Dietary methionine improves feed efficiency, growth performance, body composition and digestive enzyme activity of catfish (*Pangasianodon hypophthalmus*) fingerlings

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Abstract. Nonoptimal feed efficiency is an issue caused by limiting methionine in the aquaculture of catfish. Therefore, feed formulated with soybean as a plant-based protein source will always result in a shortage of methionine. Dietary methionine supplementation is one of the efforts to solve this problem. The present study aimed to investigate the effect of methionine supplementation in feed diets on the efficiency of feed utilization, growth, body composition, and digestive enzyme activity of catfish (*Pangasianodon hypophthalmus*) fingerlings. The study used catfish fingerlings with an average weight of 1.38±0.12 g. Experimental diets were artificial diets containing 32% crude protein and 3% lipids, added with methionine at different levels: 0 (A), 0.45 (B), 0.90 (C) and 1.35 (D) g 100 g^{-1} diet. The study found that dietary methionine supplementation had a significant ($p<0.05$) effect on fish feed efficiency, growth, body protein content, and digestive enzyme activity, but had no impact on the survival rate of catfish fingerlings and amino acid profile. Methionine at the dose of 0.45 g 100 g^{-1} diet was the best dose for increasing feed efficiency, growth, and digestive enzyme activity of catfish fingerlings.
Key Words: artificial diets, protein, supplementation, survival rate.

Introduction. Catfish (*Pangasianodon hypophthalmus*) is a high economic value species of freshwater fish in Indonesia because of its high market demand and ease of culture (Rachmawati et al 2019). The availability of high-quality feed impacts the success of intensive catfish culture. A high-quality feed contains protein that meets the needs of the fish as well as a complete amino acid profile, which increases feed utilization efficiency and fish growth performance. A complete amino acid profile is necessary to promote fish growth (NRC 2011). A lack of amino acids reduces the efficiency of feed utilization and growth (Zhou et al 2020). An insufficiency of essential amino acids in the fish diet will have a negative impact on protein utilization, growth, and feed efficiency and may change the immune response to disease in fish (Wang et al 2016).

The current problem of catfish farmers is nonoptimal feed utilization due to a low methionine concentration in plant-based protein diets (Wang et al 2016). A fish feed with soybean ingredients results in a methionine shortage (He et al 2019). A low methionine content in fish feed reduces feed efficiency, growth, and digestive enzyme activity (Elesho et al 2021). Therefore, methionine should be supplemented in a fish diet to boost fish growth and ensure optimal fish health (He et al 2019). According to Martínez et al (2017), methionine is a primary amino acid required for the metabolism and structure of proteins. Furthermore, Brosnan & Brosnan (2006) reported that methionine is a sulfur amino acid (SAA) greatly needed as a biological methyl donor for forming nucleic acids and proteins in vertebrates. Low methionine in feed intake may decrease feed utilization, impair intestinal development, and hinder the growth of poultry (Zeng et al 2015), mammals (Castellano et al 2015), and fish (Tang et al 2009). Methionine is a vital amino acid that boosts fish lysozyme activity and immunoglobulin levels, influencing the antioxidant status of immune organs that regulate fish immunity (Kuang et al 2012), protein synthesis (He et al 2019), gene modification and endocrine pathways. It is also responsible for regulating appetite (Zhou et al 2020), fish growth and the immune response of several fish species (Gao et al 2019). Methionine requirement in the fish diet differs between and within species, based on fish size and growth stage (Fang et al 2020).

Several studies on dietary methionine supplementation have been conducted in several fish species, including *Cyprinus carpio* (Kuang et al 2012), *Oncorhynchus mykiss* (Fontagné-Dicharry et al 2017), *Scophthalmus maximus* (Gao et al 2019), *Rachycentron canadum* (He et al 2019), and *Ctenopharyngodon idella* (Fang et al 2020). Information about the influence of dietary methionine supplementation on catfish fingerlings is scarce. Therefore, this study was conducted to solve problems in catfish fingerling culture. The current study aimed to investigate the influence of methionine supplementation in an artificial diet on feed utilization efficiency, growth performance, nutritional composition of the body, and enzyme activity in the digestive tract of catfish fingerlings. This study uses the method of nutrient manipulation through methionine supplementation in an artificial diet to increase the efficiency of feed utilization and suppress feed cost to increase output.

