Methionine requirement of the spotted scat *Scatophagus argus* (Linaeus, 1766) fingerling

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Abstract. Amino acids, especially methionine, play an important role in the growth and development of fingerling fish. This study aimed to determine the methionine requirement of spotted scat (*Scatophagus argus* Linaeus, 1776) fingerling (average body weight=1.5 g), a fish species with high economic value in Vietnam. The experiment was conducted for 8 weeks using 5 experimental diets containing the same protein and energy levels (38% crude protein (CP) and 21.3 MJ kg^{-1} of gross energy) with 3 replicates for each treatment. The L-D methionine content varied from 4.5 g to 12.5 g kg^{-1} CP (11.9 to 42.9 g kg^{-1} dry diet) increments. The experimental results showed that growth rate, feed conversion ratio and protein utilization efficiency were the highest at a methionine level of 8.5 g kg^{-1} CP (22.5 g kg^{-1} dry diet), while the survival rates were not significantly affected. The results of quadratic regression analysis between growth rate and dietary methionine showed that the optimal methionine content for spotted scat fingerling was at 24.4 g kg^{-1} CP or 9.21 g kg^{-1}.
Key Words: amino acid, spotted scat, methionine requirement.

Introduction. The spotted scat, *Scatophagun argus*, is a tropical euryhaline species that is a candidate for commercial coastal aquaculture in Asia because of several interesting characteristics such as good adaptation to highly fluctuating environments. There are two varieties of spotted scat fish: Scatophagus and Selenotoca (Barry & Fast 1992). However, in Vietnam there is only one species, *S. argus* (Linnaeus, 1766) (Yen 1992). *S. argus* is one of the species of economic importance, with high nutritional value and good consumer market. *S. argus* has many advantages such as having wide salinity tolerance and high vitality, and its natural food consists of mainly aquatic plants and organic humus, it can live and grow in fresh, brackish and marine environment (Phuong et al 2004). *S. argus* is distributed in many lagoon areas and coastal bays across Vietnam. It is an indigenous fish that is assessed to have promising development prospects in Thua Thien, Hue in Vietnam. However, there are still problems in its culture such as disease, variable quality of the stock, high gate price and variable quality of fish feed used (Binh et al 2022).

So far, research on *S. argus* is very scarce, especially on its nutritional aspect. The supply of nutrients must be suitable for the breeding phase and should contain optimal amounts of amino acids, lipids, vitamins and minerals, in order to promote development and growth. Knowledge of quantitative amino acid requirements of *S. argus* are indispensable. Methionine is one of the most, if not the most important amino acid for the development of fish (Hien et al 2012; Binh et al 2021). It plays a central role in protein synthesis, antioxidant metabolism and immune response that affect the overall health status of fish, and also its carbohydrate and lipid metabolism. In addition, methionine modifies the expression of genes related to the growth and health of the fish by methylation reactions of the DNA molecule (Hien et al 2012). Therefore, a study determining the quantitative need for methionine for *S. argus* is essential, the results of which will contribute to the formulation of an optimal diet containing the right amount of methionine for improved productivity and quality of breeding, leading to an increased economic efficiency.

Material and Method

Experimental design. Five experimental dietary treatments at five various methionine levels were prepared. The control treatment (NT1) (without methionine supplementation) contained 4.5 g kg^{-1} diet methionine (present in fish meal and gluten); Treatment 2 (NT2) contained 2 g kg^{-1} diet methionine, while Treatments 3 (NT3), 4 (NT4) and 5 (NT5) contained 4, 6 and 8 g kg^{-1} diet methionine, respectively. The feeding trial was conducted in a completely randomized design (CRD) which lasted for 8 weeks.

Experimental diet preparation. Following the selection of ingredients, analyses were carried out to determine the nutritional composition and amino acid composition of the ingredients, in order to balance the basal diet in terms of protein and energy. Each diet was supplemented with a mixture of crystal amino acid to ensure that the amino acid composition corresponded to the fish body's amino acids, except for the methionine. The experimental diet contained a final crude protein content of 38% and an average crude energy (CE) of 21 MJ kg^{-1}. The basal diet was composed of the following ingredients: fish meal, gluten, gelatin, dextrin, non-essential amino acid mixture, essential amino acid mixture and squid oil (Hien et al 2012; Binh et al 2021) as shown in Table 1.

