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Received: 16 February 2020 | Revised: 15 August 2020 | Accepted: 21 September 2020

DOI: 10.1111/jwas.12748

FUNDAMENTAL STUDIES

Journal of the World Aquaculture Society Open Access | WORLD AQUACULTURE Society | WILEY

Arginine supplementation in plant-rich diets affects growth, feed utilization, body composition, blood biochemical indices and gene expressions of the target of rapamycin signaling pathway in juvenile Asian red-tailed catfish (*Hemibagrus wyckoiides*)

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Funding information
Natural Science Youth Research Foundation of Yunnan Agricultural University, Grant/Award Number: 2016ZR14; Yunnan Province Agricultural Basic Research Joint Project, Grant/Award Numbers: 2017FG001-021, 2018FG001-044; Zhuangping Expert Workstation

Abstract

Arginine (Arg) is an important essential amino acid required for optimum fish growth. However, its role in plant-rich diets on growth and the required level in juvenile *Hemibagrus wyckioides* are currently unknown. This study evaluated the effects of Arg supplementation in plant-rich diets on growth, feed utilization, whole body composition, blood biochemical indicators and gene expressions of the target of rapamycin (TOR) signaling pathway in juvenile *H. wyckioides*. A total of 450 fish (6.40 ± 0.06 g) were randomized into six equal groups and fed on diets with graded

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Arg levels: 0 (control), 3, 6, 9, 12 and 15 g/kg of dry feed for eight weeks. The results revealed that specific growth rate (SGR) and feed conversion ratio of juvenile *H. wyckioides* were significantly improved and reduced, respectively in fish fed on 9 g/kg of Arg supplementation ($p < .05$). The optimum amount of dietary Arg content in plant-rich diets for *H. wyckioides* was 63.77 g/kg diet based on SGR. Feeding fish with dietary Arg supplementation ranging from 9 to 12 g/kg improved significantly ($p < .05$) whole body protein contents and decreased lipid deposition. The mRNA levels of TOR, eukaryotic translation initiation factor 4E (4EBP) and ribosomal protein S6 kinase 1 (S6K1) in muscle and liver were significantly affected by increasing dietary Arg levels. Meanwhile, the mRNA relative expression levels of TOR and S6K1 in the liver and muscle increased significantly as Arg supplementation increased. On the other hand, the mRNA relative expression levels of 4EBP was significantly higher in fish fed on the 9 g/kg Arg diet compared to those fed on the control and 15 g/kg Arg diets. In conclusion, our current results indicate that dietary Arg supplementation in plant-rich diets, might activate the TOR signaling pathway, which promotes protein synthesis and reduces lipid accumulation of *H. wyckioides*, resulting in improved growth.

KEYWORDS

arginine, blood chemistry, gene expression, *Hemibagrus wyckioides*, nutrient requirement

1 | INTRODUCTION

Asian red-tailed catfish (*Hemibagrus wyckioides*) is an omnivorous, freshwater fish, which is widely distributed throughout Southeast Asia including Yunnan province in Southern China (Kottelat, 1998). The *H. wyckioides* possess some desirable features required for aquaculture production such as tolerance to a broad range of varying water quality parameters and feeds on a wide variety of diets (i.e., prepared, frozen or live), which makes it a good candidate for aquaculture (Tippayadara, Doolgindachbaporn, & Suksri, 2016). Techniques for mass larval production of *H. wyckioides* have been developed, including successful induction of spawning and cage culture along the Mekong River Basin, especially in Yunnan province (Deng, Zhang, Bi, Kong, & Kang, 2011; Jiwyam & Nithikulworawong, 2014; Prasertwattana, Manee, & Namtum, 2005). Accordingly, its aquaculture production has increased dramatically in recent years (Hung, Binh, Thanh Truc, Tham, & Ngoc Tran, 2017).

However, research on this species has been limited. Studies conducted by Deng et al. (2011) and Hong Tham, Binh, and Hung (2013) revealed that, *H. wyckioides* requires high dietary levels of protein, between 439.0 and 441.2 g/kg. To meet the high protein level required by *H. wyckioides*, various ingredients has been used at different inclusion levels. The inclusion level of fishmeal (FM), casein and gelatin as protein sources required for optimum

growth of *H. wyckioides* were reported as 63, 29 and 8%, respectively (Deng et al., 2011). However, Xu et al. (2020) found negative effect on growth, physiological, biochemical, and intestinal health of *H. wyckioides*, when FM was replaced more than 50% by using plant protein sources. The optimal FM substitution quantity by using plant-based protein in *H. wyckioides* has been reported to range from 25 to 50% (Xu et al., 2020). Over the past several decades, FM has been the most ideal protein source for aquatic animals, but with the fast growth of the aquaculture industry, increasing demand for FM has resulted in rapid increases in prices as well as competition for this valuable feedstuff (Trushenski, Kasper, & Kohler, 2006). For these reasons, many measures have been taken in order to reduce FM consumption and enhance utilization of alternative protein sources in diets (Gatlin et al., 2007). Currently, plant proteins such as soybean meal (SBM), fermented soybean meal (FSBM), wheat gluten meal, corn gluten meal and soybean protein concentrate have all been widely used in feeds for many aquaculture species (Glencross, Booth, & Allan, 2007; He et al., 2019; Naylor et al., 2009). Feeds formulated with high levels of plant protein can limit growth potential in certain aquaculture species. This is most likely because of limitations of certain essential amino acid (EAA), as their profiles in plant protein have been shown to differ considerably compared to FM (Davies & Morris, 1997; Tulli, Messina, Calligaris, & Tibaldi, 2010). Thus, the use of high levels of plant proteins in fish feeds often results in amino acid imbalance in diets, which requires supplementation with crystalline amino acids in order to meet the EAA requirements (Figueiredo-Silva, Lemme, Sangsue, & Kiriratnikom, 2015).

Arginine (Arg) is an EAA required for optimal fish growth because of its ability to stimulate the release of various fish hormones such as insulin, growth hormone and glucagon (Mommsen, Moon, & Plisetskaya, 2001). Previous studies have shown that dietary Arg deficiency causes growth reduction and poor protein retention in several fish species such as channel catfish (*Ictalurus punctatus*) (Pohlenz, Buentello, le Helland, & Gatlin, 2014) and blunt snout bream (*Megalobrama amblycephala*) (Liang et al., 2016). However, information on the effects of dietary Arg supplementation in plant-rich diets on growth and the optimal level required for *H. wyckioides* are currently unknown. In addition, the role played by the target of rapamycin (TOR), which is involved in amino acid-sensing mechanism to promote growth, protein synthesis and hypertrophic growth in muscle of many fish (Wu et al., 2017; Zhou et al., 2019; Zou et al., 2018) is currently also not well established in *H. wyckioides* fed on plant-rich diets.