Material and Method

Experimental fish preparation and research design. The present study was conducted in the Center for Freshwater Fish Culture and Hatchery (BBIAT) Ngrajek, Magelang, Central Java, Indonesia, for 56 days. The experimental fish were catfish fingerlings (average weight of 1.38+0.12 g fish^{-1}) obtained from the Center for Freshwater Fish Culture and Hatchery (BBIAT) Muntilan, Magelang, Central Java, Indonesia. Catfish fingerlings were selected based on being physically healthy, as indicated by active swimming activity, uniformity in size, free of disease, and without injuries (Rachmawati et al 2017). Catfish fingerlings were adapted to the environment and fed for one week prior to the experiment. During adaptation, catfish fingerlings were fed methionine-free diets using the *ad satiation* method three times daily at 7.00, 12.00, and 17.00. To remove metabolic waste from the fish body, the fish was starved for one day prior to the experiment (Rachmawati et al 2022).

The average weight of experimental fish was determined before beginning the experiment. The fish were maintained in 12 fiber tanks (1.5x1x1.5 m^3) filled with 30 L of water in a recirculation system. Water quality in the experimental unit, such as temperature (25-30°C), pH (6.5-8.6), dissolved oxygen (DO) ≥3 mg L^{-1}, and ammonia <1 mg L^{-1} were set based on Boyd (2003). This study used a completely randomized design (CRD) consisting of 4 treatments with three replications. 360 catfish fingerlings were divided randomly into four groups with a stocking density of 1 fish L^{-1} for each experimental unit. Feeding was applied to satiation with a feeding frequency (FR) of 3 times daily for 56 days, at 6.00, 12.00, and 16.00. To maintain water quality, removing uneaten feed was done before feeding. The weight gain and survival rate of experimental fish were determined weekly by weighing each fish and calculating fish biomass in each experimental unit.

Experimental diets preparation. Table 1 shows the experimental diet's ingredient composition and chemical composition determined by proximate analysis. The artificial diets contained 32% crude protein and 3% lipid, which were then supplemented with methionine at 0 (A), 0.45 (B), 0.9 (C), and 1.35 (D) g 100 g^{-1} diet. The HPLC analysis of methionine in each experimental diet resulted in a dose of 0.56 (A), 0.73 (B), 1.52 (C), and 2.26 (D) g 100 g^{-1} diet, as listed in Table 2. Methionine (L-Met100) was produced by PT Cheil Jedang Indonesia, Pasuruan, East Java, Indonesia. Experimental diets were manually prepared by mixing feed ingredients until homogenous and added with oil and water (10%). The pH of the experimental diet was adjusted to 7 with 6 N sodium hydroxide (NaOH) solution (Nose et al 1974). The mixed experimental diets were printed using a 2 mm extruder (Granulating Extruder, China) to be further air-dried at room temperature for 15 min. Dry feeds were placed in a plastic bag and stored at 18°C until use. The experimental diet was chemically analyzed using the AOAC (2019) method. The Kjeldahl method was used to determine the crude protein content (NANBEI, NB-KDN, Henan, China). Crude lipid was measured by extracting ether according to the Soxhlet method (ZZKD, RE-1002, Henan, China). Moisture content was determined with the vacuum oven method.

