

## Effects of dietary protein and lipid levels on growth and feed utilization of spotted scat (*Scatophagus argus* (Linnaeus, 1766)) juveniles

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**Abstract**. A feeding trial was designed to determine the appropriate dietary protein and lipid levels for spotted scat (*Scatophagus argus*) juvenile. The experiment was conducted in a recirculating aquaculture system (RAS) with factorial 4 x 3 treatments consisting of two factors. Diets were formulated with a combination of four dietary protein levels (30, 35, 40, and 45%) and three dietary lipid levels (6, 9, and 12%). Each trial diet was fed in quadruplicate to fish groups (1.32 to 1.38 g fish<sup>-1</sup>) which were arranged in 100 L tanks at 30 fish per tank of stocking density and salinity maintained at 30‰. After 56 days of rearing, the fish survival rate in all treatments ranged from 95.0 to 98.4% and was not affected by dietary protein and lipid levels. The highest growth performance of fish was found in the treatment of 40% protein and 9% lipid (0.119 g day<sup>-1</sup>) and tended to decrease at higher protein levels 45% protein and 9% lipid (0.113 g day<sup>-1</sup>). There was an interaction (p < 0.05) between dietary protein and 9% lipid provides sufficient nutrient and energy to support acceptable growth rates and nutrient utilization of *S. argus* juveniles.

Key Words: energy source, feed utilization, nutritional requirements, Scatophagus argus.

**Introduction**. Spotted scat fish (*Scatophagus argus* (Linnaeus, 1766)) is a commercially promising species for aquarium and food fish markets in South and Southeast Asian countries (Gupta 2016). S. argus is a euryhaline species that is widely distributed in the estauries, mangrove and coastal zones where salinities can range widely from 1 to 35‰ (Sivan & Radhakrishnan 2011; Cui et al 2013; Xu et al 2020). Due to its good nutrient guality and high tolerance to environmental stress, S. argus has been much appreciated by farmers for mariculture (Cui et al 2013; Khanh et al 2014; Su et al 2019). S. argus is reported as an omnivorous feeding species and it mainly feeds on unicellular algae, detritus, mud, sand, minute broken shells of mollusks and other inorganic matter (Sivan & Radhakrishnan 2011). Therefore, it is an usual practice for this species to be integrated into large shrimp ponds for the prevention of algal and seaweed overgrowth as well as the improvement of water quality (Shao et al 2004; Viet et al 2020). As an emerging species in aquaculture, many substantial studies on morphorlogy, biology, reproductive biology, artificial breeding and culture of spotted scat fish have been carried out (Shao et al 2004; Khanh et al 2014; Gupta 2016; Su et al 2019; Xu et al 2020). Importantly, successes in using seaweed or seaweed protein as feed for S. argus has been previously recorded (Ni et al 2013; Anh et al 2014; Anh et al 2017a). These reports provide useful information for the development and optimization of culture protocols for this species.

Fish utilize nutrient and energy sources in feed for the maintenance of growth and reproduction (Prabu et al 2017). Nutritional requirements of fish vary depending on fish

species, geographic region, feeding habits, and developmental stages of fish (Bowyer et al 2013; Prabu et al 2017; Khoa et al 2019). In the nutritional profile of aquatic animal feed, proteins and lipids are two such essential nutrients in processed feed. Proteins are the main constituent of the animal body and usually the most expensive nutrient in fish diets (Khoa et al 2019). Insufficient protein amount in diets is known to lead to reduced growth and lowered feed intake in fish (De Lemos et al 2014; Khoa et al 2020). One of the main problems in optimizing dietary protein for specific species is that it is often not appropriately balanced with other essential nutrients in formulated diets (Khan et al 2019; Teles et al 2020). High protein diets also need adequate energy for its proper metabolization. Dietary supplementation of energy-yielding nutrients, mainly lipids, has been suggested as a strategy to spare or improve the efficiency of protein utilization by fish (Sankian et al 2017; Khan et al 2019). Lipids are an energy-rich nutrient and play an importante role as an energy reserve in providing the necessary energy for aquatic animals. However, if the dietary protein or lipid content is too high, the excess levels of nutrients will not be absorbed by the fish but released into the rearing environment (Li et al 2016). In this case, the energy consumption for the excess protein or lipid digestion will lead to a decrease in aquatic animal growth, an increase in feed conversion ratio thus an increase in feed cost per kg of fish produced, and an increase in environmental pollution (Tuan & Wiliam 2007; Garcia de la Serrana et al 2013; Wang et al 2013; Prabu et al 2017). Therefore, it is very important to determine the optimal dietary balance between protein and lipid for achieving maximum growth and efficient feed utilization of fish.

