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Effects of dietary levels of protein on growth, feed utilization and physiological parameters for juvenile Dabry's sturgeon, *Acipenser dabryanus*

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Abstract

Five semi-purified diets were formulated to contain graded levels of dietary protein (340, 390, 440, 490 and 540 g/kg diet), and each diet was assigned to triplicate groups of eight juvenile Dabry's sturgeon for 50 days. Results showed that specific growth rate increased linearly with increasing dietary protein levels from 340 to 440 g/kg diet, then levelled off at higher protein levels. Higher dietary protein (440 and 540 g/kg) significantly increased the feed intake and feed efficiency ratio of fish compared to lower protein diets (340 to 490 g/kg diet) ($p < .05$). Fish fed a 440 g protein kg^{-1} diet had higher protein efficiency ratio and serum lysozyme activity than other treatments. Serum ammonia content and activities of liver aminotransferase were positively correlated with dietary protein levels. No significant difference ($p > .05$) among groups was observed in glycogen content. As dietary protein level increased, protein and ash content of fish dorsal muscle were greatly enhanced, whereas lipid content was significantly reduced ($p < .05$). Based on broken-line regression analysis of SGR against dietary protein levels, the optimum dietary protein level for maximal growth of juvenile Dabry's sturgeon is 446.68 g/kg diet.

KEYWORDS
Dabry's sturgeon, dorsal muscle composition, lysozyme activity, physiological parameter, protein requirement

1 | INTRODUCTION

Dabry's sturgeon, also called Yangtze sturgeon, is an endemic freshwater fish species in the Yangtze River, China. It used to be a commercially important fish in the middle and upper reaches of the Yangtze River (Yang, 1986; Zhuang, Ke, Wei, He & Cen, 1997). However, since 1980s continued pressure from impoundment, pollution, commercial fishing and illegal poaching, has led to the dramatic decline of the population of Dabry's sturgeon (Wu & Wu, 1990). Nowadays, wild Dabry's sturgeon is rarely found in the Yangtze River and was listed as a First Class Protected Animal in 1989 (Wei et al., 1997) and as a Critical Endangered species in the 2010 IUCN RED List (IUCN, 2010 March).

Currently, Dabry's sturgeon is strictly protected by Chinese state policy (Zhuang, 1993). A series of protective measures, including establishment of natural reserves, artificial propagation programmes and legislation of fishing ban, have been taken by institutes, agencies and the government (Fan et al., 2007). In particular, artificial propagation and release has been recommended as an effective way to rehabilitate the population of Dabry's sturgeon stock in Yangtze River. Although the domestication of this fish started in the early 1970s (Xie, 1979), knowledge regarding husbandry, physiology and nutrition of Dabry's sturgeon is scarce. Lack of refined and species-specific diets is the foremost challenge in aquaculture of sturgeon, prolonged use of non-nutritionally balanced feeds may lead to poor growth, impaired immunity (Ai et al., 2007; Sayed Hassani, Mohseni, Hosseni, Yazdani Sadati & Pourkazemi, 2011), behavioural disorders (e.g. circulating running disease) and other nutritionally related deficiencies. Therefore, formulation of nutritionally balanced diet is vital for the success of intensive culture of this fish species.

In general, prior to investigating digestibility of feeds and quantitative amino acid requirements of fish, information about dietary protein requirement is critically important for any new aquaculture initiatives due to the fact that protein represents the most expensive component in fish diet and plays a major role in fish growth and health under cultured conditions (DeLong, Halver & Mertz, 1958). However, to date there has been no available information on the protein requirement of Dabry's sturgeon. The objectives of this study were to determine the optimum protein requirement of juvenile *Acipenser dabryanus* and to investigate the effects of dietary protein levels on growth performance, feed utilization and related physiological parameters of this fish. In addition, attempts to explore the relationship between fish immunity and dietary protein levels were also made.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Five experimental diets containing 340, 390, 440, 490 and 540 g crude protein kg^{-1} diet were formulated with fish oil and soybean oil as lipid sources and a fixed ratio (8:2.5:1) of white fishmeal, casein and soybean meal as protein sources. Gross energy was estimated based on published data rather than empirical determination (Table 1). Dry dietary ingredients were ground into fine powder and thoroughly mixed in a commercial mixer for 15 min, after which oil was gradually added by hand. Then water was slowly added to produce suitably textured dough, which was subsequently processed with a laboratory-use feed mill to form 2.0 mm pellets followed by air-dried for over 8 hr in a ventilated oven at 65°C. Experimental diets were stored in a cool and dry place.