Table 1

Experimental diet ingredients and proximate analysis of experimental diets

Ingredient (g 100 g^{-1})	*Experimental diet*			
	A	*B*	*C*	*D*
Fish meal	18.76	18.76	18.76	18.76
Soybean meal	18.76	18.76	18.76	18.76
Corn meal	13.65	13.65	13.65	13.65
Gelatin	4.59	4.59	4.59	4.49
Mix of amino acids[1)]	15.40	15.40	15.40	15.40
Corn oil	4	4	4	4
Fish oil	4	4	4	4
Alpha-starch	5	5	5	5
Di-calcium phosphate	1	1	1	1
Mix of vitamins and minerals [2)]	4	4	4	4
Methionine	0	0.45	0.90	1.35
Glutamine	9.84	9.44	8.99	8.54
Methylcellulose	1	1	1	1
Total	100	100	100	100
Total methionine (g 100 g^{-1})	0.56	0.73	1.52	2.26
Proximate analysis				
Protein	32.08	32.13	32.14	32.10
Fat	3.05	3.06	3.10	3.12
Ash	8.62	8.75	8.69	8.73
Fiber	2.09	2.07	2.10	2.08
Dry matter	90.82	90.52	90.96	90.72
Gross energy (KJ g^{-1}) [3)]	18.47	18.52	18.38	18.63

Note: [1)] refer to Table 2; [2)] vitamin and mineral mix kg^{-1}: magnesium (Mg) 1900 mg, calcium (Ca) 219 mg, potassium (K) 150 mg, sodium (Na) 117 mg, selenium (se) 150 mg, vitamin A 36000 I.U., vitamin B1 52 mg, vitamin B2 97 mg, vitamin B6 46 mg, vitamin B12 60 mg, vitamin D3 9000 I.U., manganese (Mn) 105 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, vitamin C (coated) 68800 mg activity, zinc (Zn) 90 mg, iodine (KI) 1.8 mg, cobalt (Co) 450 mg, pantothenic acid 93 mg, niacin 130 mg, folic acid 10 mg, inositol 225 mg, biotin 450 mg, vitamin E 187 mg, vitamin K3 19 mg; [3)] total energy: protein = 4 kcal g^{-1}, lipid = 9 kcal g^{-1}, and carbohydrates = 4 kcal g^{-1} (NRC 2011).

Observed parameters. The parameters observed in this study consisted of fish growth parameters, including weight gain (WG), relative growth rate (RGR), feed efficiency expressed by the efficiency of feed utilization (EFU), feed conversion ratio (FCR), the protein efficiency ratio (PER), protein retention (PR), and survival rate (SR). As referred to the method of NRC (2011), each parameter was calculated based on the formula as follows:

WG (g) = final body weight (g) – initial body weight (g)

EFU (%) = (final weight – initial weight) / the weight of feed consumed x 100

RGR (%) =100 × (final weight - initial weight) / (times of experiment x initial weight)

FCR = nutrient intake (g) / weight (g)

PER = 100 × (final weight – initial weight) / the amount of diet consumed x protein content of the diet

PR = 100 x [total protein gain in the body of fish (g) / total protein intake (g)]

SR (%) = 100 (final count / initial count)

Amino acid profile of experimental diets and of fish. The amino acid composition of experimental diets was determined using an Amino Acid Analyzer. A 1 mg sample was hydrolyzed in 6N HCl at 110ºC for 22 h (Ju et al 2008). The sample was then sieved through a 0.2 mm filter before being injected into a High-Speed Amino Acid Analyzer with ion-exchange columns (4.6 x 150 mm) at 53ºC. A gradient system with sodium citrate buffer solutions at pH 3.3, 4.3, and 4.9 was used to separate amino acids at a flow rate of 0.225 mL min^{-1}. Post-column of amino acids was identified using ninhydrin reagent at a flow rate of 0.3 mL min^{-1} at 570 nm and 440 nm. The amino acid profile of the experimental diets is presented in Table 2.