Despite being considered a new species for farming, the development of appropriate formulated feed for *S. argus* is still being researched. Existing literature on the nutritional requirements for *S. argus* is still limitted. Farmers usually rear this species using industrial Tilapia formulated feed and live feed. This use of nutritionally imbalanced feed often does not meet the *S. argus* fish's nutritional requirement and results in lowered production efficiency and hinders the prospective development of extensive commercial production of this species. Manh et al (2011) determined that the required protein levels for the grow out stages of *S. argus* fingerlings ranges from 30 to 35% protein. However this study did not assess the protein-energy ratio requirements for the nursery of this fish and information on this important parameter is still currently lacking. This study aims to evaluate the effects of different dietary protein and lipid levels on growth performance, feed conversion ratio, and carcass composition of *S. argus* fish. Findings from this study have the potential to assist in the optimization of feed formulations that are nutritionally balanced and which are cost-effective for farmers for the successful culture of this species.

## Material and Method

**Experimental diets**. The experimental diets were formulated to contain four different protein levels (30, 35, 40, and 45% crude protein) and each with three dietary lipid levels (6, 9, and 12% crude lipid). The diets were formulated based on common ingredients as fishmeal, soybean meal, cassava meal, fish oil, soybean oil, shrimp extract, premix, and binder (Table 1). All ingredients were finely ground, with 30% water added, pelleted to a diameter of 2 mm, dried in an oven at 45°C for 12 hours to reach a humidity of 10%. After being processed, the pellets were stored in the freezer at -20°C until feeding. The pellets were kept at room temperature at least 4 hours before feeding. The chemical composition (protein, lipid, ash, energy, amino acids) of all diet treatments were weekly sampled and measured (Table 2). The experimental design followed a 4 x 3 factorial protocol conducted in quadruplicates.

**Experimental system and maintenance**. S. argus larvae were artificially reproduced and cultured for 8 weeks (from March to April 2020) at the Wetlab of the College of Aquaculture and Fisheries, Can Tho University. Juveniles with a size range of  $1.35\pm3$  g were used for the experiment.

The experiment was conducted in a recirculating aquaculture system (RAS) connected to 100 L-tanks with continuous aeration supplied. The fish were randomly stocked at 30 fish per tank with salinity maintained at 30‰. The refreshment rate of water in the RAS system was 300% per day. The photoperiod regime was approximately 12 hours light and 12 hours dark. Water quality parameters were checked daily and maintained; temperature ( $29.5\pm1.5^{\circ}$ C), pH (7-8), oxygen concentration (higher than 5.2 ppm), alkalinity (100-120 mg CaCO<sub>3</sub> L<sup>-1</sup>). Total ammonia nitrogen TAN (0.1-0.4 mg L<sup>-1</sup>) and NO<sub>2</sub>–N (0-0.03 ppm) were weekly measured. During the experiment period, the fish were fed *ad libitum* the twelve experimental diets four times daily at 0700, 1000, 1300, and 1600. The amount of feed fed to fish was recorded and the uneaten pellets were collected 30 minutes after feeding, dried in the oven at 45°C for 8 hours, weighed, and recorded. The experiment lasted for 56 days and each dietary treatment were performed in quadruplicate.

Experimental procedure and sample analysis. Samples were collected for body composition analysis at the beginning of culture and at the end of the experimental period (5 fish per tank). Prior to the collection of the body composition samples, the fish had been dissected to collect liver, intestine, and body weight was measured to determine the hepatosomatic index (HSI) and the viscerosomatic index (VSI). All samples were homogenized by a coffee blender and stored in the freezer at -80°C until analysis. Proximate composition analysis of each diet and ingredient samples were taken during pelleting and approximately 5 g of each experimental diet were sampled weekly during the experiment. The chemical analysis were done in triplicates. According to standard laboratory methods (AOAC 2000), the dry matter (DM) content was determined by drying in the oven at 105°C until constant weight; crude protein (N  $\times$  6.25) content was measured following the Kjeldahl method; mineral (ash) content by placing in the furnace at 560°C for 4 hours; and crude fat content by solvent (diethyl ether) extraction. The carbohydrate (CHO) content in the sample on a dry matter basis was calculated as 1000 – (crude protein + crude ash + crude fat). The gross energy (kJ  $g^{-1}$ ) was calculated as  $[(crude protein \times 23.6) + (crude fat \times 39.5) + (CHO \times 17.2)] / 1000$ . Dietary amino acids constitute amino acids within the ingredients. Upscience Company, Vietnam, performed the amino acid analysis of these ingredients.

