Impact of methionine in the diet on digestive enzyme activity, protein digestibility, feed utilization efficiency, growth, and body nutrient composition in the seed stage of catfish (*Pangasianodon hypophthalmus*)

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Abstract. The soybean-based feeds commonly employed as sources of vegetable protein exhibit restricted methionine content, resulting in an inherent deficiency of this amino acid. Such methionine deficiency has been associated with compromised feed efficiency, reduced growth rates, and diminished activity of digestive enzymes. The core objective of this investigation was to assess the impact of methionine supplementation in the diet on parameters including digestive enzyme activity, protein digestibility, feed utilization efficiency, growth performance, and the nutritional composition of catfish (*Pangasianodon hypophthalmus*) during its seed stage. The research focused on catfish during their seed stage, with an average weight of 18.36±0.18 g per fish. The experimental diet was specially designed with a protein content of 32% and a fat content of 5%, and methionine was added in increasing amounts of 0.45 g/100 g feed. These incremental doses, labeled as treatments A, B, C, and D, corresponded to methionine levels of 0, 0.45, 0.90, and 1.35 g/100 g feed, respectively. The results demonstrated significant correlations ($p < 0.05$) between methionine supplementation and various parameters, such as digestive enzyme activity, protein digestibility, feed utilization efficiency, growth, and the fish's body nutritional composition. Interestingly, the study found no significant impact ($p > 0.05$) of methionine supplementation on the survival rate of catfish during the rearing stage. Of notable significance, the best methionine dose was determined to be 0.90 g/100 g feed, as it yielded the most favorable outcomes in the seed stage.
Key Words: digestibility, enzyme, feed, nutrition.

Introduction. The successful intensification of catfish (*Pangasianodon hypophthalmus*) cultivation during the seed stage is intricately linked to the availability of nutritionally balanced feed (Rachmawati et al 2023a). This form of feed encompasses not only protein in accordance with the fish's requirements but also a comprehensive array of essential amino acids, vital for achieving optimal growth performance in fish, as outlined by NRC (2012). The significance of protein and amino acids as pivotal nutrients is underscored, influencing fish's physiological well-being, immunological status, and growth performance, as corroborated by Wang et al (2023). A deficiency of any essential amino acid in the diet can lead to reduced feed utilization efficiency and stunted fish growth, thereby affecting overall growth performance and the immune response to diseases (Zhou et al 2021).

A challenge faced by catfish (*P. hypophthalmus*) farmers in the seed fish stage pertains to suboptimal feed utilization efficiency in their cultivation practices. This issue can be traced to the inherent limitation in methionine amino acid content, particularly in soybean-based feed, commonly employed as a vegetable protein source (Wang et al 2016). Methionine is crucial in plant-based diets and significantly affects fish growth and feed efficiency (Gao et al 2019), with its scarcity often observed in artificial feeds utilizing soybean (He et al 2019). Notably, methionine deficiency within the feed has been conclusively linked to reduced feed efficiency, stunted growth, and diminished digestive enzyme activity in fish (Elesho et al 2021). Consequently, supplementing methionine in feed is essential for optimal fish growth and health (He et al 2019).

Methionine plays a vital role in protein metabolism and structure (Martínez et al 2017). It acts as a sulfur amino acid (SAA) and a biological methyl donor, essential for forming nucleic acids and proteins in vertebrates (Brosnan & Brosnan 2006). Insufficient methionine in feed can cause growth retardation, poor feed utilization, impaired gut development, and weakened antioxidant defenses in fish (Tang et al 2009).

Additionally, methionine enhances lysozyme activity, immunoglobulin content, and antioxidant status in fish, thereby modulating immune responses (Kuang et al 2012; Gao et al 2019). It also influences protein synthesis, growth regulation, and appetite control (He et al 2019). The methionine requirements for fish feed span from 0.49 to 2.0 g kg^{-1} feed, varying based on species, growth stage, and the form of methionine (Wang et al 2023).

Research on methionine supplementation in feed has been conducted across diverse fish species, including *P. hypophthalmus* fingerlings (Rachmawati et al 2023a), *Ctenopharyngodon idella* (Fang et al 2021), *Scophthalmus maximus* (Gao et al 2019), *Rachycentron canadum* (He et al 2019), and *Oncorhynchus mykiss* (Fontagné-Dicharry et al 2017). However, there is limited information on the impact of methionine on the feed in seed stage of catfish (*P. hypophthalmus*). Consequently, this research assumes paramount significance as it addresses this gap, aiming to decipher the impact of methionine supplementation in artificial feed on parameters such as protein digestibility, digestive enzyme activity, feed utilization efficiency, and growth of seed stadia catfish. The urgency of this research emanates from its role as a nutritional engineering strategy to enhance feed utilization efficiency and growth among seed fish stage catfish thereby fostering increased production efficiency.