Table 2

Amino acid profile of experimental diets (g 100 g^{-1} diet)

*Amino acid**	*Experimental diet*				*Pangasianodon hypopthalmus*
	A	*B*	*C*	*D*	
Lysine	1.97	2.35	2.67	3.23	2.268
Methionine	0.36	0.73	1.52	2.26	0.755
Arginine	1.20	1.49	1.64	1.82	1.447
Isoleucine	1.34	1.98	1.32	1.32	2.019
Threonine	1.15	1.37	1.28	1.28	1.358
Tryptophan	0.17	0.29	0.37	0.39	0.283
Valine	1.46	1.79	1.67	1.57	1.805
Histidine	0.74	0.86	0.92	0.98	0.841
Leucine	3.32	3.95	2.28	2.28	4.128
Phenylalanine	1.14	1.36	1.48	1.51	1.398

Note: * - NRC (2011).

Digestive enzyme activity. The digestive enzyme activity was observed by collecting fish intestine from 5 fish, which was then centrifuged at 6000 rpm for 20 min at 4°C. The supernatant was then used to determine lipase and amylase activity (Furne et al 2005) and trypsin and chymotrypsin (Hummel et al 1959).

Statistical analysis. The growth performance and feed utilization was analyzed using ANOVA. Then, Duncan's Multiple Range Test was used to identify specific differences between means (Steel et al 1997). The Polynomial Orthogonal Test was used to determine the optimum dose of methionine in the test feed (Ju et al 2008).

Results and Discussion. The survival rate (SR) of catfish fingerlings fed experimental diets for 56 days was not affected ($p>0.05$) by dietary methionine supplementation, as shown by 100% SR in all treatments (Table 3). However, it had a significant effect ($p<0.05$) on the catfish fingerling’s WG, EFU, RGR, FCR, PER, and PR.

Table 3

The growth performances of catfish (*Pangasianodon hypophthalmus*) fingerlings fed with experimental diets with methionine

Parameter	Experimental diet			
	A	*B*	*C*	*D*
Initial body weight (g)	1.38±0.12^{a}	1.40±0.10^{a}	1.36±0.1 ^{a}	1.38±0.14^{a}
Final bodyweight (g)	13.94±0.24^{d}	20.39±0.22^{a}	17.76±0.21^{b}	15.63±0.26^{c}
WG (g/fish)	12.10±0.10^{d}	18.99±0.13^{a}	16.40±0.15^{b}	14.26±0.14^{c}
EFU (%)	59.47±0.32^{d}	75.38±0.35^{a}	70.24±0.32^{b}	64.76±0.30^{c}
RGR (%/day)	2.01±0.10^{d}	3.52±0.18^{a}	3.07±0.13^{b}	2.48±0.16^{c}
FCR	1.98±0.15^{d}	1.32±0.11 ^{a}	1.65±0.12 ^{b}	1.80±0.12^{c}
PER	2.15±0.23^{d}	3.49±0.21^{a}	2.98±0.27^{b}	2.53±0.22^{c}
PR	52.64±0.35^{d}	68.37±0.31^{a}	60.26±0.32^{b}	57.53±0.37^{c}
SR (%)	100^{a}	100^{a}	100^{a}	100 ^{a}

Note: WG - weight gain; RGR - relative growth rate; EFU - feed efficiency (efficiency of feed utilization); FCR - feed conversion ratio; PER - protein efficiency ratio; PR - protein retention; SR - survival rate; different superscripts in the same row indicate statistical differences ($p<0.05$).

Fish fed with 0.45 g 100 g^{-1} diet methionine (experimental diet B) possessed a higher WG, EFU, RGR, PER, and PR, and also a lower FCR compared to the other treatments. The control exhibited a lower WG, EFU, PER, and PR and higher FCR ($p<0.05$) than the experimental diets. Table 4 showed that all treatment groups had no notable effect ($p>0.05$) on dry matter and ash content, but had a significant effect ($p<0.05$) on protein content and lipids of catfish fingerlings. The addition of methionine in the feed also did not significantly affect ($p>0.05$) the amino acid profile of the catfish fingerlings, as indicated in Table 5.