2.2 | Experimental procedures and fish

The feeding trial was performed in an indoor flow-through water system in Rare Fish Propagation and Releasing Station of the Xiang Jia Ba and Xi Luo Du Hydropower Station (Yibin, Sichuan, China). Experimental water system comprised of 15 circular plastic tanks (diameter: 1.5 m, water level: 0.4 m) with water renewal of approximately 20 times per day. Water temperature ranged from 22.7°C to 26.1°C, and dissolved oxygen was more than 7.8 mg/L. All fish was subject to natural photoperiod.

Hatchery-produced Dabry's sturgeon juveniles (184.24 ± 6.79 g) were reared in tanks for environment acclimation. Prior to the start of the feeding trial, fish were fed an equal mixture of the five test diets for 10 days. After being starved for 3 days the fish were weighed and randomly distributed among 15 tanks. Each diet was randomly assigned in triplicate tanks stocked with eight fish per tank. Fish were hand-fed their prescribed diets to apparent satiation twice daily at 07:00 and 18:00. Feeding rations varied from 5.54% to 3.21% during the feeding trial. One hour after feeding, uneaten feed was re-collected by siphonage, dried at 105°C and re-weighed to record the amount of daily consumed feed. Given that the water stabilities of different tested pellets varied with dietary formulation, feed leaching was also studied to calibrate the actual feed intake of fish. In brief, 100 g of each feed was immersed in water for 1 hr

TABLE 1 Formulation and nutrient composition of the experimental diets (g/kg dry matter basis)

	Dietary protein levels (g/kg)				
Ingredient	340	390	440	490	540
White fishmeal[1]	310.0	360.0	420.0	455.0	500.0
Soybean meal	40.0	45.0	50.0	55.0	61.0
Casein	98.0	112.0	120.0	143.0	160.0
Wheat meal	150.0	150.0	150.0	150.0	150.0
Fish oil	52.0	50.0	48.0	46.0	44.0
Soybean oil	31.0	30.0	29.0	28.0	27.0
Soybean lecithin	10.0	10.0	10.0	10.0	10.0
Cellulose	263.0	197.0	128.0	67.0	2.0
Vitamin premix[2]	10.0	10.0	10.0	10.0	10.0
Mineral premix[3]	20.0	20.0	20.0	20.0	20.0
Ascorbic acid (35%)	2.0	2.0	2.0	2.0	2.0
Choline chloride (50%)	3.0	3.0	3.0	3.0	3.0
$Ca(H_2PO_3)_2$	5.0	5.0	5.0	5.0	5.0
Mould inhibitor[4]	1.0	1.0	1.0	1.0	1.0
Attractant[5]	5.0	5.0	5.0	5.0	5.0
Chemical composition (g/kg diet in dry matter)					
Dry matter	942.72	941.98	938.69	939.64	936.61
Crude protein	333.57	385.22	436.48	489.08	535.36
Crude lipid	104.03	103.34	107.65	107.31	105.15
Ash	109.84	116.02	131.52	139.14	156.19
Gross energy (kJ/g)[6]	18.95	19.06	19.31	19.54	19.48
CP:GE ratio (mg/kJ)[7]	17.60	20.21	22.61	25.03	27.47

