# Effect of dietary methionine level on growth performance of *Siganus guttatus* (Bloch, 1787) fingerlings

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**Abstract**. This study aimed to determine the methionine requirement in *Siganus guttatus* (Bloch, 1787), also known as Orange-spotted spinefoot fingerlings with an average weight of 5.2 g fish$^{-1}$. The experiment was conducted over 8 weeks using 5 experimental diets with the same protein and energy levels: 38% crude protein (CP) and 21.3 MJ kg$^{-1}$ of gross energy (GE), with 3 replicates for each treatment. In increments, Methionine content varied from 4.5 to 12.5 g kg$^{-1}$ CP (11.9 to 42.9 g kg$^{-1}$ dry diet). The results of the quadratic regression analysis between growth rate and dietary methionine showed that the optimal methionine content for Orange-spotted spinefoot fingerlings was 22.5 g kg$^{-1}$ CP or 8.5 g kg$^{-1}$ diet.
**Key Words**: Orange-spotted spinefoot, fingerlings, methionine requirement.

**Introduction**. *Siganus guttatus* (Bloch, 1787) is a fish commonly farmed in Vietnam. This fish has high economic value, delicious meat, and is very popular in the market (Binh et al 2022). The development of Orange-spotted spinefoot farming has contributed to the consumption of a large amount of industrial fish feed on the market. Currently, the production of industrial fish feed primarily utilizes protein from fish meal because it has a high protein content and is rich in essential amino acids, which play a crucial role in the growth and development of the aquaculture industry (Hien et al 2012). However, the increasing price of fishmeal, driven by high demand and overfishing, has resulted in a significant decline in fish catch. This has led to a trend of replacing fishmeal with other protein sources. Many studies have been conducted using plant protein sources to replace fishmeal in aquaculture feeds (Nguyen & Davis 2009). Some plant protein sources, such as soybeans and peanuts, and their by-products, are among the first choices due to their widespread, stable, and affordable supply. However, although plant protein sources can meet protein needs, they do not meet the essential amino acid needs, especially methionine (Michelato et al 2018).

Methionine is one of the most, if not the most important, amino acids for the development of fish (Jackson & Capper 1982; Lieber & Benkendorff 2007). It plays a central role in protein synthesis, antioxidant metabolism, and immune response, which collectively affect the overall health status of fish, as well as their carbohydrate and lipid metabolism. In addition, methionine regulates the expression of genes related to fish growth and health through DNA methylation reactions (Lin et al 2008; Hien et al 2012). However, the addition of methionine in *S. guttatus* feed needs to be at a sufficient level. If it is deficient compared to demand, it will affect fish growth, and excessive supplementation can lead to waste and toxicity in the fish (Thebault et al 1985; Binh et al 2021). Therefore, this study was conducted to determine the methionine requirement of Orange-spotted spinefoot by evaluating the effects of different dietary methionine levels on growth, survival, feed conversion ratio, and protein utilization efficiency of *S. guttatus* fingerlings.

## Material and Method

***Experimental design***. The experiment on methionine requirement on *S. guttatus* fingerlings was performed using the same method as that performed on spotted scat (*Scatophagus argus*) fingerlings by Binh et al 2022 because the results of nutritional analysis in these two fish species were quite similar. Based on the results of the study of the nutritional composition of *S. guttatus* muscle, the average crude protein (CP) content was 37.5%. Therefore, the diet we established for the experimental fish included 38% CP. Based on the research method of Hien et al (2012), five experimental dietary treatments with varying methionine levels were prepared. The control treatment (TM1) (without methionine supplementation) contained 4.5 g $kg^{-1}$ diet methionine (present in fish meal and gluten); Treatment 2 (TM2) contained 2 g $kg^{-1}$ diet methionine, while treatments 3 (TM3), 4 (TM4), and 5 (TM5) contained 4, 6, and 8 g $kg^{-1}$ diet methionine, respectively. The feeding trial was conducted in a completely randomized design, lasting 8 weeks.