Therefore, the current study evaluated the effects of dietary Arg supplementation in plant-rich diets on growth and determined the optimal requirement in *H. wyckioides* fed on plant-rich diets. We also explored the role of TOR signaling pathway regulation in juvenile *H. wyckioides* fed on plant-rich diets.

2 | MATERIALS AND METHODS

2.1 | Ethical considerations for the use of animals

All animal protocols were approved by the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of the People's Republic of China. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

2.2 | Source of fish and acclimatization

A total of 600 Juvenile *H. wyckioides* were obtained from a private hatchery (Zengyuan Fisheries Technology Co., Ltd., Puer, Yunnan, China). The fish were acclimatized in a cement tank (3 m × 3 m × 2 m, L: W: H) and fed with a control diet for two weeks in order to adjust to the culture environment in the aquaculture laboratory at Yunnan Agricultural University, Kunming, China.

2.3 | Formulation of experimental diets

Six iso-nitrogenous and iso-lipidic diets with graded levels of Arg supplementation (0 (control), 3, 6, 9, 12 and 15 g/kg of dry diet) were formulated by using FM, SBM, FSBM and wheat gluten meal as protein sources (Table 1). The inclusion levels plant ingredients were high (50% of the total protein) to designate plant-rich diets, based on our previous study on the same species (Xu et al., 2020). The dietary protein concentration was fixed at 44%, the reported optimum for growth of *H. wyckioides* (Deng et al., 2011; Hong Tham et al., 2013). The amount of Arg were fixed on the basis of information available on other cultured fish species (Buentello & Gatlin, 2000; Chen et al., 2012; Fagbenro, Nwanna, & Adebayo, 1999; Khan, 2012; Tu et al., 2015). Glutamic acid was used to balance the amino acid concentrations. The levels of other nutrients met the requirements for *H. wyckoiides* according to the National Research Council (NRC, 2011). Feedstuffs were sieved by using a 0.2 mm sieve and mixed thoroughly according to formulations before adding water to form a dough. The dough was then pelleted by using a 1.5 mm diameter grinder. Diets were dried under natural air at room temperature for about 48 hr. Dried pellets were sealed in plastic bags and stored at −20°C until needed for feeding fish.

TABLE 1 Formulation and proximate composition of the experimental diets (dry matter basis)

	Diets					
Ingredient composition (g/kg)	**Control**	**Arg 3**	**Arg 6**	**Arg 9**	**Arg 12**	**Arg 15**
Fish meal[a]	280	280	280	280	280	280
Fish oil	52	52	52	52	52	52
Starch	150	150	150	150	150	150
Soybean meal	120	120	120	120	120	120
Fermented soybean meal	170	170	170	170	170	170
Wheat gluten	80	80	80	80	80	80
α-Starch	60	60	60	60	60	60
Lecithin	15	15	15	15	15	15
Choline chloride	10	10	10	10	10	10
Cellulose microcrystalline	18	18	18	18	18	18
Monocalcium phosphate	10	10	10	10	10	10
Arginine	0	3	6	9	12	15
Glutamic	15	12	9	6	3	0
Mineral premix[b]	10	10	10	10	10	10
Vitamin premix[c]	10	10	10	10	10	10
Proximate composition (%)						
Dry matter	89.193	89.426	88.316	89.009	89.919	89.615
Crude protein	44.129	44.199	43.761	44.190	44.770	44.083
Crude lipid	9.142	8.835	8.921	8.863	9.036	8.866
Ash	6.865	6.884	6.843	6.894	6.877	6.989

[a]White fish meal from the United States: crude protein content of 72.37%, crude fat content of 11.22%.
[b]Mineral premix (mg/kg diet): NaF, 1; KI, 0.4; $CoCl_2{\cdot}6H_2O$, 25; $CuSO_4{\cdot}5H_2O$, 5.0; $FeSO_4{\cdot}H_2O$, 40; $ZnSO_4{\cdot}H_2O$, 25; $MnSO_4{\cdot}H_2O$, 30; $MgSO_4{\cdot}7H_2O$, 600; $Ca(H_2PO_4)_2{\cdot}H_2O$, 1,500; NaCl,50; Zeolite, 7,725.
[c]Vitamin premix (mg/kg diet): thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin B12, 0.1; vitamin K3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin, 1.20; retinal acetate, 32; cholecalciferol, 5; α-tocopherol, 120; ascorbic acid, 2,000; ethoxyquin150.

2.4 | Experimental design and fish feeding

After acclimatization, fish were fasted for 24 hr, anesthetized with 40 mg/L eugenol solution (Aladdin reagent Co., Ltd., China) to avoid stress and weighed to determine their initial weight. Then, 450 fish (initial body weight 6.40 ± 0.06 g) were randomly distributed into 18 tanks (1.0 m × 0.5 m × 0.6 m), each tank had 25 juveniles *H. wyckioides*. Three tanks were randomly assigned to one of the six dietary treatments. Fish were cultured in a closed-water recirculating system, with biological and mechanical filters consisting of high-density polyester screens to remove particulate materials and automatic oxygen supplementation. Fish were hand-fed with the test diets to apparent visual satiation, twice daily at 8:00 and 4:30 hr for eight weeks. Initial and weekly weights of feeds were recorded and all uneaten feeds were collected by siphoning. Water quality parameters were maintained within suitable ranges for juvenile *H. wyckioides* growth and survival. During the experiment, the water temperature was maintained by using a heating rod. The water temperature ranged from 26 to 30°C, while the contents of ammonia nitrogen, nitrites, and dissolved oxygen were less than 0.20 mg/L, less than 0.01 mg/L, and more than 7.0 mg/L, respectively. The pH ranged from 7.4 to 7.8 in all culture tanks.