[1]Imported from Fishery Kolkhoz Company (Russia) by Qihao Biotechnology Co., Ltd. (Qingdao, China) (Crude protein: 669.1 g/kg, Crude lipid: 70.5 g/kg).
[2]Mineral premix (mg/kg diet): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 7500; $NaH_2PO_4 \cdot 2H_2O$, 12 500; KH_2PO_4, 16 000; $Ca(H_2PO_4)_2 \cdot H_2O$, 10 000; $FeSO_4$, 1250; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750; $ZnSO_4 \cdot 7H_2O$, 176.5; $MnSO_4 \cdot 4H_2O$, 81; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.5; KI, 1.5; Starch, 225.
[3]Vitamin premix (mg/kg diet): vitamin A, 1.83; vitamin D, 0.5; vitamin E, 10; vitamin K, 10; niacin, 100; riboflavin, 20; pyridoxine, 20; thiamin, 20; D-calcium pantothenate, 50; biotin, 0.1; folacin, 5; vitamin B_{12}, 20; inositol, 100.
[4]Purchased from Shanghai Bangcheng Bioengineering Co., Ltd. (benzoic acid ≥3%, sodium diacetate ≥10%, sodium benzoate, synergistic agent, SiO_2).
[5]Attractant: Dimethyl-β-propiothetin (DMPT): inosinic acid: betaine: Glycyrrhizic Acid = 1:1:1:1
[6]Gross Energy of g^{-1} diet = 17.2 kJ/g × crude protein g^{-1} diet + 23.6 kJ/g × protein g^{-1} diet + 39.5 kJ/g × lipid g^{-1} diet.
[7]Crude Protein to Gross Energy.

and then re-collected, dried at 105°C for 24 hr and re-weighed. The leaching coefficient was calculated as: DMi = [1-(Wi/Wf)], where: DMi = percentage of dry matter leaching (%); Wf = dry feed weight before immersion in water (g); Wi = dry feed weight after immersion in water (Carvalho & Nunes, 2006).

2.3 | Sample collection and analysis

At the termination of the feeding trial, each fish was weighed and harvested after a 24 hr starvation period. Growth performance and feed utilization in terms of specific growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio (PER) were assessed. The application for fish usage was approved by local fishery supervision administration and handling procedures with experimental fish were performed according to the Guidelines for the Use of Fishes in Research (Nickum et al., 2004). Three fish per tank were randomly selected for biological sample collection and dispatched with a lethal blow to the head. Blood collected from the caudal vein was placed under 4°C to obtain the serum sample which was subsequently stored at −20°C for further analysis. After dissection, liver was weighed and sampled for enzyme analysis. Dorsal muscle was sampled and stored at −20°C to determine the proximate chemical composition. The calculation formulas were as follows (NRC, 1993):

$$\text{FI: Feed intake(g/fish)} = \text{total amount of diet fed to a tank for 50 days/the fish number in the same tank;}$$

$$\text{SGR: Specific growth rate}(\%\text{d}^{-1}) = 100 \times \text{Ln(final body weight/initial body weight)/days;}$$

$$\text{FER: Feed efficiency ratio} = \text{weight gain (g)/feed intake (g);}$$

$$\text{PER: Protein efficiency ratio} = \text{weight gain (g)/protein intake (g);}$$

$$\text{SR: Survival rate}(\%) = 100 \times \text{final number of fish/initial number of fish}$$

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$$\text{VSI: Viscerosomatic index}(\%) = 100 \times \text{(Viscera weight)/(whole-body weight);}$$

$$\text{HSI: Hepatopancreas index}(\%) = 100 \times \text{(Liver weight)/(whole-body weight).}$$

2.4 | Chemical analysis

Proximate chemical composition of feed ingredients, experimental diets and fish muscle were determined by standard methods of AOAC (1990). The samples of diets and fish muscle were dried to a constant weight at 105°C to determine the dry matter content. Protein was determined by measuring nitrogen ($N \times 6.25$) using the Kjeldahl method (Kjeltec 2300 Protein Analyzer, Denmark); lipid by ether extraction using Soxhlet system (B-801, Switzerland); and ash by burning samples in a muffle furnace at 550°C.