***Experimental diet preparation***. Proximate composition and amino acid content of the ingredients were analyzed. They formed the basis for the formulation of the diets in terms of gross energy (GE), CP, lipid, and methionine level. A mixture of crystalline amino acids was added to ensure balance with the body's amino acids, except for the methionine levels. The experimental diet was so formulated to contain CP of 38% and GE 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 2022) as shown in Table 1. Following the selection of ingredients, analyses were carried out to determine the nutritional composition and amino acid composition of the ingredients, to balance the basal diet in terms of protein and energy.

Table 1

Ingredients of experimental fish diets

| *Ingredients* | *Treatments* | | | | |
|---|---|---|---|---|---|
| (g $kg^{-1}$ diet) | *TM1* | *TM2* | *TM3* | *TM4* | *TM5* |
| Fish meal | 200 | 200 | 200 | 200 | 200 |
| Gluten | 150 | 150 | 150 | 150 | 150 |
| Dextrin | 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 |

Available cystine content is 2.15 g $kg^{-1}$ diet; 5.6 g $kg^{-1}$ CP.

Table 2

Chemical composition of the diet after analysis

| *Ingredients* | *Treatments* | | | | |
|---|---|---|---|---|---|
| | *TM1* | *TM2* | *TM3* | *TM4* | *TM5* |
| 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 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 |

***Experimental fish and facility***. The experiment was conducted in a recirculating water system comprising 15 composite tanks with a total volume capacity of 4 $m^3$ and containing 3.5 $m^3$ of water. Additionally, the facility included storage tanks, settling tanks, chlorine reduction tanks, aerators, water pumps, circulating water purifiers, electrical systems, and freshwater systems. Orange-spotted spinefoot fingerlings (initial average body weight of 5.2 g $fish^{-1}$) were procured from the Center for Aquaculture Research, Application and Technology Transfer, University of Agriculture and Forestry, Hue University. Fish were transported to the laboratory and acclimatized in composite tanks for 3 weeks. During acclimation, Orange-spotted spinefoot were fed with commercial feed (Uni-President company's commercial fish feed with 38% protein) for 2 weeks. The fish diet was modified with the experimental diet by initially feeding a mixture of commercial and corresponding experimental feed for 1 week, with the test feed being gradually increased in proportion until it became 100% pure formulated test diet. This point was considered the start of the experiment, following the random distribution of fingerling scats to their respective experimental units, at 30 fish × $tank^{-1}$. The fish were fed the experimental diet twice daily, at 8 am and 4 pm, at a rate of 5% 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, and $NH_3$ were monitored twice a week. Fifteen to 20 fish in each tank were randomly caught, batch-weighed, and individual total lengths measured.

***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 2022).

Weight gain (WG, %):

$$WG\ (\%) = \frac{W\text{-}_2 - W_1}{W_1} \times 100$$

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, %):

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

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 using 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 using 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 was conducted when significant differences were found ($p < 0.05$). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 20). Percent data were transformed into arcsine values (variance-stabilizing transformation) before analysis. The methionine needs of fish were determined using the quadratic regression curve method between the WG and the methionine content in the feed.

## Results

***Water quality***. Water environmental parameters play a crucial role and directly impact the life of fish (Wang et al 2016). In this experiment, factors such as water temperature (°C), pH, DO (mg $L^{-1}$), salinity (S‰), and $NH_3$ (mg $L^{-1}$) were closely monitored throughout the experiment. The results of water environment parameter fluctuations in the experimental batches are shown in Table 3. The statistical analysis results also showed that there was no difference between the treatments in terms of fluctuations in these environmental parameters. In addition, these parameters were also favorable for the experimental Orange-spotted spinefoot.