Table 1

Lipid	Drotoin	Ingredients (%)							
(%)	Protein (%)	Fish meal	Soybean meal	Cassava	Fish oil	Soybean oil	Premix*	Binder	Shrimp extract
6	30	19.4	30.0	41.3	1.9	2.9	2.0	1.0	1.5
	35	26.7	30.0	34.4	1.5	2.9	2.0	1.0	1.5
	40	33.9	30.0	27.7	1.0	2.9	2.0	1.0	1.5
	45	41.3	30.0	20.7	0.6	2.9	2.0	1.0	1.5
9	30	19.4	30.0	38.3	3.4	4.4	2.0	1.0	1.5
	35	26.7	30.0	31.4	3.0	4.4	2.0	1.0	1.5
	40	33.9	30.0	24.7	2.5	4.4	2.0	1.0	1.5
	45	41.3	30.0	17.7	2.1	4.4	2.0	1.0	1.5
12	30	19.4	30.0	35.3	4.9	5.9	2.0	1.0	1.5
	35	26.7	30.0	28.4	4.5	5.9	2.0	1.0	1.5
	40	33.9	30.0	21.7	4.0	5.9	2.0	1.0	1.5
	45	41.3	30.0	14.7	3.6	5.9	2.0	1.0	1.5

Amounts (%) of ingredients used in the experimental diets

Ca Mau Fishmeal, Argentina soybean meal, \*Premix: Bio-premix C-1 AG: vitamin A (6.000.000 IU), vitamin D3 (2000.000 IU), vitamin E (50.000 mg), vitamin K<sub>3</sub> (6000 mg), vitamin B1 (11.000 mg), vitamin B2 (7000 mg), vitamin B6 (8000 mg), vitamin C Stay (50.000 mg), inositol (5000 mg), folic acid (3000 mg), biotin (500 mg), pantothenic acid (35.000 mg), niacine (60.000 mg), Cu (34.000 mg), Fe (50.000 mg), Mn (12.000 mg), Zn (125.000 mg), I (500 mg), Co (250 mg), Se (200 mg).

Chemical compositions of experimental diets (%, as dry matter basis)

	30P-6L	35P-6L	40P-6L	45P-6L	30P-9L	35P-9L	40P-9L	45P-9L	30P-12L	35P-12L	40P-12L	45P-12L
Protein	30.3	35.4	40.3	45.4	30.3	35.2	40.3	45.3	30.4	35.3	40.1	45.3
Lipid	6.38	6.27	6.24	6.48	9.23	9.25	9.39	9.47	12.3	12.3	12.4	12.5
Ash	9.81	10.8	11.2	13.1	9.90	10.5	11.6	13.4	9.80	10.3	11.6	13.4
Energy (KJ g⁻¹)	19.1	19.2	19.4	19.5	19.7	19.9	20.0	20.1	20.4	20.6	20.7	20.7
Protein/Energy ratio	15.8	18.4	20.8	23.4	15.4	17.7	20.1	22.6	14.9	17.1	19.4	21.9
				The p	roximal ai	mino acid	s (%)					
Methionine	0.60	0.75	0.89	1.04	0.60	0.75	0.89	1.04	0.60	0.75	0.89	1.04
Cysteine	0.35	0.40	0.44	0.49	0.35	0.40	0.44	0.49	0.35	0.40	0.44	0.49
Methionine + Cysteine	0.96	1.16	1.35	1.55	0.96	1.16	1.35	1.55	0.96	1.16	1.35	1.55
Lysine	2.08	2.48	2.88	3.28	2.08	2.48	2.88	3.28	2.08	2.48	2.88	3.28
Threonine	1.21	1.43	1.65	1.86	1.21	1.43	1.65	1.86	1.21	1.43	1.65	1.86
Tryptophan	0.37	0.43	0.48	0.54	0.37	0.43	0.48	0.54	0.37	0.43	0.48	0.54
Arginine	2.03	2.33	2.64	2.94	2.03	2.34	2.64	2.94	2.03	2.34	2.64	2.94
Isoleucine	1.37	1.59	1.82	2.05	1.37	1.59	1.82	2.05	1.37	1.60	1.82	2.05
Leucine	2.31	2.70	3.10	3.49	2.31	2.70	3.10	3.49	2.31	2.71	3.10	3.49
Valine	1.48	1.75	2.01	2.27	1.49	1.75	2.01	2.28	1.49	1.75	2.01	2.28
Histidine	0.77	0.89	1.02	1.14	0.77	0.89	1.02	1.14	0.77	0.89	1.02	1.14
Phenylalanine	1.40	1.61	1.83	2.04	1.40	1.62	1.83	2.04	1.41	1.62	1.83	2.04