2.5 | Physiological parameters assays

Lysozyme activity in serum, Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity together with glycogen content in liver were determined using commercial reagent kits (Nanjing Jiancheng Bioengineering Co., Ltd, Nanjing, China). As described by the kits instructions, frozen liver was homogenized in ice-cold 0.7% saltwater followed by centrifugation (3200 × *g*, 20 min, 4°C), and the activities of AST and ALT in resultant liver supernatants were measured at an absorbance of 505 nm according to the reported method (Panteghini, 1990). Lysozyme activity in serum was determined as described by Ellis (1990). Glycogen content in liver was determined following the Trinder's glucose-oxidase method (Trinder, 1969). Serum ammonia determination was performed using commercial assaying kits. All assays were reproducible.

2.6 | Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) in SPSS 13.0 for Windows. Differences between the means were tested by Tukey's multiple range test and were considered significant when probability (*p*) values <.05 were obtained, results are expressed as means ± *SE*. Broken line regression analysis (Robbins, Norton & Baker, 1979) was used to determine the optimum protein requirement. The equation used in the model is $Y = L + U (R\text{-}X_{LR})$, where Y is the parameter (SGR) chosen to estimate the requirement, L is the ordinate and R is the abscissa of the breakpoint. R is taken as the estimated requirement, X_{LR} represents X less than R and U is the slope of the line for X_{LR}. By definition, $R\text{-}X_{LR} = 0$ when $X > R$.

3 | RESULTS

3.1 | Growth performance and feed utilization

No mortality occurred during the feeding trail. Growth performance and feed utilization of juvenile Dabry's sturgeon are presented in Table 2. As dietary protein levels increased from 340 to 440 g/kg diet, SGR increased significantly ($p < .05$) before levelling off at higher dietary protein levels. Fish fed on the 490 g protein kg^{-1} diet had the highest SGR. Similar trends were also observed for both FER and PER. As dietary protein level increased, FER tended to increase and PER was significantly higher in the 440 g protein kg^{-1} diet treatment ($p < .05$). FI was significantly enhanced as dietary protein levels increased ($p < .05$). Based on broken-line regression analysis of SGR against dietary protein level, the optimum dietary protein level for maximal growth of the fish was 446.68 g/kg (Figure 1).

Results shown in Table 2 indicate that VSI values deceased markedly with increasing dietary protein levels, with the highest value (5.86 ± 0.18) and lowest values (4.90 ± 0.180.33) were

TABLE 2 Growth performance and feed utilization of Dabry's sturgeon fed diets with graded levels of protein

	Experimental diets				
Dietary protein Level	340	390	440	490	540
IBW[1]	183.60 ± 3.26	185.35 ± 3.16	180.82 ± 2.20	179.01 ± 3.02	181.63 ± 3.72
FBW[2]	306.14 ± 9.06[a]	320.55 ± 9.40[a]	384.73 ± 3.60[b]	382.22 ± 4.31[b]	381.60 ± 8.17[b]
FI[3]	152.07 ± 6.26[ab]	146.16 ± 3.89[a]	167.75 ± 0.37[bc]	184.48 ± 2.31[c]	182.15 ± 3.91[c]
SGR[4]	0.98 ± 0.07[a]	1.09 ± 0.07[a]	1.51 ± 0.01[b]	1.54 ± 0.05[b]	1.48 ± 0.04[b]
FER[5]	0.73 ± 0.04[a]	0.93 ± 0.10[ab]	1.14 ± 0.01[b]	1.05 ± 0.03[b]	1.03 ± 0.06[b]
PER[6]	2.35 ± 0.14[ab]	2.39 ± 0.17[ab]	2.72 ± 0.02[b]	2.28 ± 0.08[ab]	2.05 ± 0.11[a]
SR[7]	100	100	100	100	100
VSI[8]	5.86 ± 0.18[a]	5.54 ± 0.13[ab]	5.41 ± 0.16[ab]	4.90 ± 0.33[b]	5.08 ± 0.15[ab]
HSI[9]	2.10 ± 0.07	2.27 ± 0.14	2.12 ± 0.25	2.01 ± 0.16	1.99 ± 0.15