Table 1

Ingredients, chemical composition (% DM) and energy (MJ kg^{-1}) by treatment

Ingredients	*Treatments*				
	NT1	*NT2*	*NT3*	*NT4*	*NT5*
Fish meal	200	200	200	200	200
Gluten	150	150	150	150	150
Destrin	300	300	300	300	300
Gelatin	10.0	10.0	10.0	10.0	10.0
Essential amino acid mixture	67.8	67.8	67.8	67.8	67.8
Non-essential amino acid mixture*	64.2	62.2	60.2	58.2	56.2
L-D methionine	0.0	2.0	4.0	6.0	8.0
Carboxylmethyl cellulose	103	103	103	103	103
Squid oil	50	50	50	50	50
Premix vitamin	20	20	20	20	20
Mineral premix	20	20	20	20	20
Vitamin C	10	10	10	10	10
Cholin	5	5	5	5	5
Chemical composition (%)					
Ingredients	Treatments				
	NT1	NT2	NT3	NT4	NT5
Crude protein	37.3	37.8	38.0	37.5	38.0
Crude lipids	6.8	7.6	7.5	6.8	7.5
Ash	8.2	7.3	7.4	8.5	8.6
Crude fiber	1.20	1.13	1.09	1.19	1.03
NFE	46.5	46.2	53.4	46.0	44.9
Energy (MJ kg^{-1})	20.9	21.4	21.4	21.0	21.6
Methionine content					
Methionine g (kg $diet^{-1}$)	4.5	6.5	8.5	10.5	12.5
Methionine (g kg^{-1} CP)	11.9	17.2	22.5	27.7	32.9

* Available cystine content is 2.15 g (kg $diet^{-1}$)(5.6 g kg^{-1} CP)

Experimental fish and facility. *S. argus* fingerlings (average body weight of 1.5 g fish^{-1}, 2-4 cm total length) were purchased from the Aquaculture Research Center, Faculty of Fisheries, University of Agriculture and Forestry, Hue University. *S. argus* were transported to the laboratory and acclimatized in composite tanks for 3 weeks. During acclimation, scats were fed with commercial feed for 2 weeks, then weaned with the experimental diet. Weaning was done by feeding initially mixed commercial and corresponding experimental feed for 1 week, with the proportion of test feed gradually increased to 100% of the formulated test diet; this point was considered the start of the experiment. Following the random distribution of fingerling scats at 30 fish tank^{-1}, *S. argus* were fed twice daily at 08:00 and 16:00 h, at 5% of their body weight. Feeding was closely monitored to ensure that the fish consumed the daily ration completely. Water quality parameters such as temperature, salinity, pH, DO, NH_3, $NH4^+$ were monitored twice in a week. The experiment was conducted in a recirculating water system consisting of 15 composite tanks with a total volume capacity of 4 m^3 and containing 3.5 m^3 of water. Additionally, there were also storage tanks, settling tanks, chlorine reduction tanks, aerators, water pumps, circulating water purifiers, electrical systems, fresh water systems.

Calculations*.* At the end of the experiment, the following performance and feed efficiency indices were evaluated: weight gain (WG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), and protein efficiency ratio (PER) according to the equations below (Hien et al 2012; Binh et al 2021).

Weight gain (WG %)

$$WG\ (\%) = \frac{W_{-2} - W_1}{W_1} \times 100$$

Specific growth rate (SGR; % day^{-1})

$$SGR\ (\%\ day^{-1}) = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \times 100$$

Where:
W_1 - average weight at start of the experiment, t1;
W_2 - average weight at termination of the experiment, t2;
t_2 - t_1 - feeding experiment period.