Table 3

Fluctuation of environmental factors during experimental Orange-spotted spinefoot culture

| *Treatments* | *TM1* | *TM2* | *TM3* | *TM4* | *TM5* |
|---|---|---|---|---|---|
| | | | *min-max* / *Mean ± SEM* | | |
| Temperature (°C) | 25.5-28.35 / 27.0±0.66$^a$ | 25.4-28.58 / 27.15±0.71$^a$ | 25.57-28.47 / 27.15±0.66$^a$ | 25.8-28.5 / 27.04±0.61$^a$ | 26.0-28.4 / 26.97±0.57$^a$ |
| DO (mg $L^{-1}$) | 3.1-5.62 / 4.45±0.86$^a$ | 3.12-5.67 / 4.42±0.85$^a$ | 3.15-5.55 / 4.39±0.82$^a$ | 3.13-3.55 / 4.43±0.84$^a$ | 3.05-5.57 / 4.71±0.86$^a$ |
| pH | 7.0-8.45 / 7.6±0.23$^a$ | 7.1-8.50 / 7.5±0.4$^a$ | 7.16-8.52 / 7.6±0.21$^a$ | 7.1-8.50 / 7.6±0.23$^a$ | 7.14-8.52 / 7.5±0.21$^a$ |
| $NH_3/NH_4$ (mg $L^{-1}$) | 0.009-0.03 / 0.002±0.007$^a$ | 0.0095-0.03 / 0.002±0.008$^a$ | 0.0098-0.03 / 0.002±0.008$^a$ | 0.01-0.03 / 0.002±0.007$^a$ | 0.095-0.03 / 0.023±0.017$^a$ |
| Salinity (‰) | 14.75-16.21 / 15.9±1.9$^a$ | 15.0-16.30 / 16.0±1.9$^a$ | 14.85-16.30 / 16.0±1.9$^a$ | 14.95-16.35 / 16.0±1.95$^a$ | 14.9-16.35 / 16.0±1.95$^a$ |

***Growth performance and feed efficiency***. The growth of Orange-spotted spinefoot was significantly different between the treatments (Table 4).

Table 4

Effect of methionine levels on weight gain, specific growth rate, food conversion ratio, and protein efficiency ratio of *Siganus guttatus*

| *Indicators* | *Treatments* | | | | | *p-value* |
|---|---|---|---|---|---|---|
| | *TM1* | *TM2* | *TM3* | *TM4* | *TM5* | |
| Initial body weight (IBW) (g) | 5.12±0.01 | 5.11±0.01 | 5.26 ±0.17 | 5.24±0.14 | 5.28±0.16 | 0.39 |
| Final body weight (FBW) (g) | 22.67±0.15[a] | 23.80±0.44[b] | 25.83±0.38[c] | 24.37±0.32[b] | 23.00±0.44[a] | <0.00 |
| Weight gain (WG) (%) | 343.09±6.7[a] | 365.44±7.94[b] | 391.69±13.0[c] | 365.24±13.7[b] | 335.51±13.3[a] | <0.001 |
| Specific growth rate (SGR) (% day-1) | 3.11±0.00[a] | 3.14±0.00[b] | 3.25±0.00[c] | 3.07±0.00[b] | 3.00±0.00[a] | <0.001 |
| Feed conversion ratio (FCR) | 1.867±0.02[c] | 1.762±0.03[ab] | 1.702±0.06[a] | 1.747±0.08[ab] | 1.845±0.06[c] | 0.018 |
| Protein efficiency ratio (PER) | 4.698±0.06[a] | 4.979±0.09[abc] | 5.159±0.18[c] | 5.027±0.22[bc] | 4.757±0.15[ab] | 0.02 |

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

The experimental results showed that, compared to the initial weight of the fish, the weight of the fish after the experiment increased significantly, and there was a difference between the experimental tanks corresponding to different levels of methionine. From Table 4, it can be seen that the growth rate of Orange-spotted spinefoot corresponding to different methionine-supplemented diets was different; TM5 gave the lowest WG (335.51%), followed by TM1, TM4, and TM2, respectively, and reached the highest in the TM3 diet, reaching 391.69%. When comparing TM3 and other treatments, we found a statistically significant difference ($p < 0.05$).

