *M. salmoides*, while increasing the dietary carbohydrate level from 10% to 13% had no significant difference on growth performance, after only 8 weeks of trial. Generally, a level of ≤15%–25% digestible carbohydrate was appropriate for marine and carnivorous fish [21]. The results of this study indicated that 10%–13% dietary wheat starch level provided a comparable growth for juvenile *M. salmoides*, which is in agreement with the results obtained by Lin *et al*. [20]. One of the major functions of the liver is to extract glucose from the bloodstream and store it as glycogen and, if necessary, to mobilize it through glycogen hydrolysis [22]. Hepatosis in *M. salmoides* had become the first limiting factor for the sustainable development of the species [23]. However, no definite pathological trend in liver of the experimental fish in current study was observed and the survival was all higher

than 90% among the dietary treatments. The muscle composition of *M. salmoides* was scarcely modified by the dietary conditions, with fish fed the 42% protein diets exhibiting the lowest protein content. The body composition of Atlantic halibut (*Hippoglossus hippoglossus*) [24] and Senegalese sole (*Solea senegalensis*, Kaup 1858) [25] remained unchanged when the dietary protein/carbohydrate ratio decreased. But European seabass (*Dicentrarchus labrax*) [26] and gilthead sea bream (*Sparus aurata*, L.) [27] increased lipid deposition, suggesting a species-dependent metabolic efficiency. The protein-sparing effect of carbohydrate was suggested in some species only when the fish were fed diet with a suboptimal protein level [28]. In the present study, diets contain 45% protein level with two different carbohydrate level seem to be satisfied juvenile *M. salmoides* in current culture conditions, although the protein-sparing effect by carbohydrate was not obvious. This recommended protein level was relatively lower than the previous studies in this fish species, such as 47% by Tidwell *et al* [10], or 48%-51% by Huang *et al*. [29]. On the other side, extruded feed was used in current study. Compared with a pelleted diet, an extruded diet has a higher stability in water and a better nutrient utilization, which leads to lower nitrogen and phosphorus loads during fish culture [30, 31, 32]. Therefore, it is also hypothesized that the protein requirement of *M. salmoides* can be reduced slightly if the diet is extruded due to the improved nutrients digestibility and utilization.

As the principal organ of glucose homeostasis, the liver plays a critical role in regulating intermediary metabolism in response to nutritional status [33]. To evaluate the response of physiological and biochemical processes to dietary starch levels, the activities of a number of key carbohydrate metabolic enzyme, including HK, PK and PEPCK, were examined in the present study. HK and PK activities appear to be induced by either dietary carbohydrate levels or protein levels. The response of PK of *M. salmoides* is generally stronger than that of HK, suggesting that PK may play a more important role in adaptation to carbohydrate diets than HK. In contrast, the current results found that the hepatic gluconeogenic enzyme activity of PEPCK decreased significantly with increasing starch levels. The results suggested that endogenous glycolysis in *M. salmoides* was enhanced while gluconeogenesis was depressed by an increase in the dietary starch level, the similar results were found in European seabass [34] and golden pompano (*Trachinotus ovatus*) [35], and also in agreement with the results in previous study for *M. salmoides* fed with high starch diet [20]. The fish fed the highest protein level diets, the most expensive component in the diet, showed an increase in hepatic GPT and GOT activities, suggesting that amino acids were metabolized and contributed to providing energy. The similar results were found in surubim cachara (*Pseudoplatystoma reticulatum*) [36] and *Labeo rohita* (Hamilton) [37], means that amino acids acquired from a high protein diet were used, rather than carbohydrate, for energy production.

Digestion of food is one of the most important functions in the physiology of organism to obtain nutrients for various body activities, such as growth, maintenance, motion and reproduction [38]. The level of digestive enzymes in fish may be influenced by type of feeding, biochemical composition of food and onset of sexual maturity. Amylase is one of the major carbohydrases which hydrolyzes glycosidic bonds between sugar residues in large carbohydrate molecules. Amylase specifically breakdowns starch into glucose molecules. In the present study, *M. salmoides* juveniles fed with 13% carbohydrate level exhibited high amylase activity in stomach regardless of dietary protein levels. This indicated that changes in amylase enzyme activities did not occur in *M. salmoides* when subjected to dietary high protein level but carbohydrate level. Proteases are digestive enzymes which hydrolyzes peptide bonds between the adjacent amino acids in the proteins. The pepsin level of *M. salmoides* was lower when fed with low protein diet, the similar results were observed in black seabream (*Acanthopagrus schlegelii*) [39], hybrid Clarias catfish (*Clarias batrachus* × *Clarias gariepinus*) [40] and Chinese rice field eel (*Monopterus albus*) [41]. The relatively low pepsin activity in fish might be due to the lower availability of dietary protein as substrate for protease activity. Besides, lipase activity remained constant among the six groups, corresponding to the equal lipid concentrations in all the experiment diets. Further integrated studies combining enzyme regulation, nutrient digestibility and growth performance in relation to diets are required for this fish species.

5. Conclusion

In conclusion, the data generated from present study demonstrates that feeding with 45% protein level at 10% or 13% carbohydrate levels in extruded feed are both applicable for *M. salmoides* juveniles without any significant negative effects when compared with 48% protein 10% carbohydrate level diet treatment. The results may provide opportunities for development of new aqua-feed useful for formulating low-cost and efficient commercial feed for this fish species.

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7. References

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