**Calculations.** The initial mean weight (Wi) and final mean weight (Wf) of individual fish were measured. The survival rate (SR), daily weight gain (DWG), feed intake (FI), feed conversion ratio (FCR), the protein efficiency ratio (PER), net protein utilization (NPU), hepatosomatic index (HSI), and viscerosomatic index (VSI) were calculated as follows (where t = time in days):

SR (%) = (number of fish at the end of experiment) / (number of initial fish) × 100 DWG (g day<sup>-1</sup>) = (Wf - Wi) / t FI (% body weight day<sup>-1</sup>) = consumed feed / ((Wf × Wi)/2) × t FCR = amount of consumed feed in dry matter / weight gain PER = (Wf - Wi) / protein intake NPU = (protein of final fish - protein of initial fish) / protein intake HSI = liver weight (g) / total body weight (g) VSI = viscera weight (g) / total body weight (g)

**Statistical analysis.** The mean values of growth and feed efficiency and its standard deviation were calculated using Microsoft Excel 2010 (Microsoft Office 2010). Statistical differences in means between dietary treatments were analyzed via two-way Analysis of Variance (ANOVA) test followed by post-hoc DUNCAN test at a significant level of 0.05. All statistical analysis were conducted using SPSS Version 16.0 (SPSS for Windows 2007, Chicago, SPSS Inc.).

## Results

**Survival rate and growth**. After 56 days of culture, survival rates were observed to be high  $(96.7\pm1.7\%)$  (Figure 1). There were no significant differences in survival observed between diet treatments nor factors.

The mean final fish weight reached 6.83-7.98 g fish<sup>-1</sup>, concomitant with the growth rate of 0.099-0.119 g day<sup>-1</sup> (Table 3). There were no interactions observed between the dietary protein and dietary lipid in the growth. However, the independent effect in each factor (p < 0.05) of the two factors was found in the fish growth performance. Growth rates were observed to increase from 0.105 g day<sup>-1</sup> with the increase of dietary protein (from 30% protein), reached the highest value (0.115 g day<sup>-1</sup>) at 40% protein, then decreased to 45% protein (Table 3). Moreover, *S. argus* growth increased with the increase of dietary lipid in the feed (0.106-0.114 g day<sup>-1</sup>).

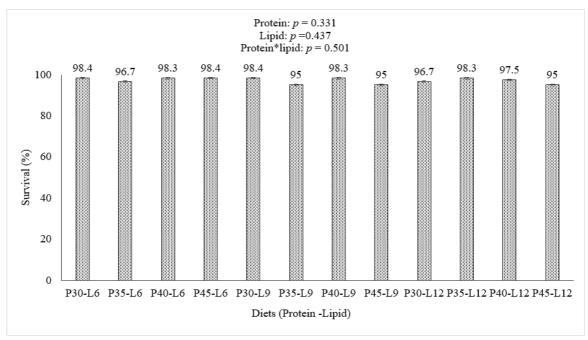


Figure 1. The survival rate of experimental *S. argus* after 56 days.