Table 4

Nutritional composition (g kg^{-1}) of catfish (*Pangasianodon hypophthalmus*) fingerlings after the experiment

Composition	Treatment				
	Initial	*A*	*B*	*C*	*D*
Dry matter	81.4±0.35^{a}	81.6±0.26^{a}	80.8±0.22^{a}	81.5±0.32^{a}	80.7±0.31^{a}
Crude protein	40.6^{c}±0.24^{e}	44.8±0.21^{a}	43.5±0.25^{b}	42.2±0.28^{c}	41.6±0.23^{d}
Crude lipid	13.2±0.27 ^{a}	11.7±0.32^{b}	11.8±0.25^{b}	11.7±0.34^{b}	11.6^{a}±0.26^{b}
Ash	18.84±0.34^{a}	18.22±0.23^{a}	18.37±0.25^{a}	18.30±0.22^{a}	18.29±0.24^{a}

Note: different superscripts in the same row indicate statistical differences ($p<0.05$).

Trypsin and amylase activity in the intestine of catfish fingerlings increased significantly ($p<0.05$) with the addition of methionine (up to 0.45 g 100 g^{-1} diet). However, there were no significant differences among treatments B, C and D. Chymotrypsin and lipase activity increased significantly ($p<0.05$) with increasing methionine doses in experimental diets (Table 6).

Dietary methionine supplementation increased WG, EFU, RGR, PER, and PR of catfish fingerlings ($p<0.05$), and FCR gradually decreased with methionine addition. These results indicated that catfish fingerlings could utilize methionine for growth effectively. Catfish fingerlings fed with a methionine-deficient diet (experimental diet A) exhibited a low WG, apparent digestibility of protein (ADCp), EFU, RGR, PER, and PR, while dietary methionine excess could not support further growth performance (experimental diet D). Moreover, the high values of WG, EFU, RGR, PER, and PR, and the lowest FCR were found in fish fed with experimental diet B. This occurred due to the similarity of the feeds' essential amino acid profiles. He et al (2019) mentioned that feed is considered suitable for fish if it contains a similar amino acid profile to that of the fish.

Table 5

Amino acid profile of catfish (*Pangasianodon hypophthalmus*) fingerling (g 100 g^{-1}) fed with experimental diets

Amino acid	*Treatment*			
	A	*B*	*C*	*D*
Essential amino acids (EAA)				
Lysine	5.74±0.18[a]	5.64±0.15[a]	5.68±0.10[a]	5.70±0.12[a]
Methionine	1.73±0.12[a]	1.76±0.05[a]	1.78±0.13[a]	1.75±0.04[a]
Arginine	3.96±0.19[a]	3.82±0.13[a]	3.78±0.24[a]	3.80±0.18[a]
Isoleucine	3.40±0.04[a]	3.39±0.22[a]	3.42±0.15[a]	3.45±0.10[a]
Threonine	2.54±0.14[a]	2.49±0.06[a]	2.53±0.04[a]	2.51±0.04[a]
Tryptophan	0.68±0.01[a]	0.64±0.03[a]	0.63±0.03[a]	0.66±0.03[a]
Valine	2.73±0.03[a]	2.78±0.08[a]	2.75±0.04[a]	2.72±0.03[a]
Histidine	1.43±0.04[a]	1.49±0.03[a]	1.52±0.02[a]	1.48±0.04[a]
Leucine	4.75±0.15[a]	4.68±0.09[a]	4.73±0.05[a]	4.70±0.11[a]
Phenylalanine	2.64±0.07[a]	2.73±0.04[a]	2.69±0.07[a]	2.70±0.07[a]
EAA	29.30±0.10	29.42±0.09	29.50±0.08	29.47±0.08
Non-essential amino acids (NEAA)				
Tyrosine	0.35±0.03[a]	0.32±0.02 [a]	0.29±0.04[a]	0.30±0.02[a]
Glycine	4.28±0.15[a]	4.30±0.23 [a]	4.32±0.17[a]	4.29±0.14[a]
Proline	3.87±0.20[a]	4.02±0.25 [a]	3.92±0.13[a]	4.10±0.22[a]
Alanine	4.63±0.06[a]	4.53±0.03 [a]	4.03±0.04[a]	4.01±0.06[a]
Serine	3.11±0.19[a]	3.08±0.04 [a]	3.15±0.02[a]	3.09±0.10[a]
Aspartate	6.42±0.26[a]	6.37±0.18 [a]	6.29±0.14[a]	6.35±0.17[a]
Glutamate	9.67±0.31[a]	9.54±0.12 [a]	9.58±0.26[a]	9.62±0.30[a]
NEAA	32.33±0.17	32.16±0.87	31.58±0.11	31.76±0.14
EAA/NEAA	0.91±0.01	0.91±0.02	0.93±0.01	0.93±0.01