2.5 | Estimation of survival rate, growth performance, feed efficiency, and organ indices

At the end of the feeding trial, the fish were fasted for 24 hr and anesthetized with 40 mg/L eugenol solution (Aladdin reagent Co., Ltd., China). All the survived fish were collected by using a scoop net and counted to determine survival rate using the formula:

$$\text{Survival rate}\,(\%) = \left(\frac{\text{Final number of fish}}{\text{Initial number of fish}}\right) \times 100$$

Afterwards, the obtained fish from each tank were weighed to determine their final weight and for calculation of growth performance by using the formula:

$$\text{Specific growth rate}\left(\text{SGR}, \%\text{day}^{-1}\right) = \left[\frac{\ln(\text{Final weight}) - \ln(\text{Initial weight})}{\text{Time}\,(\text{days})}\right] \times 100$$

The amount of feeds fed and uneaten feeds collected from each tank were used to calculate feed intake by using the formula:

$$\text{Feed intake}\,(\text{FI}, \text{g}) = \sum\left[\frac{\text{Total daily dry feed consumption per tank}\,(\text{g})}{\text{Number of fish} \times \text{Time}\,(\text{days})}\right]$$

The amount of feed fed and the weight gained by fish in each tank were used to calculate feed conversion ratio by using the formula:

$$\text{Feed conversion ratio}\,(\text{FCR}) = \frac{\text{Dry feed intake}\,(\text{g})}{\text{Wet weight gain of fish}\,(\text{g})}$$

After weight measurements and counting, six fish from each tank were sampled, weighed individually and sacrificed for liver and visceral mass samples collection for body indices calculation by using the following formulae:

$$\text{Hepatosomatic index (HSI, \%)} = \left[\frac{\text{Liver weight (g)}}{\text{Individual fish weight (g)}}\right] \times 100$$

$$\text{Visceral somatic index (VSI, \%)} = \left[\frac{\text{Visceral weight (g)}}{\text{Individual fish weight (g)}}\right] \times 100$$

2.6 | Biochemical analyses of feeds and fish

Triplicate samples of the diets were taken and six *H. wyckioides* per tank were randomly collected, weighed and stored at −20°C for proximate composition analyses by using standard methods of the Association of Official Analytical Chemists (AOAC, 1995), as described in our previous study (Rong et al., 2020). The dry matter was determined by drying samples to constant weight at 105°C for 24 hr, crude protein was analyzed by using Kjeldahl method (N × 6.25), crude lipid was extracted by using the Soxhlet method (without acid hydrolysis) and ash was obtained after incinerating the samples in a muffle finance at 550°C for 18 hr. The dietary amino acid profiles of feeds (Table 2) were analyzed by using a high performance liquid chromatography Ultimate 3000 (Thermo Scientific Dionex) based on a national standard method (GB/T 18246–2000) as described previously in our study (Rong et al., 2020).

Another six fish from each tank were sampled for blood samples collection. The fish were anesthetized as described above and their blood was drawn from the caudal vein of individual fish by using 2 ml syringes (Klmedical, China). The serum sample was obtained after centrifugation (4,000 g for 10 min) at 4°C and then stored at −20°C until needed for analysis. The serum obtained was used for analyzing biochemical indicators by using specific Diagnostic Reagent Kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions, except for phosphorus. Therefore, triglyceride (TG; Art. No. A110-2), total cholesterol (T-CH; Art. No. A111-2), calcium (Art. No. C004-2) and catalase (CAT; Art. No. A007-1) were measured in the serum. Phosphorus was measured by using a Diagnostic Reagent Kit (Beijing Solarbio Science & Technology Co., Ltd.; Art. No. BC1650) according to the manufacturer's instructions. The glutamic oxalacetic transaminase (GOT; Art. No. C010-2), glutamate pyruvate transaminase (GPT; Art. No. C009-2), alkaline phosphatase (AKP; Art. No. A059-2), total protein (TP; Art. No. A045-4), albumin (AIB; Art. No. A028-2), serum urea nitrogen (SUN; Art. No. C013-2), serum ammonia (SA; Art. No. A086-1) were also measured in serum by using specific Diagnostic Reagent Kits (Nanjing Jiancheng Bioengineering Institute).

2.7 | Analysis of metabolism-related genes expression in muscle, liver and intestine

Four fish from each tank were sampled for tissue samples (liver, muscles and intestines) and immediately placed into liquid nitrogen and then stored at −80°C for total RNA extraction and real-time quantitative polymerase chain reaction (RT-PCR) analysis. The total RNA isolation and RT-PCR analysis were performed as described recently in our previous study (Rong et al., 2019). Briefly, total RNA was extracted from liver, intestine and muscle of *H. wyckioides* following the manufacturer's instructions (RNAiso plus Kit, Takara, Dalian, Liaoning, China). Potential genomic DNA contamination was eliminated by DNase I digestion (Takara). The cDNAs were synthesized using a TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech, Beijing, China). The specific primers were designed with Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). All the primers used in this study are shown in Table 3. The PCR fragments were sequenced (The Beijing Genomics Institute, Shenzhen city) and verified by comparison to those existing in the National Center for Biotechnology Information. The RT-PCR for each gene was performed according to standard protocols on a Roche Light Cycler® 480 System. The β-actin gene was used as a housekeeping gene for normalization. The relative quantification of genes was calculated by using the ΔΔCT method (Livak & Schmittgen, 2001).

TABLE 2 Amino acid contents of experimental diets (g/kg protein)

	Diets					
Amino acid contents	**Control**	**Arg 3**	**Arg 6**	**Arg 9**	**Arg 12**	**Arg 15**
EAA						
Arginine, Arg	44.51	51.71	56.89	64.15	71.36	80.32
Histidine, His	17.23	17.34	17.05	17.02	17.14	17.44
Valine, Val	34.04	34.37	33.64	33.60	33.80	34.39
Phenylalanine, Phe	31.48	31.51	30.90	30.77	30.76	31.44
Leucine, Leu	53.64	53.91	52.38	52.45	52.32	53.71
Isoleucine, Ile	29.81	30.00	29.21	29.61	29.93	30.60
Threonine, Thr	29.30	29.28	28.70	28.74	28.98	29.58
Methionine, Met	13.64	13.58	14.51	14.19	16.90	13.95
Lysine, Lys	45.10	45.40	44.51	44.43	44.71	45.54
Tryptophan Trp	9.08	9.27	8.89	8.80	8.97	8.97
NEAA						
Aspartic, Asp	42.97	41.47	43.76	41.48	42.96	41.92
Serine, Ser	32.27	30.74	28.65	29.85	32.09	31.27
Glutamic, Glu	130.77	124.51	113.68	103.98	96.40	89.67
Alanine, Ala	125.76	111.08	122.71	123.73	123.08	126.41
Glycine, Gly	98.28	102.68	95.97	101.54	100.34	97.17
Tyrosine, Tyr	59.11	58.53	56.33	53.22	58.60	57.90
Proline, Pro	40.24	36.70	38.99	36.86	41.04	39.00

Abbreviations: EAA, essential amino acid; NEAA, nonessential amino acid.