Table 3

Protein (%)	Lipid (%)	W <sub>i</sub> (g fish⁻¹)	$W_f(g fish^{-1})$	DWG (g day <sup>-1</sup> )		
30			6.83±0.08	$0.099 \pm 0.003$		
35	35 6		7.24±0.18	$0.106 \pm 0.004$		
40	6	$1.37 \pm 0.02$	7.56±0.11	$0.111 \pm 0.002$		
45	6	$1.34 \pm 0.06$	7.51±0.13	$0.110 \pm 0.002$		
30	9	1.33±0.04	7.38±0.42	0.108±0.007		
35	9	$1.32 \pm 0.05$	7.73±0.14	$0.115 \pm 0.004$		
40	9	$1.34 \pm 0.04$	7.98±0.26	$0.119 \pm 0.005$		
45	9	$1.37 \pm 0.04$	7.71±0.17	$0.113 \pm 0.003$		
30	12	1.35±0.06	7.42±0.35	$0.108 \pm 0.006$		
35	12	$1.32 \pm 0.07$	7.66±0.20	$0.113 \pm 0.003$		
40	12	$1.31 \pm 0.06$	7.81±0.11	$0.116 \pm 0.002$		
45	12	$1.38 \pm 0.07$	7.78±0.12	$0.114 \pm 0.002$		
p va	lues					
Prot	ein	0.292	0.001	0.001		
Lip	bid	0.955	< 0.001	0.001		
Protein	Protein*Lipid		0.526	0.530		
	Stati	stical analysis in e	each factor			
Dietary protein						
30			7.21±0.39 <sup>a</sup>	0.105±0.007 <sup>a</sup>		
35			7.54±0.28 <sup>b</sup>	$0.111 \pm 0.005^{b}$		
40			7.78±0.24 <sup>c</sup>	0.115±0.005 <sup>c</sup>		
45			7.66±0.17 <sup>bc</sup>	0.112±0.003 <sup>bc</sup>		
	Dietary lipid					
	6		$7.29\pm0.32^{A}$ $0.106\pm0.006^{A}$			
	9		$7.67 \pm 0.33^{B}$ $0.113 \pm 0.006^{B}$			
	12		$7.69\pm0.25^{B}$ $0.114\pm0.004^{B}$			

Growth performance of S. argus juvenile fed experimental diets

Values were represented as mean $\pm$ standard deviation. Different letters in the same column represented a significant difference (p < 0.05).

The size distribution of the experimental fish (Figure 2) observed in this study highlights that this species has a high range of size distribution. The 30% dietary protein supplementation treatment showed the highest percentage of the smallest group size (< 5 g) while the treatments with 9 and 12% lipid in the diet expressed a high percentage of the largest group size (> 11 g) (Figure 2).

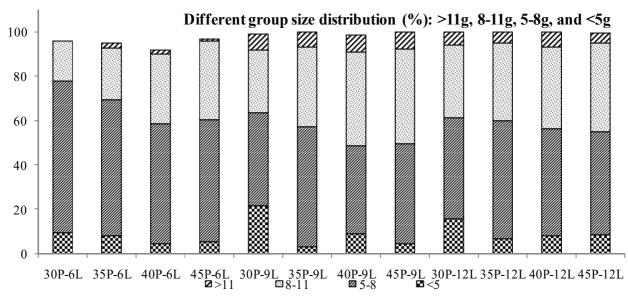


Figure 2. The size distribution of the experimental S. argus fish.

**Feed utilization**. Our results show that there are significant differences (p < 0.05) in FI, FCR, and PER between the dietary protein and lipid (protein:lipid) in *S. argus* (Table 4). The lowest FCR (2.15±0.14) was found at 35P:9L ratio, while the highest FCR (2.61±0.10) was at 30P:6L ratio. Moreover, at 30P-6L ratio, the highest feed intake (6.30±0.08%) was found. High values of PER (1.25-1.4) were observed in diet treatments with low levels of protein (30 and 35%) (Table 4).