Survival rate (SR, %)

$$SR\ (\%) = \frac{\text{The total number of fish at the end of the experiment}}{\text{The number of fish initially stock in the experiment}}\ X\ 100$$

Feed conversion ratio (FCR)

$$FCR = \frac{\text{The amount of feed the fish has consumed (kg)}}{\text{The weight gain (kg)}}$$

Protein efficiency ratio (PER)

$$PER = \frac{\text{Weight gain of fish (g)}}{\text{Amount of protein consumed (g)}}$$

Statistical analysis. Data were analyzed according to a completely randomized design and presented as means ± standard deviation (SD; n=3). All data were tested for normality, using the Kolmogorov-Smirnov test, and for homoscedasticity, with the Levene's test. Subsequently, a one-way analysis of variance (ANOVA) was applied to determine significant differences among dietary treatments, and a Tukey's ranking test when significant differences were found ($p<0.05$). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 20). Percent data were transformed into arcsine values (variance-stabilizing transformation) prior to analysis. Methionine needs of fish were determined using the quadratic regression curve method between the growth rate (WG) and the methionine content in the feed.

Results

Water quality. The mean water quality parameters during the experiment are presented in Table 2. No major fluctuations occurred in water temperature (°C), pH, DO (mg L^{-1}), salinity (S‰) and NH_3 (mg L^{-1}) and their values were within the recommended range for the spotted scat.

Table 2

Fluctuation of environmental factors during experimental *Scatophagun argus* culture

Treatments/ Factors	NT1	NT2	NT3	NT4	NT5
			min-max / Mean ± SEM		
Temperature (˚C)	25.3-27.6 / 26.49 ± 0.42[a]	25-28 / 26.52 ± 0.51[a]	25.5-27.5 / 26.44 ± 0.5[a]	25.55-27.69 / 26.49 ± 0.4[a]	25-27.7 / 26.46 ± 0.5[a]
DO (mg L^{-1})	3.2÷5.65 / 4.47 ± 0.84[a]	3.12÷5.7 / 4.42 ± 0.84[a]	3.2÷5.55 / 4.41 ± 0.8[a]	3.12÷5.5 / 4.42 ± 0.81[a]	3.05÷5.85 / 4.42 ± 0.86[a]
pH	7-8 / 7.6 ± 0.21[a]	7-8 / 7.54 ± 0.19[a]	7.2-8 / 7.56 ± 0.17[a]	7.14-8 / 7.6 ± 0.2[a]	7-7.91 / 7.5 ± 0.15[a]
NH_3/NH_4 (mg L^{-1})	0.009-0.124 / 0.02 ± 0.014[a]	0.009-0.124 / 0.023 ± 0.02[a]	0.01-0.124 / 0.022 ± 0.02[a]	0.01-0.126 / 0.02 ± 0.02[a]	0.01-0.125 / 0.03 ± 0.03[a]
Salinity (‰)	20-221 / 21.25 ± 0.7[a]	20-22.5 / 21.38 ± 0.74[a]	20-22.5 / 21.2 ± 0.75[a]	20-22 / 21.1 ± 0.6[a]	20-22.01 / 20.9 ± 0.64[a]

Values with the same letter ([a]) represent statistically insignificant differences (p> 0.05).

Growth performance and feed efficiency. The growth (WG) of spotted scat was significantly different between the treatments (Table 3). Fish fed with the diet without methionine supplementation exhibited the lowest WG followed by those fed with the diet supplemented with 2 g kg^{-1} diet methionine and the highest value was exhibited by those fed diet with methionine at 4 g kg^{-1} diet. The WG of fish fed the diet containing 6 and 8 g kg^{-1} diet decreased significantly from those supplemented with 6 g kg^{-1} diet.