SGR values of Orange-spotted spinefoot also exhibited significant differences among treatments ($p < 0.05$). TM5 (8 g $kg^{-1}$ diet methionine) yielded the lowest SGR (3% $day^{-1}$), increasing from TM4 (3.07% $day^{-1}$) and reaching its highest value in TM3 (3.25% $day^{-1}$). After that, SGR tended to decrease from TM2 (3.14% $day^{-1}$) gradually, and the TM1 without methionine supplementation was 3.11% $day^{-1}$ (Table 3). TM5 gave the lowest SGR, in our opinion, because the methionine content (8 g $kg^{-1}$ diet methionine) in the diet exceeded the special growth needs of young Orange-spotted spinefoot. TM3 (4 g $kg^{-1}$ diet methionine) yielded the highest SGR in the experimental diets, as the feed quality had a suitable methionine content, fully meeting the special growth needs of *S. guttatus*. And when the fish reached the maximum SGR, it stopped increasing and began to decrease gradually again.

The value of the FCR was significantly lowest (1.702) in the methionine 4 g $kg^{-1}$ diet TM3 and differed statistically from all other treatments ($p < 0.05$). The diet without methionine exhibited the highest FCR (1.867). The efficiency of protein utilization in fish fed different diets showed statistically significant differences ($p < 0.05$). The diet without methionine yielded the lowest PER value of 4.698, followed by TM 2 (4.979). The PER value was highest in TM3 (5.159) and decreased in TM4 (5.027) and TM5 (4.757), respectively (Table 4).

The SR of the Orange-spotted spinefoot in diets with different methionine contents is shown in Figure 1.

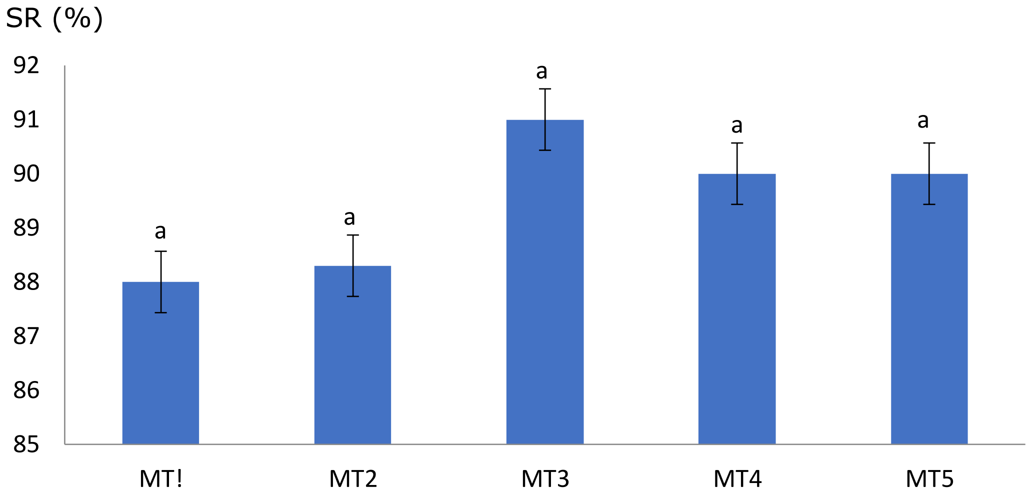


Figure 1. Effects of supplemental methionine levels in feed on the survival rate of Orange-spotted spinefoot fingerlings.

Fingerlings of Orange-spotted spinefoot fed with various methionine levels exhibited statistically similar survival rates, ranging from 88% to 91%, with the TM3 (4 g $kg^{-1}$ diet group) displaying the highest rate (91%). In comparison, the group without methionine supplementation exhibited the lowest rate (88%). Statistical analysis results showed no difference in SR between treatments ($p > 0.05$). Thus, the amount of methionine added to the food did not affect the SR of the experimental fish (Figure 1).