Table 4

Protein	Lipid	FI (%BW day⁻¹)	FCR	PER				
30	6	6.30±0.08 <sup>d</sup>	2.61±0.10 <sup>c</sup>	1.27±0.05 <sup>e</sup>				
35	6	$5.39\pm0.24^{a}$	2.18±0.13 <sup>ab</sup>	1.30±0.08 <sup>e</sup>				
40	6	5.83±0.21 <sup>bc</sup>	2.36±0.07 <sup>b</sup>	1.05±0.03 <sup>bc</sup>				
45	6	$5.63 \pm 0.19^{abc}$	2.26±0.09 <sup>ab</sup>	0.97±0.04 <sup>ab</sup>				
30	9	$5.76 \pm 0.15^{abc}$	2.33±0.06 <sup>ab</sup>	$1.42 \pm 0.10^{f}$				
35	9	$5.45 \pm 0.30^{ab}$	$2.15\pm0.14^{a}$	1.32±0.16 <sup>e</sup>				
40	9	$5.55 \pm 0.07^{abc}$	$2.18 \pm 0.01^{ab}$	$1.14 \pm 0.01^{d}$				
45	9	$5.71 \pm 0.29^{abc}$	2.29±0.13 <sup>ab</sup>	$0.96 \pm 0.05^{a}$				
30	12	$5.78 \pm 0.16^{abc}$	2.34±0.05 <sup>ab</sup>	$1.41 \pm 0.03^{f}$				
35	12	5.72±0.31 <sup>abc</sup>	2.27±0.14 <sup>ab</sup>	1.25±0.08 <sup>e</sup>				
40	12	5.87±0.17 <sup>c</sup>	$2.31 \pm 0.10^{ab}$	1.08±0.04 <sup>cd</sup>				
45	12	5.82±0.32 <sup>bc</sup>	2.33±0.15 <sup>ab</sup>	$0.95 \pm 0.06^{a}$				
p values	p values							
Protein		0.001	< 0.001	< 0.001				
Lipid		0.072	0.072 0.021					
Protein*Lipi	id	0.020	0.025	0.029				

Values were represented as mean $\pm$ standard deviation. Different letters in the same column represented a significant difference (p < 0.05).

**Hepatosomatic index (HSI)**, **viscerosomatic index (VSI)**. Significant differences (p < 0.05) were only found in the VSI with juveniles fed different dietary lipid levels. The VSI increased with the increase of lipid levels in the diet. While the HSI did not show any difference or interaction (p > 0.05) (Table 5).

Henatosomatic index (HSI) and viscerosomatic index (VSI)

Table 5

пераю		) and viscerosomatic index	(VSI)					
Protein	Lipid	HSI	VSI					
30	6	0.030±0.002	0.117±0.007					
35	6	0.032±0.004	$0.115 \pm 0.006$					
40	6	0.031±0.002	$0.113 \pm 0.004$					
45	6	0.034±0.002	0.115±0.005					
30	9	0.033±0.003	0.117±0.004					
35	9	0.028±0.003	$0.121 \pm 0.011$					
40	9	0.028±0.004	$0.113 \pm 0.007$					
45	9	0.031±0.004	$0.118 \pm 0.006$					
30	12	0.029±0.005	$0.126 \pm 0.001$					
35	12	$0.030 \pm 0.001$	$0.125 \pm 0.004$					
40	12	0.033±0.002	$0.125 \pm 0.005$					
45	12	0.029±0.002	$0.118 \pm 0.005$					
p va	p values							
Prot		0.360	0.236					
Lip	oid	0.365	0.001					
Protein	*Lipid	0.434	0.437					
Statistical analysis in each factor								
Dietary lipid								
6 0.115±0.00								
9 0.117±0.007ª								
	12		0.124±0.005 <sup>b</sup>					

Values were represented as mean $\pm$ standard deviation. Different letters in the same column represented a significant difference (p < 0.05).

**Chemical composition analysis of fish body**. Significant differences between the dietary protein and lipid were not observed in the chemical composition analysis of the whole body of *S. argus* (Table 6). However, it was observed that moisture content in the fish body decreased with the increase of the dietary lipid while the opposite effect was observed in the lipid content of the fish body. Dietary protein levels were observed to significantly affect (p < 0.05) the protein and ash contents of the fish body (Table 6). The protein content in the fish body increased with the increase of the dietary protein levels were observed to significantly affect (p < 0.05) the protein and ash contents of the fish body (Table 6). The protein content in the fish body increased with the increase of the dietary protein levels while the highest ash content was found at 35% protein. Total energy of the fish body was affected by both dietary protein and lipid (Table 6). The lowest fish body energy (20.07±0.06 KJ g<sup>-1</sup>) was found at 35% protein in the diet while fish body energy increased with the rise of dietary lipid levels.