Table 3

Effect of methionine levels on initial body weight (IBW), final body weight (FBW), weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) of *Scatophagus argus*

Factors	*Treatments*					*p-value*
	NT1	*NT2*	*NT3*	*NT4*	*NT5*	
IBW (g)	3.24±0.06	3.18±0.08	3.14±0.16	3.26±0.1	3.22±0.14	0.23
FBW (g)	13.75±0.73[a]	14.38±0.08[bc]	16.74±0.47[d]	14.32±0.24[c]	14.43±0.15[b]	<0.001
WG (%)	324.93± 22.01[a]	352.1± 17.23[bc]	433.62± 19.39[d]	399.41± 21.22[c]	348.77± 18.39[b]	<0.001
SGR (% d^{-1})	2.95±0.12[a]	3.08±0.08[bc]	3.42±0.15[d]	3.02±0.09[c]	3.06±0.09[b]	<0.001
FCR	1.9±0.04[b]	1.87±0.04[b]	1.74±0.05[a]	1.87±0.03[b]	1.87±0.06[b]	0.012
PER	4.62±0.1[a]	4.7±0.1[a]	5.05±0.13[b]	4.7±0.06[a]	4.7±0.16[a]	0.008

Different characters ([a, b, c]) in the same row show statistically significant differences (p<0.05). ± indicates the standard deviation.

Specific growth rate values (SGR) of spotted scat also exhibited significant differences among treatments (P<0.05). The diet without methionine supplementation resulted in the lowest SGR value (2.95% day^{-1}) and this value gradually increased in the 2 g kg^{-1} methionine group (3.08% day^{-1}) and peaked in the 4 g kg^{-1} diet group (3.42% day^{-1}). The SGR values decreased in the 6 and 8 g kg^{-1} diet methionine groups (3.02% day^{-1}) and 3.06% day^{-1}, respectively (Table 3). The value of the feed conversion ratio (FCR) was significantly the lowest (1.74) in the methionine 4 g kg^{-1} diet treatment and was statistically different from all the other treatments (P<0.05). The diet without methionine exhibited the highest feed conversion ratio (1.9). The efficiency of protein utilization of fish fed with the different diets exhibited statistically significant differences (P<0.05). The diet without methionine gave the lowest PER value of 4.62, followed by the 2 g kg^{-1} diet methionine group (4.7); the PER value was highest (5.05) in the 4 g kg diet^{-1} group (5.05) and decreased in the 6 g kg^{-1} diet and 8 g kg^{-1} diet groups (4.7), respectively (Table 3). The survival rate of the spotted scat in diets with different methionine contents are shown in Figure 1.

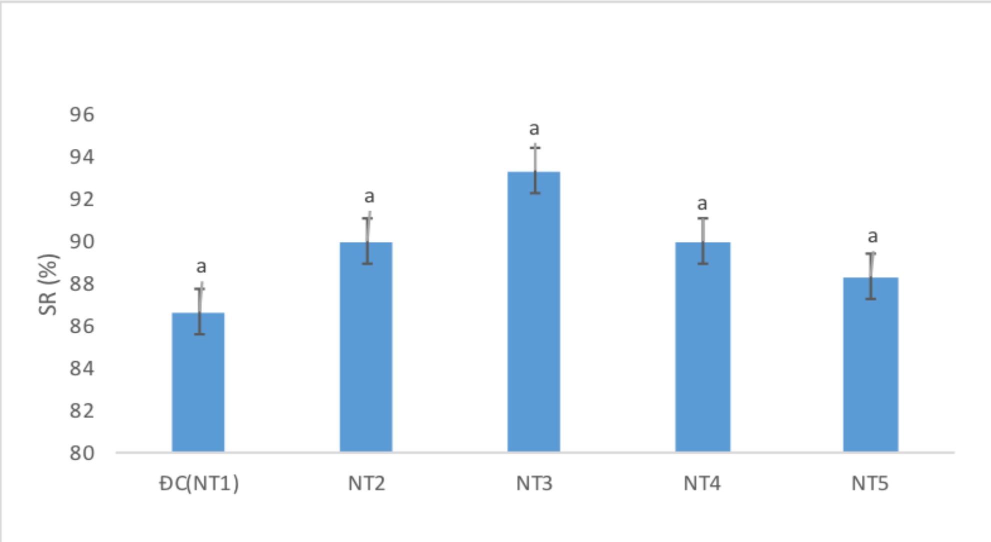

Figure 1. Effects of supplemental methionine levels in feed on the survival rate of *Scatophagus argus* fingerling.