TABLE 3 The primers sequences used for real-time PCR primer sequences in the present study

Name	Sequences of primers		Annealing temperature	Product length	Accession number
m-TOR	Forward	5′-CCCATCATTGATATTACTCCTA-3′	58	248	NM_001077211
	Reverse	5′-CGAATGCACATTCGTAGAATCA-3′			
4E-BP	Forward	5′-CCAGATGACCACTACCAAGG-3′	60	163	NM_199683
	Reverse	5′-CGACACTGCAGCAGGAACTTT-3′			
S6K1	Forward	5′-ACGAGGATGTGAGCGAGTTTG-3′	59	138	NM_213076
	Reverse	5′-TTCTAGGACTGTTGGAGCAAC-3′			
β-Actin	Forward	5′-GGTTACTCCTTCACCACCACAG-3′	58	147	GU584189.1
	Reverse	5′-TCCGTCGGGCAGCTCATA-3′			

Abbreviations: PCR, polymerase chain reaction; TOR, target of rapamycin, 4E-BP, eukaryotic translation initiation factor 4E, S6K1, ribosomal protein S6 kinase 1.

2.8 | Statistical analyses

The results are expressed as means ± *SEM*. All data were tested for normality by using the Kolmogorov–Smirnov test and homogeneity of variances was determined by using Levene's test. All the mean values for each measured parameter were compared by using one-way analysis of variance (ANOVA). Differences among treatment means were

determined by Tukey's multiple comparisons test. Results with a $p < .05$ were considered significant. All statistical analyses were conducted by using Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL). The optimum dietary Arg supplementation for juvenile *H. wyckioides* based on SGR was estimated by using broken-line regression analysis.

3 | RESULTS

3.1 | Growth performance and related parameters

Feeding *H. wyckioides* with diets supplemented with Arg for eight weeks affected the growth performance and feeding utilization efficiency but not survival rate and organ indices (Table 4). The fish fed on the 9 g/kg Arg diet had significantly higher SGR than those fed on the control, 3, and 15 g/kg Arg diets ($p < .05$). The fish fed on fed on the control, 3, 6, 12 and 15 g/kg Arg diets had similar SGR values ($p > .05$). Equally, fish fed on the 9 and 12 g/kg Arg diets had statistically comparable SGR values ($p > .05$). The FI of fish fed on the control diet was significantly higher ($p < .05$) than fish on fed diets containing 3, 6, and 15 g/kg Arg. However, the fish fed on the control, 9, and 12 g/kg Arg as well those fed on the 3, 6, and 15 g/kg Arg diets had statistically similar FI values ($p > .05$). The FCR of fish fed on the control diet was not significantly different compared with fish fed on the other diets, except for fish fed on the diet containing 9 g/kg Arg ($p < .05$). Feeding the fish with diets supplemented with Arg at different levels did not affect SR, HSI and VSI ($p > .05$).

Further analysis on SGR showed that, it initially increased followed by a decrease with increasing Arg content of diets, with the maximum SGR obtained in fish fed on 9 g/kg Arg diet. Broken-line regression analysis showed that the maximum SGR of fish was obtained at a content of 63.77 g/kg Arg in the plant-rich diets for juvenile *H. wyckioides* (Figure 1).

3.2 | Proximate composition of whole body fish

Supplementing the plant-rich diets with Arg affected the proximate composition of the fish body (Table 5). The crude protein content of the fish fed on the 9 and 12 g/kg Arg diets was significantly higher than those fed on the control

TABLE 4 Growth performance of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine

	Diets					
	Control	Arg 3	Arg 6	Arg 9	Arg 12	Arg 15
Survival ratio (SR, %)	88.00 ± 10.58	98.67 ± 2.31	92.00 ± 6.93	98.67 ± 2.31	96.00 ± 4.00	96.00 ± 4.00
Initial weight (g)	6.40 ± 0.00	6.41 ± 0.01	6.40 ± 0.00	6.40 ± 0.00	6.39 ± 0.01	6.40 ± 0.00
Final weight (g)	15.09 ± 0.53	15.59 ± 0.47	16.52 ± 0.84	17.28 ± 0.24	16.10 ± 0.65	15.35 ± 0.43
Specific growth rate (SGR, %day^{-1})	1.53 ± 0.06a	1.58 ± 0.05a	1.69 ± 0.09ab	1.77 ± 0.03b	1.65 ± 0.02ab	1.56 ± 0.05a
Feed intake (FI, g)	18.96 ± 0.97b	17.28 ± 0.90a	17.57 ± 0.42a	18.24 ± 0.67ab	17.86 ± 0.43ab	17.27 ± 0.79a
Feed conversion ratio (FCR)	2.21 ± 0.20b	1.90 ± 0.12ab	2.04 ± 0.14ab	1.64 ± 0.06a	1.82 ± 0.11ab	1.95 ± 0.15ab
Hepatosomatic index (HSI, %)	1.89 ± 0.12	1.72 ± 0.09	1.82 ± 0.12	1.57 ± 0.13	1.77 ± 0.10	1.78 ± 0.12
Viscerosomatic index (VSI, %)	5.08 ± 0.17	5.14 ± 0.25	5.11 ± 0.25	4.71 ± 0.26	5.29 ± 0.21	5.17 ± 0.13

Note: Values are presented as means ± *SEM*, $n \geq 18$. Means in the same row with different letters are significantly different from each other ($p < .05$).

diet ($p < .05$). The fish fed on fed on the control, 3 and 6 g/kg Arg diets had similar protein content in the body ($p > .05$). Equally, the fish fed on the 9, 12 and 15 g/kg Arg diets had statistically comparable protein content ($p > .05$). The crude lipid of fish fed on the 9 and 12 g/kg Arg diets was lower than the other diets ($p < .05$). However, no significant differences in moisture and ash contents were obtained among the fish fed on the Arg dietary treatments.