Table 6

Protein	Lipid	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Energy (KJ g⁻¹)
30	6	69.54±0.64	14.32±0.37	$11.68 \pm 0.44$	4.31±0.14	20.25±0.10
35	6	69.80±0.88	$14.44 \pm 0.44$	$11.03 \pm 0.34$	4.55±0.21	20.07±0.06
40	6	67.56±0.96	$15.17 \pm 0.15$	12.31±0.82	4.75±0.23	20.36±0.20
45	6	68.73±0.56	15.45±0.28	11.49±0.22	4.31±0.16	20.28±0.06
30	9	68.68±2.11	14.19±0.95	12.35±0.98	4.53±0.38	20.35±0.20
35	9	68.08±1.01	15.10±0.40	11.75±0.42	4.67±0.34	20.25±0.11
40	9	68.17±0.49	15.36±0.28	11.93±0.62	4.43±0.08	20.35±0.15
45	9	68.25±1.39	15.36±0.79	$11.90 \pm 0.58$	4.45±0.09	20.34±0.17
30	12	68.37±0.04	14.57±0.30	12.64±0.30	4.87±0.21	20.49±0.06
35	12	67.52±0.80	14.78±0.40	12.63±0.59	4.62±0.17	20.39±0.13
40	12	67.46±1.22	14.85±0.52	12.92±0.77	4.62±0.16	$20.50 \pm 0.17$
45	12	67.32±0.80	15.12±0.57	13.05±0.64	4.48±0.32	20.57±0.16
p val	lues					
Prot	ein	0.062	< 0.001	0.127	0.003	0.022
Lip	id	0.007	0.567	< 0.001	0.660	< 0.001
Protein	*Lipid	0.365	0.393	0.342	0.097	0.801
		St	atistical analysi	is in each factor	-	
Dietary	protein					
30	)		14.36±0.58ª		4.36±0.28ª	20.36±0.16 <sup>b</sup>
35	5		14.77±0.47 <sup>ab</sup>		4.70±0.24 <sup>c</sup>	20.24±0.16 <sup>ª</sup>
40	)		15.13±0.39 <sup>bc</sup>		4.60±0.22 <sup>bc</sup>	20.40±0.17 <sup>b</sup>
45			15.31±0.55 <sup>c</sup>		4.42±0.55 <sup>ab</sup>	20.39±0.18 <sup>b</sup>
Dietar	y lipid					
6		68.91±1.14 <sup>B</sup>		11.63±0.66 <sup>A</sup>		20.24±0.15 <sup>A</sup>
9		68.29±1.26 <sup>AB</sup>		11.98±0.65 <sup>A</sup>		20.32±0.15 <sup>A</sup>
12	2	67.67±0.86 <sup>A</sup>		12.81±0.57 <sup>B</sup>		20.49±0.14 <sup>B</sup>

Chemical composition analysis of the whole body

Values were represented as mean $\pm$ standard deviation. Different letters in the same column represented a significant difference (p < 0.05).

**Discussion**. The formulation of economically and biologically viable and environmentfriendly diets is currently gaining popularity in the aquaculture industry (Khan et al 2019). Several studies have been carried out to optimize feed formulations and feeding approaches, focusing on the balance of dietary protein and energy levels (Teles et al 2020), whilst simultaneously attempting to maximize fish performance, decrease economic feed costs and reduce negative environmental impacts.

In this study, the survival rates of *S. argus* were not affected by the dietary treatments and this parameter was high (95.0-98.4%, Figure 1) perhaps also accounting for the hardiness of this species. Similar findings have also been observed in other marine omnivorous fish species such as mullet (*Liza subviridis*) and *Pseudapocryptes elongatus* at different protein levels (Viet et al 2010a, b; Be & Hien 2014). The survival rates of other species such as the yellowfin pompano (*Trachinotus ovatus*) and rainbow

trout (*Oncorhynchus mykiss*) have also been known to not be affected by protein and lipid contents in the diet (Wang et al 2013; Ahmed & Ahmad 2020).

In the present study, the best results were obtained with the feed with a protein content of 40%. According to Pesta & Samuel (2014), if the dietary protein is too high, the excess protein is not be absorbed by the fish's body to synthesize new protein, but this protein will be used for conversion into energy or excreted. Furthermore, a study by Manh et al (2011) on the effects of different protein contents on the growth of *S. argus* in the commercial stage highlighted that the fish body has to allocate energy directed towards digesting the excess protein rather than growth. Protein takes more energy to digest and the fishes fed high protein levels might also have the satiating effect of very high protein intakes decreasing actual energy intake. Thus, the growth of the fish's body will decrease as was reflected in our study (Table 3).