Fingerlings of spotted scat fed with various methionine levels exhibited statistically similar survival rates, ranging from 86.67% to 93.33%, with the 4 g kg^{-1} diet group displaying the highest rate (93.33%), while the group without methionine supplementation exhibited the lowest rate (86.67%). Thus, the amount of methionine added to the food did not affect the survival rate of the experimental fish.

Discussion. The quality and composition of amino acids in feed are of great significance for maximizing the development in shrimp and fish and for increasing the productivity and quality of shrimp and fish (Ahmed & Khan 2004). Therefore, changing the amino acid content in the feed, specifically changing the L-D methionine content in the feed, will affect the growth of fingerling spotted scat. The weight gain (WG) of the spotted scat increased when the fish were fed with increased methionine diets and at a suitable level of methionine supplementation of 4 g kg^{-1} diet. But when the methionine content in the diet exceeded the fish's needs, the WG tended to decrease. Fish fed with the diet without methionine supplementation, exhibiting the lowest WG, were deficient, considering the needs of potted scats. WG of fish gradually increased at methionine levels of 2 g kg^{-1} diet, displaying the highest growth rate, superior to all fish fed with the other diets and then gradually decreased in the 6 g kg^{-1} diet and 8 g kg^{-1} diet groups, respectively. This indicates that the methionine for the maintenance and growth of fish fed with the 2 g kg diet-1 was closer to the optimal level than the other diets. Continuing to increase the methionine level beyond the optimal amount proves to be not economical.

WG rates of *S. argus* in the present study had similar patterns to those of rockfish fingerlings *(Scarus schelegeli),* which also increased when the methionine content in the diet increased and decreased when the methionine content increased. *S. schelegeli* grew maximally when methionine levels in the diet were increased from 1.3 to 1.58% (Yan et al 2007). Similar results were also recorded in carp *(C. carpio)*: the WG rate increased when the methionine content in the feed increased to 0.85% of the diet, and then fish weight also decreased when the methionine content in the feed increased beyond 0.85% of the diet (Wang et al 2016). The WG of seabass fingerlings *(Dicentrachus labrax)* also increased when the dietary methionine content increased to 1.30%. If the methionine levels continue to increase beyond the optimum, the fish growth rate will decrease (Thebault et al 1985). In order to evaluate the correlation between the methionine content and fish growth rate (WG), we conducted a regression analysis between the growth rate (Y), and the methionine content (X) using a quadratic regression curve, of the form $Y = aX^2 + bX + c$. The results of the analysis are presented in Figure 2.