**Discussion**. In formulating feeds for aquaculture species, information on individual essential amino acid (EAA) requirements is crucial for replacing or complementing animal protein sources with plant-based sources to reduce costs (Stites et al 2022). Protein source and quality in amino acid profiles of diets are essential for maximizing fish growth (Ahmed & Khan, 2004; Binh et al 2022). Among the sulfur-containing amino acids (S), methionine and cysteine are notable; the relationship between these two amino acids is similar to the relationship between phenylalanine and tyrosine. For fish and aquatic animals, cysteine is a non-essential amino acid, and this amino acid can be synthesized from the essential amino acid methionine. If there is a deficiency of cysteine in the feed, part of the methionine is used for protein synthesis, and part is converted to cysteine. If the feed has enough cysteine, the methionine content can be reduced. Because of this relationship, fish have a greater need for the total sulfur-containing amino acids than for methionine alone. For this reason, many studies on methionine requirements for fish have been conducted. Methionine is an essential amino acid that is often lacking in the diet due to the use of plant protein sources (Lieber & Benkendorff 2007).

Incorporating dietary methionine at an adequate level can enhance growth performance (Santiago & Lovell 1988). In the current study, growth indices (WG% and SGR) improved when fish were fed diets with increased methionine levels and at a suitable methionine supplementation level of 4 g $kg^{-1}$ diet (TM3). 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 *S. guttatus*. WG of fish gradually increased at methionine levels of 4 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 $kg^{-1}$ and 8 g $kg^{-1}$ diet groups, respectively. Thus, supplementing the diet of Orange-spotted spinefoot fry with the appropriate amount of methionine will help the fish grow well. However, supplementing at a higher level (6 or 8 g kg diet$^{-1}$) will be wasteful because it does not increase the fish growth rate. The results of this study are similar to those of Binh et al (2024), who conducted a study to determine the methionine requirement in spotted scat fingerlings. Our research results are quite similar to those of Hien et al (2012), who found that increasing the methionine content too much in the feed reduces the growth of Striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings. Yan et al (2007) indicated that the Yellowband parrotfish fingerlings *(Scarus schelegeli)* exhibited increased growth when the methionine content in the diet increased, and decreased growth when the methionine content decreased. Yellowband parrotfish grew maximally when methionine levels in the diet were increased from 1.3 to 1.58%. The study by Wang et al (2016) showed similar results, indicating that the optimal methionine content for common carp *(Cyprinus carpio)* is 0.85%. If the methionine content increases beyond this level, the growth dynamics of the fish decrease. This result is similar to the study on spotted scat fingerlings, where the optimal methionine content in the diet is 4 g kg$^{-1}$; exceeding this level, fish growth decreases (Binh et al 2024).

To evaluate the correlation between the methionine content and fish growth rate, we conducted a regression analysis between the WG (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.

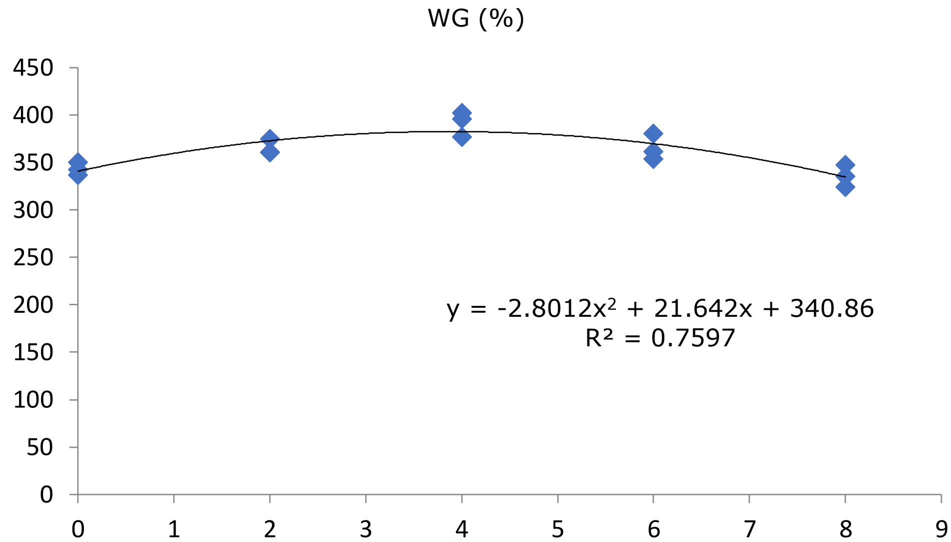


Figure 2. Correlation between methionine content (g kg$^{-1}$ CP) and weight gain.