The results of previous studies showed that when nursing *S. argus* fish, juveniles often have a very large size variation although in the same care regime (Khanh et al 2010; Khanh 2018). In addition, similar to many other aquaculture species, the stocking density of *S. argus* is known to often greatly affect its size distribution during rearing. When stocking density is low, the fish tend to be larger and inversely when stocked at high-density (Viet et al 2020).

Ni et al (2013) showed that the feed consumption coefficient in *S. argus* rearing was relatively high, ranging from 2.60 to 2.83. Manh et al (2011) studied the effect of different dietary protein content on the growth and survival of *S. argus* and highlighted that the FCR in the experiment ranged from 2.39 to 2.92. Tu et al (2014) determined the protein requirements of goby fry (*P. elongatus*) with 4 protein levels (30, 35, 40, 45%) and 2 energy levels (20 and 18 KJ  $q^{-1}$ ). In the present study, results showed that the lowest FCR (2.15±0.14) of the fish was in the treatment with 35P-9L (Table 4). The highest PER was found in the treatment diets of 30P-9L and 30P-12L (Table 4). For the animal-fed fish group, Ahmed & Ahmad (2020) reported that the FCR and PER of the rainbow trout (Oncorhynchus mykiss) were improved gradually with the increase in the protein content of the feed and the best FCR (1.12) and PER (1.99) were found in 45% protein treatment. In their study, it was observed that fish which were fed a diet containing 50 and 55% protein had increased FCR but decreased PER. However, a study by Sankian et al (2017) on Siniperca scherzeri fish fed diets with different protein and lipid contents in the feed did not show any effect on the feed efficiency (FE) and PER, which highlights the fact that effects of dietary protein, lipid levels and their respective ratios vary strongly among species.

The results of this study are similar to previously published works reported on Trichogaster trichopterus (Mohanta et al 2013), Oplegnathus fasciatus (Kim et al 2016), and Cirrhinus mrigala (Alam et al 2020). When the dietary protein was high, it was also reflected in the protein content in fish body where it was also observed to be increased. Similar trends were also found between the dietary lipid and the lipid content in the fish body (Table 6). When the dietary lipid level was the same, the sea buckthorn's (Nibea *iaponica*) body protein increased with the increase in protein in the feed (36, 40, 44, 48, and 52%) (Li et al 2016). When the dietary protein level was the same, the lipid and energy content of the croaker's body increased but the moisture content of the fish reduced with an increase of the dietary lipid from 9 to 15%, and the ash content of the croaker's body was unaffected by the diets (Chai et al 2013). Lan et al (2014) reported that the chemical compositions (moisture, protein, lipid, and ash) of Chitala chitala was not affected by the interaction between protein and lipid in the diet. The authors concluded that the dietary protein and lipid did not affect the chemical composition of that fish body. Anh et al (2014) reported the spotted scat fed only the commercial pellet had a higher lipid content in the body than the fish fed a combination of commercial pellet and algae. For the yellowfin pompano (Trachinotus ovatus), the fish's body protein tended to decrease with an increase of the dietary lipid, but there was no difference in moisture, lipid, ash, and energy in the body (Wang et al 2013). Research by Ahmed & Ahmad (2020) found that the moisture content of rainbow trout decreased gradually with the increase of the dietary protein from 30 to 45%. It demonstrates that fish fed the dietary protein beyond the optimum level is useless for body protein synthesis or tissue building in fish (Siddiqui & Khan 2009). Therefore, further studies are required to optimize the protein-energy ratio for specific fish species.

**Conclusions**. The survival rate of the experimental spotted scat juveniles in all treatments ranged from 95.0 to 98.4% and was not affected by different dietary protein and lipid levels, which also highlights the hardiness of this species. The highest fish growth, the best feed efficiency was found in the treatment diet 40% protein and a 9% lipid supplementation in the diet. The dietary lipid reduced the moisture content but increased the lipid content in the whole fish body. The dietary protein increased the protein content and influenced the ash content in the whole fish body. The dietary lipid increased the viscerosomatic index (VSI) of the experimental fish. Results obtained from this study could assist in the formulation of diets, which are cost-effective and nutritionally practical, for spotted scat juveniles.

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**Conflict of interest**. The authors declare that there is no conflict of interest.

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