3.3 | Biochemical indicators of lipid deposition, hepatotoxicity and antioxidant parameters in the serum

The levels of lipid metabolism indicators and calcium and phosphorus in the serum of juvenile *H. wyckioides* fed on high-plant protein diets were affected with the levels of dietary Arg supplementation ($p < .05$; Table 6). Both the TG and calcium levels showed a similar increasing trend with increasing dietary Arg levels. The fish fed on the 9 g/kg Arg diet had higher TG content than those fed on the control diet ($p < .05$). However, the fish fed on the control diet and those fed on 9 g/kg Arg diet had similar TG levels to the other diets ($p > .05$). The fish fed on the 9 and 15 g/kg Arg diets had higher calcium levels than those fed on control, 3 and 6 g/kg Arg diets ($p < .05$). The T-CH of fish fed on the 12 g/kg Arg diet was significantly lower than that of fish fed on the 3 g/kg Arg diet ($p < .05$). However, there was no significant difference in the serum phosphorus level among the dietary treatments ($p > .05$).

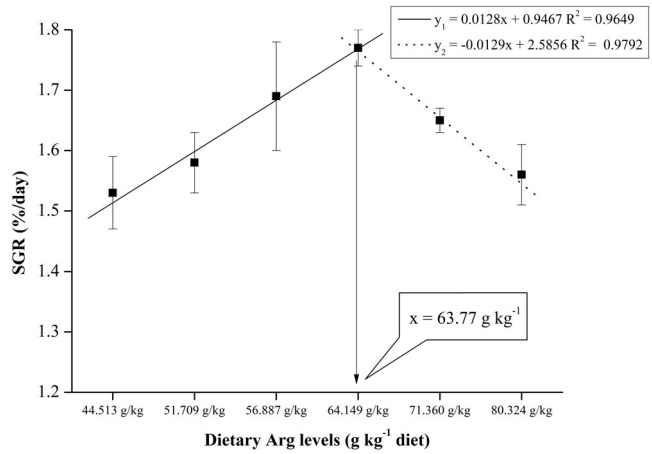


FIGURE 1 The broken-line regression analysis of specific growth rate (SGR, %/d) based on dietary arginine levels in diets of juvenile *Hemibagrus wyckioides*. ($y_1 = 0.0128x + 0.9467$ $R^2 = .9649$ and $y_2 = -0.0129x + 2.5856$ $R^2 = .9792$). Content of dietary arginine estimated from SGR was 63.77 g/kg. Each point represents the mean of three groups of *H. wyckioides* with 25 fish per treatment.

TABLE 5 Proximate composition (% wet matter basis) of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine

	Diets					
Proximate composition	**Control**	**Arg 3**	**Arg 6**	**Arg 9**	**Arg 12**	**Arg 15**
Moisture (%)	71.06 ± 0.78	71.32 ± 0.64	71.08 ± 0.77	71.88 ± 0.38	71.33 ± 0.36	71.24 ± 0.67
Crude protein (%)	15.26 ± 0.57a	15.42 ± 0.54ab	15.73 ± 0.48ab	15.92 ± 0.33b	16.00 ± 0.40b	15.86 ± 0.45ab
Crude lipid (%)	9.02 ± 0.23b	8.86 ± 0.48ab	8.82 ± 0.35ab	8.31 ± 0.49a	8.37 ± 0.38a	8.86 ± 0.41ab
Ash (%)	3.04 ± 0.04	3.05 ± 0.10	3.10 ± 0.06	3.06 ± 0.13	3.00 ± 0.03	3.04 ± 0.06

Note: Values are presented as means ± *SEM*, $n = 18$. Means in the same row with different letters are significantly different from each other ($p < .05$).

The use of Arg supplementation in plant-rich diets did not affect significantly the hepatotoxicity indicators such as GOT, GPT and AKP activities in the fish serum (Table 7). Feeding the fish with various Arg levels affected the antioxidant capacity and protein metabolites in the serum of juvenile *H. wyckioides* (Table 8). The CAT activity in fish serum generally increased significantly ($p < .05$) with increasing Arg content of the diets. The fish fed on the control diet had lower CAT activity than those fed on the 9, 12 and 15 g/kg Arg diets ($p < .05$). However, the fish fed on the control had similar CAT activity to those fed on the 3 and 6 g/kg Arg diets ($p > .05$). Similarly, the fish fed on the 9 g/kg Arg diet had similar CAT activity to those fed on the 12 and 15 g/kg Arg diets ($p < .05$). Contrary, feeding fish with dietary Arg levels generally decreased the levels of SUN and SA, with the lowest levels obtained in the serum *H. wyckioides* fed on the 12 g/kg Arg diet. There was no significant difference in other protein metabolism parameters (TP and AIB) fed on the various levels of dietary Arg.

3.4 | Expression of genes related to the mTOR signaling pathway

The expression of genes related to the mTOR pathway such as TOR (Figure 2), S6K1 (Figure 3) and 4EBP (Figure 4) in liver, intestines and muscle of juvenile *H. wyckioides* fed on high-plant protein experimental diets with different supplements of dietary Arg levels were significantly affected ($p > .05$). The relative expression of TOR and S6K1 in fish tissues showed a similar trend. For example, in liver and muscle, the mRNA levels of TOR and S6K1 increased significantly with increasing dietary Arg supplementation ($p < .05$), but there were no significant differences in the levels of these genes in intestine at different dietary Arg supplementation ($p > .05$). The expression the 4EBP gene in intestine did not change significantly at different dietary Arg supplementation, similar to that of TOR and S6K1 in the intestine. In the liver and muscle, the expression of 4EBP was significantly affected by dietary Arg levels

TABLE 6 The lipid deposition indicators, calcium and phosphorus levels in the serum of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine

	Diets					
	Control	Arg 3	Arg 6	Arg 9	Arg 12	Arg 15
Triglyceride (TG, mmol/L)	6.35 ± 0.42a	6.71 ± 0.65ab	6.66 ± 0.78ab	7.34 ± 0.25b	6.98 ± 0.32ab	7.13 ± 0.44ab
Total cholesterol (T-CH mmol/L)	7.24 ± 0.60ab	7.41 ± 0.74b	6.89 ± 0.70ab	6.82 ± 0.65ab	6.27 ± 0.80a	6.69 ± 0.17ab
Calcium (mmol/L)	3.08 ± 0.14a	3.11 ± 0.13a	3.16 ± 0.17ab	3.92 ± 0.25c	3.75 ± 0.30bc	3.94 ± 0.46c
Phosphorus (mmol/L)	5.55 ± 0.57	5.53 ± 0.50	4.95 ± 0.19	4.96 ± 0.54	5.09 ± 0.32	5.41 ± 0.60

Note: Values are presented as means ± *SEM*, $n = 18$. Means in the same row with different letters are significantly different from each other ($p < .05$).