Note: Different superscripts in the same row indicated statistical difference ($p<0.05$).

Table 6

Digestive enzyme activity in the intestine of catfish (*Pangasianodon hypophthalmus*) fingerlings fed methionine-supplemented diets at various doses

Digestive enzyme (U g^{-1} tissue)	*Treatment*			
	A	*B*	*C*	*D*
Trypsin	0.76±0.02[a]	1.32±0.04[b]	1.40±0.03[b]	1.42±0.06[b]
Chymotrypsin	5.86±0.43[a]	7.53±0.18[b]	7.66±0.15[c]	7.89±0.17[d]
Lipase	676±66[a]	1459±10[b]	1487±66[c]	1502±13[d]
Amylase	1562±12[a]	1826±10[b]	1828±11[b]	1829±14[b]

Note: different superscripts in the same row indicate statistical differences ($p<0.05$).

An increase in the efficiency of feed utilization is directly proportional to the increasing relative growth rate and in line with the decreasing FCR. Catfish fingerlings fed experimental diet B possessed the highest EFU (75.38±0.35%), followed by the highest RGR (3.52±0.18% day^{-1}) and the lowest FCR (1.32±0.11). According to Aliu & Omenogor (2021), feed efficiency is inversely proportional to feed conversion. A lower feed conversion incurs a more efficient feed utilization for body tissue development (growth). Nwanna et al (2012) stated that feed efficiency is directly proportional to fish body weight gain, and higher feed efficiency means a better feed utilization for growth. Higher protein intake, particularly methionine, in fish bodies will also increase amino acid deposition, protein retention, and energy retention to body protein. This will further reduce body fat accumulation and boost growth. Zeng et al (2015) reported that increased methionine concentration in the body due to increasing protein consumption could enhance energy retention as body protein, while energy retention as body fat will decrease and fish will grow faster. Dietary methionine supplementation is expected to improve fish appetite and produce less uneaten feed. Methionine contains sulfur that plays a role as a methyl group donor that is converted by S-adenosylmethionine (SAM) into homocysteine and used for protein synthesis (Zhou et al 2020). It is easier for fish to utilize feed for growth, thus increasing feed efficiency (Elesho et al 2021). An increase in fish growth is related to the fact that methionine supplementation in the diet will increase feed efficiency (Kuang et al 2012; Gao et al 2019; Fang et al 2020; Elesho et al 2021). The orthogonal polynomial test on EFU yielded a coefficient of determination R^2=0.8469, with the equation $Y=-26.407x^2 + 38.034x + 60.505$ (Figure 1). R^2 shows that 84.69% of the efficiency of feed utilization was influenced by methionine supplementation in artificial diets, while other factors had an influence of 15.31%. Following the equation, the optimal dose of methionine supplementation in an artificial diet would be 0.72 g 100 g^{-1} diet, which would produce a maximum EFU of 74.20% day^{-1}.