Values are presented as means ± *SE* of triplicate groups ($n = 3$). Means in the same column with different superscripts are significantly different from each other ($p < .05$).
[1]IBW: Initial body weight (g);
[2]FBW: Final body weight (g);
[3]FI: Feed intake (g/fish) = total amount of diet fed to a tank for 50 days/the fish number in the same tank.
[4]SGR: Specific growth rate (% d^{-1}) = 100 × Ln (final body weight/initial body weight)/days;
[5]FER: Feed efficiency ratio (FER) = weight gain (g)/feed intake (g);
[6]PER: Protein efficiency ratio (PER) = weight gain (g)/feed intake (g);
[7]SR: Survival rate (%) = 100 × final number of fish/initial number of fish.
[8]VSI: Viscerosomatic index (%) = 100 × (viscera weight)/(whole-body weight);
[9]HSI: Hepatopancreas index (%) = 100 × (liver weight)/(whole-body weight).

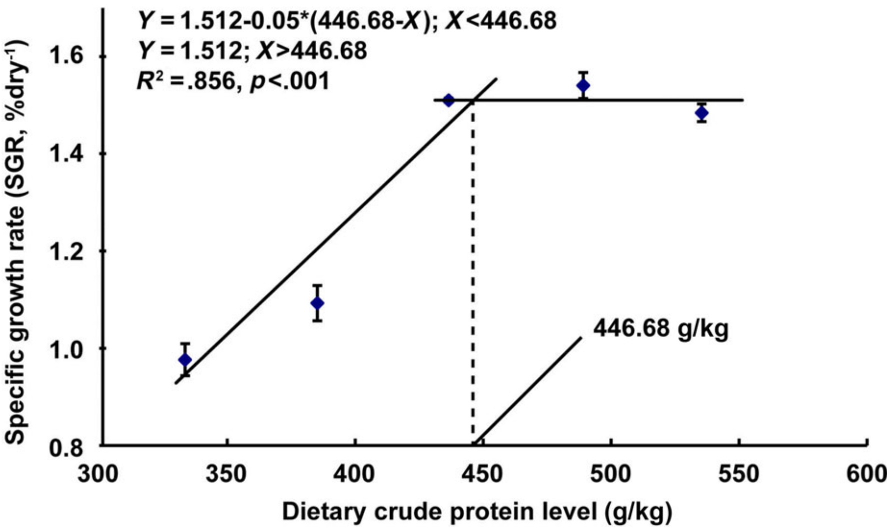

FIGURE 1 Relationship between dietary protein level and specific growth rate of Dabry's sturgeon fed diets with graded levels of protein [Colour figure can be viewed at wileyonlinelibrary.com]

recorded in 340 and 490 g protein kg^{-1} diet treatments respectively. There were no significant differences in HIS among dietary treatments.

3.2 | Dorsal muscle composition

The dorsal muscle composition of sturgeon juveniles is presented in Table 3. Crude protein content increased significantly with increasing dietary protein levels ($p < .05$). A similar tendency was observed for ash content. In contrast, lipid content was inversely related to dietary protein ($p < .05$). High protein diets tended to result in higher moisture, but no significant difference between treatments was observed ($p > .05$).

3.3 | Physiological parameters

As dietary protein levels increased, ALT activities in fish liver markedly increased ($p < .05$), whereas AST activities were not significantly affected by dietary protein, although an enhancing trend was observed ($p > .05$). Fish fed higher protein diets had significantly higher serum ammonia levels compared to those fed lower protein diets ($p < .05$). Liver glycogen content did not significantly differ

TABLE 3 Proximate composition (g/kg wet matter basis) of the dorsal muscle of Dabry's sturgeon fed diets with graded levels of protein