TABLE 7 Biochemical parameters in serum of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine

	Diets					
	Control	Arg 3	Arg 6	Arg 9	Arg 12	Arg 15
Glutamic oxalacetic transaminase (GOT, U/ml)	6.56 ± 0.95	6.74 ± 1.51	7.47 ± 0.89	7.97 ± 0.64	6.83 ± 0.41	6.52 ± 1.61
Glutamate pyruvate transaminase (GPT, U/ml)	4.45 ± 0.08	4.46 ± 0.20	4.50 ± 0.25	4.20 ± 0.09	4.25 ± 0.15	4.25 ± 0.14
Alkaline phosphatase (AKP, U/ml)	2.79 ± 1.03	3.10 ± 0.54	3.25 ± 0.39	2.42 ± 1.05	2.30 ± 0.39	2.53 ± 0.86

Note: Values are presented as means ± *SEM*, $n = 18$.

TABLE 8 Antioxidant capacity, immune parameters and protein metabolites in serum of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine

	Diets					
	Control	Arg 3	Arg 6	Arg 9	Arg 12	Arg 15
Catalase (CAT, U/ml)	5.24 ± 0.09a	5.57 ± 0.14ab	5.84 ± 0.45abc	6.47 ± 0.53cd	6.30 ± 0.67bcd	6.65 ± 0.10d
Total protein (TP, g/L)	25.97 ± 2.28	29.90 ± 0.19	30.21 ± 0.27	25.54 ± 4.33	28.80 ± 0.96	25.47 ± 5.65
Albumin (AIB, g/L)	9.86 ± 2.62	11.31 ± 1.31	10.58 ± 3.15	8.70 ± 1.90	9.35 ± 2.39	11.17 ± 2.06
Serum urea nitrogen (SUN, mmol/L)	5.24 ± 0.05b	5.57 ± 0.07b	4.77 ± 0.23ab	3.88 ± 1.30a	3.47 ± 1.04a	4.71 ± 0.39ab
Serum ammonia (SA μmol/L)	146.75 ± 1.34ab	156.13 ± 2.01b	133.57 ± 6.37ab	108.78 ± 6.37a	97.16 ± 9.14a	132.01 ± 10.78ab

Note: Values are presented as means ± *SEM*, $n = 18$. Means in the same row with different letters are significantly different from each other ($p < .05$).

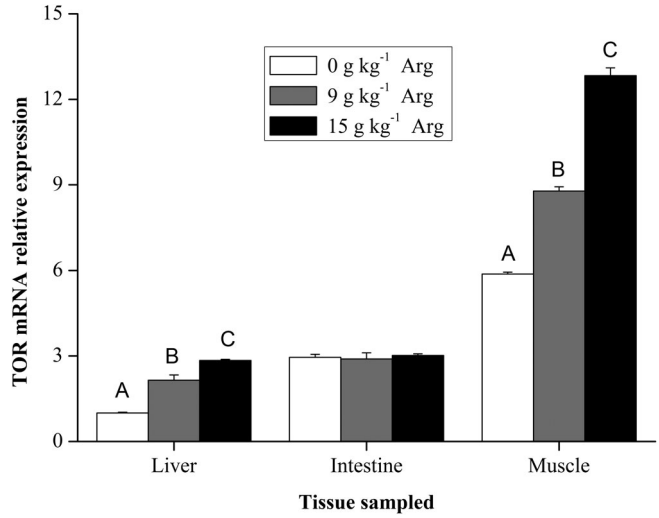


FIGURE 2 Relative expression of target of rapamycin (TOR) mRNA of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine. Values are means ± *SEM* ($n = 12$). Mean values with unlike capital letters were significantly different among the dietary treatments in the same tissues ($p < .05$). Mean values with unlike lowercase letters were significantly different among the different tissues compared to control diet ($p < .05$).

($p < .05$), with the highest 4EBP expression in liver and muscle found in fish fed on diets with 9 g/kg Arg diet ($p < .05$).

4 | DISCUSSION

Plant proteins are potential ingredients, which have been suggested to replace FM in aquafeed formulations. However, they have intrinsic limitations because most of them are deficient in some bioactive molecules. Many plant proteins lack one or more EAA such as taurine (Wang et al., 2014), hydroxyproline (Liu et al., 2014), Arg (Liang et al., 2016; Tu et al., 2015) and methionine (Figueiredo-Silva et al., 2015; He et al., 2019), which are important for animal growth. Amino acid imbalances may also occur when excess plant proteins are used in diet formulations,

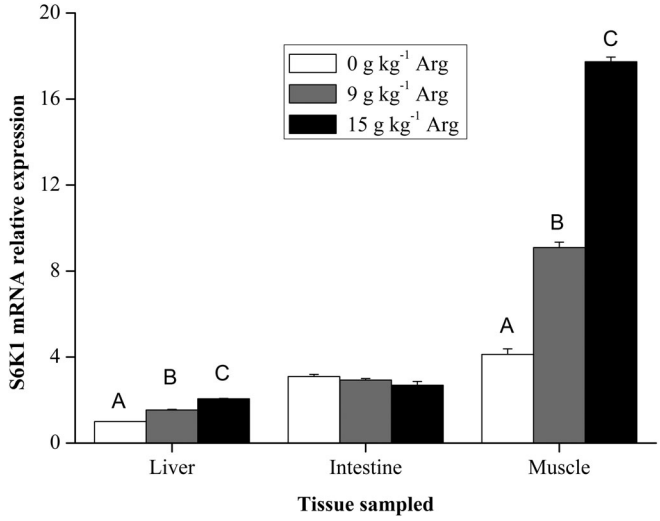


FIGURE 3 Relative expression of ribosomal protein S6 kinase 1 (S6K1) mRNA of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine. Values are means ± *SEM* (*n* = 12). Mean values with unlike capital letters were significantly different among the dietary treatments in the same tissues ($p < .05$). Mean values with unlike lowercase letters were significantly different among the different tissues compared to control diet ($p < .05$).