The results of the linear equation analysis: Y = -2.8012X2 + 21.642X + 340.86 showed that the regression correlation between methionine content and WG had a very close correlation between the experimental rations with the value ($R^2$ = 0.7597 or R = 0.8706). The breaking point of the graph occurred at the methionine content of 4 g kg$^{-1}$ CP ($X_{max}$ g kg$^{-1}$ diet) and corresponded to the maximum growth rate of Orange-spotted spinefoot fingerlings at $Y_{max}$ = 391.69%.

Figure 2 also shows that the growth rate of Orange-spotted spinefoot fry in diets containing different methionine levels ranges from 335.51 to 391.69%, reaching its highest value in diets with methionine levels of 4 g kg$^{-1}$ CP, equivalent to 22.5 g kg$^{-1}$ CP or 8.5 g kg$^{-1}$ diet, as calculated. That means the optimal methionine requirement of Orange-spotted spinefoot fingerlings is 22.5 g kg$^{-1}$ CP or 8.5 g kg$^{-1}$ diet.

The results of the study on methionine requirements in Orange-spotted spinefoot are quite similar to those of spotted scat fingerling, as shown by Binh et al (2024). The methionine requirement for spotted scat fingerling is 24.4 g kg$^{-1}$ CP or 9.21 g kg$^{-1}$. This result is also quite similar to that of *P. hypophthalmus*, which was of 10.1 g kg$^{-1}$ diet (26.7

g $kg^{-1}$ CP) (Hien et al 2012). However, this methionine level is higher than the methionine level determined to be 170 g $kg^{-1}$ CP in Florida pompano *(Trachinotus carolinus)* (Corby et al 2024).

The amount of methionine supplemented in the diet also affects the PER, which is the WG of fish per unit of protein intake. PER varies with the amount and type of protein consumed, as well as the protein content of the feed (Hien et al 2012). The results of this study showed that the highest protein efficiency was achieved in the 4 g $kg^{-1}$ diet supplemented, reaching 5.159 g. Meanwhile, the diet without methionine supplementation had the lowest protein efficiency (4.698g) (Table 3). This result is similar to those reported on in some other fish species such as spotted scat by Binh et al (2024), on the Nile tilapia *(Oreochromis niloticus)* by Liebert & Benkendorff (2007) and, on the rainbow trout *(Oncorhynchus mykiss)* by Nguyen & Davis (2009) and on the striped catfish *(P. hypophthalmus)* by Hien et al (2012).

The FCR of Orange-spotted spinefoot between experimental diets with different methionine content was statistically significant ($p < 0.05$). The diet without methionine had the highest FCR (1.867), and the 4 g $kg^{-1}$ diet supplemented with methionine had the lowest FCR. However, when supplemented at 8 g $kg^{-1}$ diet, the FCR increased without any difference compared to the diet without methionine (TM1) (Table 4). This shows that supplementing a reasonable level of methionine will reduce FCR and increase the economic efficiency of farmed fish. This result is similar to that recorded in Redbelly tilapia *(Coptodon zillii)*: when the methionine content was 0.50% of the diet, the FCR decreased significantly, compared to other methionine levels (Polat 1999).

**Conclusions**. The methionine requirement of Orange-spotted spinefoot fingerlings was determined in this study. After an experimental period with different levels of methionine supplementation in the diet, 4.5 g to 12.5 g $kg^{-1}$ CP (11.9 to 42.9 g $kg^{-1}$ dry diet). 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 or 22.5 g $kg^{-1}$ dry diet. In contrast, the survival rates were not significantly affected. The results of the quadratic regression analysis between growth rate and dietary methionine showed that the optimal methionine content for *S. guttatus* fingerlings was 22.5 g $kg^{-1}$ CP or 8.5 g $kg^{-1}$ diet.

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

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