Dietary protein Level (g/kg)	Dry matter	Crude protein	Crude lipid	Crude ash
340	225.27 ± 5.13	153.69 ± 1.91^{a}	53.47 ± 5.76^{a}	8.89 ± 1.05^{a}
390	223.75 ± 3.26	159.71 ± 2.82^{ab}	46.65 ± 4.14^{b}	9.24 ± 0.78^{ab}
440	217.08 ± 3.09	164.09 ± 5.09^{bc}	37.74 ± 3.07^{c}	9.35 ± 0.50^{ab}
490	213.14 ± 4.20	165.60 ± 5.38^{bc}	32.89 ± 4.67^{d}	10.25 ± 0.89^{ab}
540	211.97 ± 1.89	169.88 ± 8.85^{c}	28.94 ± 7.50^{e}	11.64 ± 0.71^{b}

Values are presented as means ± *SE* of triplicate groups (n = 3). Means in the same column with different superscripts are significantly different from each other (p < .05).

among diet groups (p > .05). Serum lysozyme activities were markedly influenced by dietary protein levels, with fish fed 440 g protein kg^{-1} diet showing the significantly higher lysozyme activity compared to other treatments (Table 4).

4 | DISCUSSION

4.1 | Growth performance

The optimum dietary protein for juvenile Dabry's sturgeon between 160 and 400 g calculated by broken-line model analysis and second-order polynomial regression analysis were 446.68 g and 532 g protein kg^{-1} diet respectively. Although the coefficient of determination was improved when SGR data were fitted to the second-order polynomial regression analysis (R^2 = .891) rather than broken-line model analysis (R^2 = .856), from a practically economic point of view, protein requirement determined by broken-line model may be more appropriate than the other method. This value is somewhat higher than earlier reports on juvenile White Sturgeons (Moore, Hung & Medrano, 1988), juvenile Siberian Sturgeon (Kaushik, Luquet, Blanc & Paba, 1989) and juvenile Persian Sturgeon (Mohseni, Sajjadi & Pourkazemi, 2007), which estimated the dietary protein level for maximum growth of juvenile sturgeons to be around 36%–42%. Irrespective of culture conditions (feeding manipulation, stocking density and water temperature, etc.), the quality of protein sources used in the experimental diets are as good as fish meal and casein with balanced EAA composition and high digestibility, the higher protein requirement estimated in this study is probably due to the carnivorous species, Dabry's sturgeon juveniles, need higher levels of protein to maintain the fast growth at this life stage compared to other sturgeon species.

4.2 | Feed utilization

Although initial body weight was similar across treatments, FI varied with dietary protein levels. There was obvious increase in FI with the increased dietary protein inclusions. Comparable results were observed in tilapia (De Silva & Gunasekera, 1989) and channel catfish (Page & Andrews, 1973). One reason that fish reduced their voluntary pellet intake was largely due to the poorer palatability of lower protein diets (Giri, Sahoo, Paul, Mohanty & Sahu, 2011). However, studies on rabbit fish (El-Dakar, Shalaby & Saoud, 2011) and grass carp (Du et al., 2005) indicated that FI decreased as dietary protein levels increased. In addition, Kim and Lee (2009) reported that the FI of the juvenile tiger puffer was independent of protein levels in diets. A considerable number of studies have clearly indicated that FI varies with fish species.

FER values in this study ranged from 0.73 to 1.14, and the highest value was found in 440 g/kg dietary protein treatment. Usually, fish fed diets with higher protein levels showed significantly higher FER than those fed lower protein diets. This effect has also been reported on other fish species (Kaushik, Breque & Blanc, 1991; Mohseni et al., 2007; Siddiqui, Howlader & Adam, 1988). PER tended to increase from 2.31 to 2.70 with dietary protein levels increasing from 340 to 440 g/kg diet, then declined markedly at higher protein levels. Comparably, previous literatures also reported that excessive dietary proteins lead to decreased PER of fish, and similar PER trends have been noted for catfish (Giri et al., 2011), carp (Ogino &

TABLE 4 Physiological parameters of Dabry's sturgeon fed diets with graded levels of protein