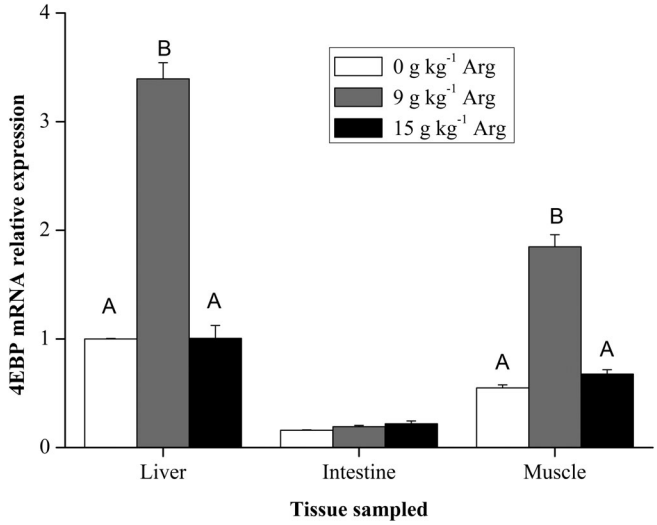


FIGURE 4 Relative expression of eukaryotic translation initiation factor 4E binding protein (4EBP) mRNA of juvenile *Hemibagrus wyckioides* fed on diets with varying levels of dietary arginine. Values are means ± *SEM* (*n* = 12). Mean values with unlike capital letters were significantly different among the dietary treatments in the same tissues ($p < .05$). Mean values with unlike lowercase letters were significantly different among the different tissues compared to diet ($p < .05$).

especially for fish with high requirements for dietary Arg (Li, Mai, Trushenski, & Wu, 2009). Recently, Arg supplementation in fish diets has been shown to affect fish flesh quality (Wang et al., 2015), growth and protein deposition (Chen et al., 2012; Khan, 2012; Pohlenz et al., 2014; Ren et al., 2013) as well as antioxidant parameters (Hoseini, Yousefi, Hoseinifar, & Van Doan, 2019; Liang, Ji, et al., 2018; Liang, Mokrani, et al., 2018). The present study explored the importance of dietary Arg supplementation in high plant-based protein experimental diets for the normal growth of juvenile *H. wyckioides*.

The results obtained revealed that dietary Arg supplementation improved the growth performance of juvenile *H. wyckioides*, similar to results reported by Chen et al. (2018) in turbot. The promotion of fish growth performance by the appropriate dietary Arg supplementation is ascribed to enhanced FI and decreased FCR, which increased feed utilization efficiency and protein deposition. These results indicate that adequate Arg supplementation is indispensable in feeds for optimum growth of *H. wyckioides* when fed with plant-rich diets. In this study, the dietary Arg supplementation for optimum growth was estimated as 63.77 g/kg (6.38% of the diet) for *H. wyckioides* fed on high-

plant protein diets. Therefore, feeding *H. wyckioides* with insufficient or excessive dietary Arg caused nutritional stress, which resulted in the depression of fish growth and feed utilization as reported previously (Fauzi, Haga, Kondo, Hirono, & Satoh, 2019; Fournier et al., 2003; Ren et al., 2013; Zhou et al., 2010; Zhou, Jin, Elmada, Liang, & Mai, 2015; Zhou, Zeng, Wang, Xie, & Zheng, 2012). We hypothesize that, supplementing dietary Arg in *H. wyckioides* excessing the optimum level may cause extra energy expenditure toward deamination of unbalanced dietary amino acids. In general, the dietary Arg required for optimum growth obtained in this study is similar to that reported in Nile tilapia (*Oreochromis niloticus L.*) (6.24% of dietary protein) (Yue et al., 2015). However, the optimum Arg level obtained in this study is relatively higher compared to other fish species such as rohu (*Labeo rohita*) (3.05–3.47% of dietary protein) (Abidi & Khan, 2009), Japanese flounder (*Paralichthys olivaceus*) (4.08% of dietary protein) (Alam, Teshima, Koshio, & Ishikawa, 2002), hybrid catfish (*Clarias gariepinus* × *Clarias macrocephalus*) (4.45–5.0% of dietary protein) (Singh & Khan, 2007) and *I. punctatus* (3.3–3.8% of dietary protein) (Buentello & Gatlin, 2000). These results suggest that, the Arg requirement for optimum growth varies among fish species. Our findings provide important information for the development of feeds in *H. wyckioides* required for optimum growth performance. However, the role of Arg on growth performance in fish feeds with balanced dietary amino acids in *H. wyckioides* should be elucidated in future studies.

Protein synthesis as a key component of growth response is related to amino acids balance in the diet. The current study revealed that the protein content in whole fish body increased significantly with increasing dietary Arg levels. However, the protein content decreased when the optimal Arg requirement was exceeded, similar to results reported in yellow grouper (*Epinephelus awoara*) (Zhou et al., 2012), catfish (*Heteropneustes fossilis*) (Khan, 2012) and hybrid sturgeon (*Acipenser schrenckii* ♀ × *Acipenser baerii* ♂) (Wang et al., 2017). These results suggest that, the optimum Arg supplementation obtained possibly balanced amino acids to promote protein synthesis and retention, which increased growth performance as indicated in the previous section. On the other hand, the dietary Arg supplementation reduced SA and SUN contents, which are the main end products of amino acid catabolism. These results suggest that, the optimal Arg supplementation balanced the amino acid profiles as reported previously by Fournier et al. (2003). This is consistent with previous studies in rainbow trout (*Oncorhynchus mykiss*) (Cho, Kaushik, & Woodward, 1992), sea bass (*Dicentrarchus labrax*) (Tibaldi, Tulli, & Lanari, 1995) and *P. olivaceus* (Alam et al., 2002). Furthermore, the dietary Arg supplementation did not only affect significantly protein retention but also lipid deposition as indicated by TG and T-CH. The TG and T-CH are the main intermediate products of lipid metabolism, which are considered as good indicators of lipid catabolism (Deng et al., 2013). In this study, the fat deposition decreased significantly with increasing dietary Arg, but increased when the level of Arg exceeded the optimal requirement. Jobgen, Fried, Fu, Meininger, and Wu (2006) reported that Arg regulates metabolism of energy substrates, especially fatty acids. Thus, lipids were probably catabolized in order to get rid of the excess nitrogenous load from the body under the amino acid balance following supplementation of Arg (Khan, 2012), which resulted in a decrease in body fat deposition.