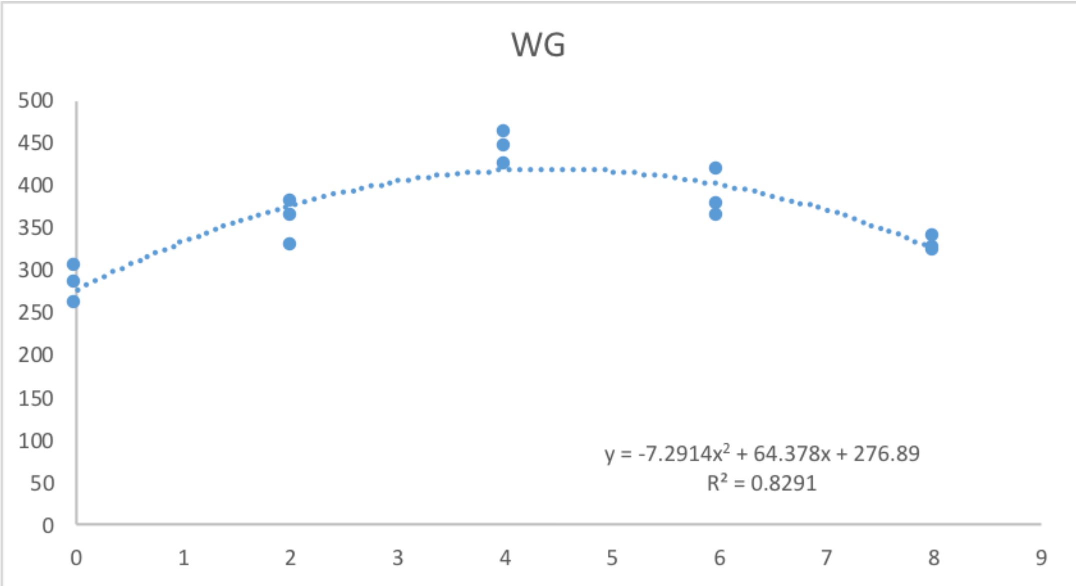

Figure 2. Correlation between methionine content (g kg^{-1} CP) and WG.

The equation $Y=-7.2914X^2+64.387X+276.89$ describes a close correlation between the experimental treatments (methionine concentrations) and the WG rates ($R^2=0.829$) or $R=0.91$. Thus, we can conclude that the need for methionine is necessary for the absolute growth rate of the spotted scat fingerling.

From the equation above mentioned, we have determined the maximum point at the coordinates $X_{max}=4.34$ g kg^{-1} diet and $Y_{max}=427\%$. We found that the maximum on the regression curve occurred at the methionine content of 24.4 g kg^{-1} CP or 9.21 g kg^{-1} diet. Thus, the growth rate of spotted scat, for diets containing different methionine concentrations, ranges from 284.38 to 459.6%. Growth was the highest in diets with a methionine content of 4 g kg^{-1} CP. That means the optimal methionine requirement of juvenile brown fish is 24.4 g kg^{-1} CP or 9.21 g kg^{-1} diet. The methionine requirement of spotted scat is quite similar to that of striped catfish *(Pangasianodon hypophthalmus)*, which catfish was of 10.1 g kg^{-1} diet (26.7 g kg^{-1} CP) (Hien et al 2012).

In addition, different methionine supplementation treatments also caused differences in the SGR and FCR parameters. It can be seen that for an increase of the methionine content in the treatments, the FCR tended to decrease in the present study, but increasing the methionine amount too high caused the FCR to increase. This result is similar to that recorded in tilapia *(Coptodon zillii)*: when the methionine content was 0.50% of the diet, the FCR decreased significantly, compared to other methionine levels (Polat 1999). Besides, diets that do not use methionine have the lowest protein utilization efficiency, in our opinion because the methionine content is still low, not fully meeting the nutritional needs of fish.

In general, PER increased by increasing methionine content in the diet and reached the highest level at a methionine diet of 4 g kg^{-1} diet and then decreased. Data on the survival rate of experimental *S. argus* (Figure 1) indicated no statistically significant differences among treatments (p>0.05). This shows that methionine content in food did not affect the survival rate of experimental *S. argus* at all. This result is similar to those reported on in some other fish species such as striped tilapia *(Oreochromis niloticus)* by Liebert et al (2007) and, on the rainbow trout *(Oreochromis mykiss)* by Nguyen & Davis (2009) and on the striped catfish *(Pangasianodon hypophthalmus)* by Hien et al (2012). These experiments showed that the methionine content in diets did not affect the survival rates of these fish species when performing experiments.

Conclusions. After 8 weeks of experiments with different levels of L-D mithionine supplementation (5 treatments) ranging from 4.5 g to 12.5 g kg^{-1} CP (11.9 to 42.9 g kg^{-1} dry diet), the results showed that methionine content in feed affects growth, feed conversion ratio and feed efficiency of *S. argus* fingerlings. Experimental results showed that growth rate, feed conversion ratio and protein utilization efficiency were the highest at a methionine level of 8.5 g kg^{-1} CP (22.5 g kg^{-1} dry diet, while the survival rates were not significantly affected. The optimal methionine concentration required to meet the growth needs of *S. argus* fingerling was 9.21 g kg^{-1} diet or 24.4 g kg^{-1} CP.

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Conflict of interest. The authors declare no conflict of interest.

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