Dietary protein Level (g/kg)	AST (U/gprot)	ALT (U/gprot)	Glycogen (mg/g)	Serum ammonia (μmol/L)	Lysozyme (unit/ml)
340	36.48 ± 2.84	32.45 ± 0.29^{a}	28.81 ± 1.62	310.65 ± 7.48^{a}	182.32 ± 5.03^{abc}
390	36.56 ± 5.05	41.27 ± 4.47^{ab}	24.80 ± 2.61	339.74 ± 26.25^{a}	188.40 ± 7.67^{bc}
440	36.37 ± 2.03	43.55 ± 3.37^{ab}	27.82 ± 0.81	362.81 ± 6.44^{ab}	214.49 ± 7.52^{c}
490	40.45 ± 3.31	46.62 ± 6.61^{ab}	27.41 ± 2.03	429.21 ± 17.68^{b}	147.83 ± 10.04^{a}
540	45.68 ± 4.89	59.42 ± 1.45^{b}	32.38 ± 0.61	416.80 ± 14.63^{b}	153.62 ± 10.44^{ab}

Values are presented as means ± *SE* of triplicate groups (n = 3). Means in the same column with different superscripts are significantly different from each other (p < .05).

Saito, 1970), gilthead seabream (Santinha, Gomes & Coimbra, 1996) and Mahseer (Ng, Abdullah & De Silva, 2008), which may be attributed to the imbalanced protein/energy (P:E) ratio and additional energy costs by deamination, amino acids absorption (De Silva & Perera, 1985; Mohanta, Mohanty, Jena & Sahu, 2008; Santinha et al., 1996; Shiau & Huang, 1989).

4.3 | Composition of dorsal muscle

The crude protein of dorsal muscles responded positively to dietary protein level in a dose-dependent manner. Studies on tilapia (Siddiqui et al., 1988), Japanese flounder (Kim, Wang & Bai, 2002) and Chinese sucker (Zhang, Gong, Yuan, Chu & Yuan, 2009) showed similar trends of body protein content with the increase in dietary protein. However, studies on juvenile marbled rabbitfish (El-Dakar et al., 2011), white sturgeon (Moore et al., 1988) and turbot (Lee, Cho, Park, Kim & Lee, 2003) showed that body protein content of turbot was not affected by dietary protein levels. And Khan, Ang, Ambak and Saad (1993) reported that although body protein content of Malaysian catfish was enhanced by increasing protein levels, it decreased when dietary protein levels surpassed a certain level. To the contrary, lipid content was inversely related to dietary protein levels, agreeing well with earlier reports (Jauncey, 1982; Kim et al., 2002; Ng, Soon & Hashim, 2001). However, this result is contrary to previous studies by Sá, Pousão-ferreira and Oliva-teles (2008) and Kim and Lee (2009) who found that lipid levels in fish increased with dietary protein levels, possibly due to excessive dietary protein being deaminated and stored as body fat (Driedzic & Hochachka, 1978). As regards the disagreement concerning the influences of various levels of dietary protein on fish muscle composition, Shearer (1994) has pointed out that the proximate composition of fish was influenced by both endogenous factors such as fish species, size, age and sex as well as exogenous factors such as diet composition and culture environment.