Blood parameters are reliable indicators of physiological conditions and health status of fish. For instance, GOT and GPT are the most important enzymes in amino acid catabolism, hence are used as indicators of normal liver function in vertebrates (Metón, Mediavilla, Caseras, Cantó, & Baanante, 1999). In addition, the AKP is an enzyme required for phosphate hydrolysis and absorption of nutrients, whereby high concentration indicates hepatic disorder (Malambugi et al., 2020). In the present study, dietary supplementation with Arg had no significant effect on serum GOT, GPT and AKP in juvenile *H. wyckioides*, suggesting no adverse effects on liver function. The lack of hepatotoxicity after Arg supplementation was further indicated by the insignificant influence on HSI and VSI. These results suggest that, supplementing Arg at the levels used in this study do not cause hepatotoxicity contrary to the results reported by Tibaldi, Tulli, and Lanari (1994), where optimal dietary Arg level decreased HSI in sea bass (*D. labrax*). The variations in the obtained results might be because of different experimental animals and developmental stages as well as experimental diets, considering that this study used diets rich in plant protein (50% diet protein). Nevertheless, the underlying reasons require further investigation.

There is growing evidence that Arg scavenges oxygen free radicals and enhances antioxidant capacity (Coutinho et al., 2016). In the present study, dietary supplementation with Arg increased the CAT activity in serum, which suggest

that exogenous addition of Arg improves antioxidant capacity. Similar results have been reported in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) (Wu et al., 2018) and yellow catfish (*Pelteobagrus fulvidraco*) (Zhou et al., 2015). In addition, many studies have indicated that Arg metabolites, such as polyamines and nitric oxide, might offer the beneficial effects in transport of dissolved nutrients (Fauzi et al., 2019). Our study revealed that TP and AIB levels were relatively constant, suggesting that the dietary Arg supplementation did not impair oncotic pressure of the blood for efficient transport of dissolved components in the body (Hassaan et al., 2020). These results indicate that, supplementation of Arg at levels used in this study does not interfere with transport of blood fluid components.

The activation TOR signaling pathway and its role on amino acids regulation is one of the recent research hotspots. The TOR signaling pathway is regarded as an important potential regulator of growth and protein synthesis in fish, because it is sensitive to food intake and amino acids present in the diet (Chen et al., 2012; Seiliez et al., 2008; Tu et al., 2015; Wang, He, Mai, Xu, & Zhou, 2016). Indeed, recent studies have shown that dietary Arg supplementation promotes protein synthesis in fish by increasing TOR signaling activity (Liang et al., 2016; Wu et al., 2018). However, the role of TOR signaling pathway in fish fed on diets rich in plant protein was not known before our study. In this study, the transcript levels of TOR and S6K1 in liver, intestine and muscle of juvenile *H. wyckioides* showed a similar expression pattern with increasing dietary Arg supplementation. For instance, the mRNA levels of TOR and S6K1 in the intestine were relatively stable, and were not affected significantly by dietary Arg levels. However, the levels of TOR and S6K1 in liver and muscle increased significantly with increasing dietary Arg levels, similar to results obtained in *M. amblycephala* (Liang et al., 2016) and grass carp (*Ctenopharyngodon idella*) (Wang et al., 2015). These results suggest that, the up-regulation of S6K1 expression in liver and muscle by the appropriate Arg levels might partly be related to the up-regulation in TOR expression, because TORC1 is believed to control cell growth and protein synthesis via S6K1 and 4EBP, which phosphorylates into mTORC1 targets (Ma & Blenis, 2009). In fact, Tu et al. (2015) suggested that, supplementing diets with Arg balances the amino acids and activates the TOR signaling pathway, which promotes protein synthesis in gibel carp (*Carassis auratus gibelio*). In this study, 4EBP showed a different pattern compared with TOR or S6K1. The mRNA expression of 4EBP in liver and muscle were highest in fish fed on 9 g/kg Arg diet, while the mRNA expression level in intestine was not affected significantly by dietary Arg supplementation. These results are similar to those obtained in Jian carp (*Cyprinus carpio* var. Jian) (Chen et al., 2012) and *C. auratus gibelio* (Tu et al., 2015). In general, these results indicate that transcript levels of genes involved in TOR signaling pathway such as TOR, S6K1 and 4EBP have different expression patterns in different tissues or organs after dietary Arg supplementation. Currently, the amino acids signaling by the mTORC1 pathway is complex and the mechanisms on involvement of TOR signaling pathway by Arg are still not clear in fish, which warrant further studies to investigate.

5 | CONCLUSION

The results of the present study revealed that dietary Arg supplementation in plant-rich diets improved significantly the growth performance by enhancing feed utilization efficiency. The amount of Arg required for optimum growth in *H. wyckioides* was estimated to be 63.77 g/kg diet. This amount increases protein synthesis but decreases lipid accumulation, which improve antioxidant capacity thereby ensures optimum liver function and blood biochemical transport in the body of *H. wyckioides*. The ability of Arg supplementation to affect *H. wyckioides* growth is possibly regulated through TOR signaling pathway activation, which promotes protein synthesis in *H. wyckioides*.

ACKNOWLEDGMENTS

This study was supported by the Natural Science Youth Research Foundation of Yunnan Agricultural University (Grant No. 2016ZR14) and Yunnan Province Agricultural Basic Research Joint Project (Grant No. 2018FG001-044; 2017FG001-021). We are grateful to Zhuangping Expert Workstation, and all laboratory members for technical advice and valuable help during the feeding trial and sample analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Gu D, Zhao J, Limbu SM, et al. Arginine supplementation in plant-rich diets affects growth, feed utilization, body composition, blood biochemical indices and gene expressions of the target of rapamycin signaling pathway in juvenile Asian red-tailed catfish (*Hemibagrus wyckoiides*). *J World Aquacult Soc*. 2022;53:133–150. https://doi.org/10.1111/jwas.12748