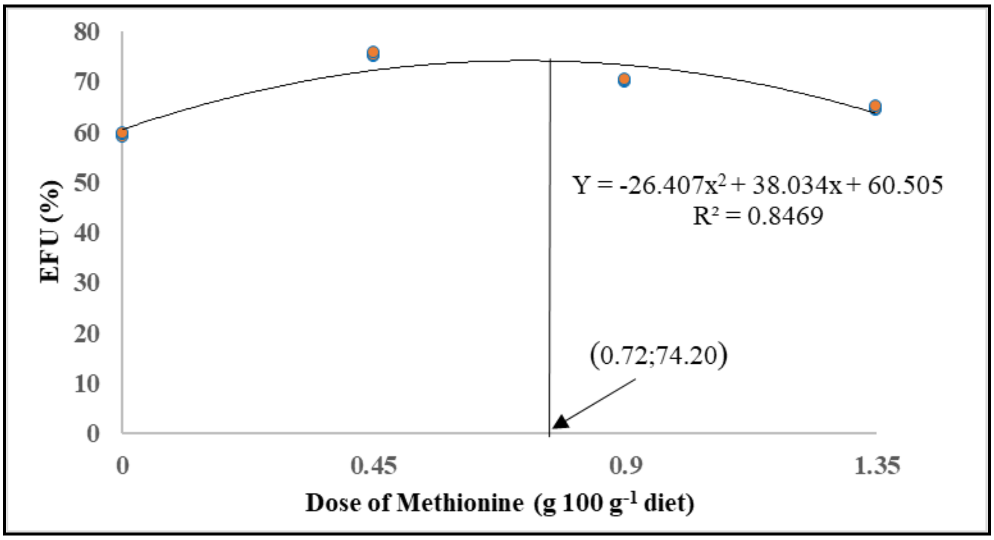

Figure 1. Correlation between dietary methionine supplementation and efficiency of feed utilization (EFU).

Methionine supplementation in an artificial diet at different doses significantly affected ($p<0.05$) the FCR of catfish fingerlings. The best FCR of 1.32±0.11 was obtained by catfish fingerlings fed experimental diet B, followed by C, D and A. Methionine was able to decline the FCR of catfish fingerlings, and also other fish such as cobia (*R. canadum*) (Wang et al 2016), African catfish (*Clarias gariepinus*) juveniles (Elesho et al 2021) and turbot (*S. maximus*) (Gao et al 2019). According to NRC (2011), the optimum FCR in fish ranges between 0.8-1.8. Therefore, FCR obtained from methionine supplementation in this study is considered good, within the optimum range. However, the FCR of fish given the diet without methionine supplementation exceeded the optimum range.

The supplementation of methionine exhibited better RGR. Methionine is the precursor of carnitine synthesis, which serves as a carrier for long-chain fatty acids into the mitochondrial membrane for the β-oxidation process of fatty acids (Zeng et al 2015). He et al (2019) mentioned that methionine addition in artificial diets successfully increased the formation of carnitine, thus enhancing the β-oxidation process of fatty acids to produce high energy in the form of $FADH_2$ and NADH for growth. High carnitine could also improve protein synthesis and increase protein in fish bodies for body tissue development. He et al (2019) reported that methionine is the precursor of carnitine synthesis in the liver. Espe et al (2008) found that carnitine increased the growth of *Salmo salar* and successfully increased protein synthesis and retention to promote body tissue development.

The present study showed that dietary methionine supplementation increased protein synthesis, thus increasing PER and PR, which eventually enhanced the growth of catfish fingerlings. The highest RGR was obtained in catfish fingerlings fed diet B (3.52±0.18), followed by diet C (3.07±0.13), diet D (2.48±0.16), and diet A (2.01±0.10). Catfish fingerlings fed experimental diet B possessed high RGR, since the dose given was under the requirement of catfish fingerlings to support growth. Based on the orthogonal polynomial test on RGR, the coefficient of determinant $R^2=0.8379$, with equation $Y = -2.5926x^2 + 3.7133x + 2.101$ (Figure 2) was obtained. R^2 shows that 83.79% of the relative growth rate was influenced by methionine supplementation in artificial diets, while the influence of other factors was 16.21%. Following the equation, the optimal dose of methionine supplementation in an artificial diet was 0.72 g 100 g^{-1} diet, which produced a maximum RGR of 3.43% day^{-1}.