4.4 | Physiological conditions

In this study, VSI but not HSI was effected by dietary protein, VSI tended to decrease with increasing dietary protein levels, suggesting a reduction in fat deposition and advantageous carcass performance. It is of note that VSI of Dabry's sturgeon was much lower than that of other sturgeons, including juvenile Russian sturgeon (Wang et al., 2016), Chinese sturgeon (Xiao et al., 2011) and Amur sturgeon (Ni et al., 2016), but was comparable to the VSI of turbot (Zhu et al., 2014) and flounder (Li et al., 2015). In this study, two critical enzymes ALT and AST in liver regarded as reliable indicator of dietary protein availability, were found to correlate positively with the increase in dietary protein content. The results are in partial agreement with the results obtained for ALT and AST activities in sea bream (Metón et al., 1999), ALT activities in rainbow trout (Sánchez-Muros, García-Rejón, García-Salguero, de la Higuera & Lupiáñez, 1998) and ALT and AST activities in tilapia (Abdel-Tawwab, Ahmad, Khattab & Shalaby, 2010). Moreover, the rise of AST and ALT activities in this study also denoted the use of excessive dietary amino acids, resulting in the enhanced production of serum ammonia, the end product of amino acids catabolism in teleostean. Furthermore, the deamination and transamination actions of amino acids likely provided substrate for gluconeogenesis which could be evaluated by the glycogen content in fish liver (BibianoMelo, Lundstedt, Metón, Baanante & Moraes, 2006; Moyano, Cardenete & De la Higuera, 1991). However, the present results showed that glycogen content seemed to be unaffected by dietary protein. Earlier studies on gilthead sea bream (Couto, Enes, Peres & Oliva-Teles, 2008) and European sea bass (Peres & Oliva-Teles, 2002) have established the positive correlation between HIS, hepatic glycogen and dietary carbohydrate level, thus the constable digestible carbohydrate in experimental diets may explain the results. In addition, lysozyme activity has been reported to be an important indicator for evaluating fish health status, reflecting the capacity of fish to resist alien pathogens and bacteria (Ellis, 1990). Similar to findings in previously reported studies (Kiron, Watanabe, Fukuda, Okamoto & Takeuchi, 1995), the present work showed that serum lysozyme activity increased with increasing dietary protein levels, but declined when fish were fed diets containing excessive protein.

4.5 | Protein to energy ratio

The importance of P:E ratio for species-specific fish pellets lies in the facts that an imbalanced P:E ratio tends to result in poor utilization of protein and consequent environmental nitrogen stress (Sá et al., 2008; Somsueb & Boonyaratpalin, 2001). Given that digestibility of diets is a major factor affecting the usefulness of diet as energy sources, digestible energy and digestibility of individual nutrients should be used for formulation of fish diet. The DP (digestible protein) to DE(digestible energy) ratios generally considered to be optimum for fish growth range between17–26 mg DP kJ^{-1} DE (NRC, 1993), which was comparable to previous reports in Siberian sturgeon (Médale, Gorraze & Kaushik, 1995) and Chinese sturgeon (Wen, Ku & Luo, 2003). However, due to a lack of sufficient information on DP and digestibility of feed ingredients for some fish species, values of GE and crude nutrient are still commonly used in fish diet formulation (Bureau, Kaushik & Cho, 2003). In a recent review authored by Hung (2017), juvenile sturgeons appear to be able to utilize a wide range of P:E ratios on condition that amino acids are not limiting and dietary GE approach or exceed 14.7 kJ/g diet. Using experimental diets with crude protein (CP) to GE ratio ranging from 17.6 to 27.4 mg protein kJ^{-1}, this study is the first report to document that juvenile Dabry's sturgeon fed 436 g CP and 19.31 kJ GE g^{-1} diet had the best SGR and PER compared to other dietary treatments, nonetheless, additional research is still needed to help to understand the digestible (or metabolic) energy requirement and corresponding P:E ratios for Dabry's sturgeons.

5 | CONCLUSION

From this study, it was concluded that juvenile Dabry's sturgeons reached maximum growth when fed a semi-purified diet containing

436.48 g protein kg^{-1} diet with a CP:GE ratio of 22.61 mg protein kJ^{-1}. Moreover, dietary protein levels could significantly modify feed intake and enzyme activities, and might thus affect the utilization and metabolism of dietary protein as well as the chemical composition of dorsal muscle. As expected, the optimal level of dietary protein emphasized a beneficial effect on the immune response of fish. The results herein are expected to serve as a basis for further investigations into, among others, the digestibility coefficients of various dietary ingredients as well as the dietary energetic needs, the protein to energy requirements and the amino acids requirements of Dabry's sturgeon.

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