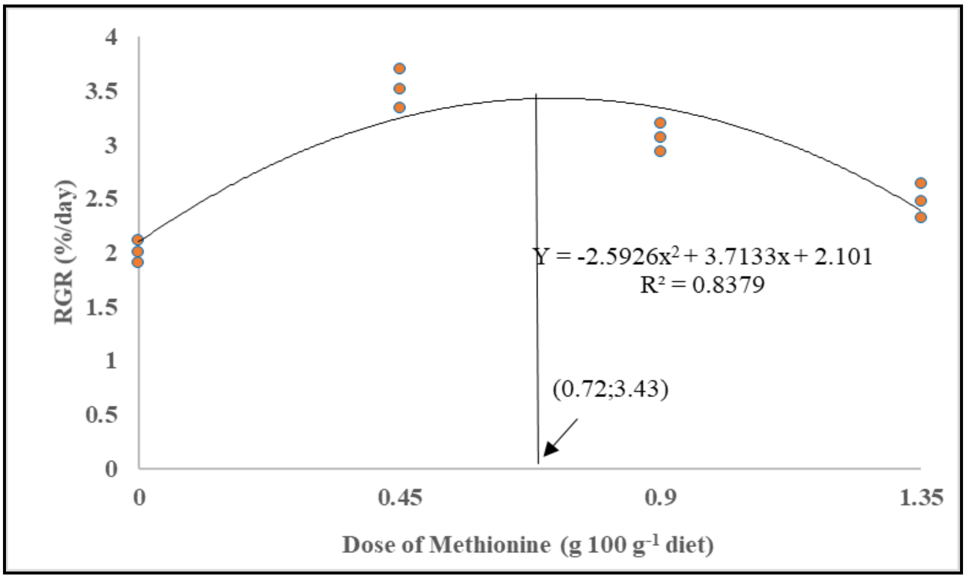

Figure 2. Correlation between dietary methionine supplementation and relative growth rate.

Dietary supplementation of methionine also influenced the body composition of catfish fingerlings. After being fed with methionine, the body composition of catfish fingerlings showed a linear decrease in whole body fat (lipid) along with the increasing dose of methionine in feed. Yet, it was inversely proportional to the increase in protein concentration in the whole body of catfish fingerlings due to dietary methionine supplementation. This is probably related to methionine function in body protein synthesis and lipid metabolism. Methionine should be present because it serves as a sulfur source and methyl group donor (Espe et al 2008) and plays an important role in lipid metabolism (Brosnan & Brosnan 2006). Methionine is an essential amino acid that plays a role in protein synthesis and other metabolic functions, ensuring normal fish growth (Alam et al 2001; Luo et al 2005; Zhou et al 2006; El-Wahab et al 2016).

Wen et al (2009) mentioned that digestive enzyme activity in the digestive tracks of fish could directly reflect fish digestibility. Dietary methionine supplementation significantly affected ($p<0.05$) the digestive enzyme activity of catfish fingerlings. The activity of trypsin, chymotrypsin, lipase, and amylase in the intestine of catfish fingerlings increased significantly with increasing doses of methionine in experimental diets. Supplementation of methionine in the diet is expected to improve the digestive enzyme activity of catfish fingerlings. The pancreas is assumed to secrete many digestive enzymes into the intestine, including protease, amylase, and lipase. A similar result was obtained in a study on adding glutamine and lysine to feed (Yan & Qiu-Zhou 2006; Zhou et al 2008).

Conclusions. This study concluded that methionine supplementation can improve feed efficiency, growth performance, body composition and digestive enzyme activity of catfish fingerlings. The dose of methionine of 0.45 g 100 g^{-1} is the best dose out of the tested doses to increase feed efficiency, growth, and digestive enzyme activity of catfish fry.

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

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