Material and Method

Preparation of test fish. The study involved 360 seed stadia catfish each weighing an average of 18.36±0.18 g. These test fish were procured from the Freshwater Fish Farming Center (BBIAT) located in Muntilan, Magelang, Central Java, Indonesia. A seven-day acclimatization period was set, during which the test fish were introduced to the rearing environment and provided with artificial feed. This adjustment period was implemented to facilitate the fish's adaptation to the new feed source and its surrounding conditions. Throughout the adaptation phase, the fish were fed commercial feed three times daily, ensuring satiation. The subsequent selection of test fish was meticulous, focusing on achieving homogeneity in terms of size, absence of anomalies, intact body organs, and overall physical well-being, substantiated by active swimming behavior, as outlined by Rachmawati et al (2023b). On the day prior to the commencement of the study, the test fish were fasted. This step was undertaken with the intention of clearing any residual metabolic activity within the fish's body (Rachmawati et al 2022).

Experimental procedure and test feed formulation. The research was conducted over 63 days, from June to August 2023, in the Wet Laboratory of the Department of Aquaculture at Diponegoro University, Semarang, Central Java, Indonesia. A completely randomized design with four different methionine treatments was employed, each with three replicates. The composition of the test feed ingredients, as well as the outcomes of proximate analysis, are meticulously detailed in Table 1. The artificial feed used in this study had a crude protein content of approximately 32% and energy content of about 348 KJ g^{-1} (Jayant et al 2018). This base feed was supplemented with methionine at an incrementally escalating dosage, spanning 0.45 g/100 g feed, namely 0; 0.45; 0.90; and 1.35 g/100 g feed as treatments A, B, C and D respectively. The methionine content within each feed was ascertained through high-performance liquid chromatography (HPLC) analysis, yielding values of 0.61 g/100 g feed (A), 0.82 g/100 g feed (B), 1.65 g/100 g feed (C), and 2.38 g/100 g feed (D), as summarized in Table 1. The feed was manually blended to ensure uniformity, with oil and water (10%) added to the mix. The pH of the dough was adjusted to 7 using a 6 N sodium hydroxide solution (NaOH), adhering to the procedure detailed by Nose et al (1974). The dough was then processed using a granulating extruder (China) fitted with a 2 mm nozzle. The ensuing dough was subjected to air drying at room temperature for a duration of 15 minutes, following which the desiccated feed was packaged within plastic bags and stored at 18°C, awaiting utilization during the study period.

Table 1

Composition of test feed ingredients and proximate analysis results

Ingredient (g 100 g^{-1})	*Test feed*			
	A	*B*	*C*	*D*
Fish meal	26.54	26.54	26.54	26.54
Soybean meal	26.54	26.54	26.54	26.54
Corn meal	15.88	15.43	14.98	14.53
Gelatin	3.29	3.29	3.29	3.29
Mix of amino acid[1)]	14.25	14.25	14.25	14.25
Corn oil	4	4	4	4
Fish oil	4	4	4	4
Mix of vitamin-mineral[2)]	4	4	4	4
Methionine	0	0.45	0.90	1.35
Methylcellulose	1	1	1	1
Cr_2O_3	0.5	0.5	0.5	0.5
Total	100	100	100	100
Total methionine	0.61	0.82	1.65	2.38
Proximate analysis				
Protein (%)	32.12	32.09	32.08	32.09
Fat (%)	4.16	4.14	4.16	4.15
Ash (%)	7.58	7.63	7.49	7.68
Fiber (%)	3.05	3.11	3.09	3.10
Dry matter (%)	89.63	89.70	89.59	89.74
Gross energy (KJ g^{-1})[3)]	18.74	18.68	18.46	18.59

Notes: [1)] Refer to Table 2; [2)] Vitamin and mineral mix kg^{-1}: sodium (Na) 117 mg, manganese (Mn) 105 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, pantothenic acid 93 mg, Vit. C (coated) 68,800 mg activity, Zinc (Zn) 90 mg, iodine (KI) 1.8 mg, niacin 130 mg, folic acid 10 mg, inositol 225 mg, biotin 450 mg, Vit. E 187 mg, Vit. K3 19 mg, selenium (Se) 150 mg, Vit. A 36,000 I.U., Vit. B1 52 mg, Vit. B2 97 mg, magnesium (Mg) 1.900 mg, calcium (Ca) 219 mg, potassium (K) 150 mg, Vit. B6 46 mg, Vit. B12 60 mg, Vit. D3 9,000 I.U., cobalt (Co) 450 mg; [3)] Total energy:protein = 4 kcal g^{-1}, lipid = 9 kcal g^{-1}, and carbohydrates = 4 kcal g^{-1} (NRC 2012).

Feed digestibility evaluation. Adapted seed stage of catfish, initially weighing 18.36±0.18 g were systematically placed into fiber tanks filled with 30 liters of water, achieving a stocking density of one fish per liter. Over a span of 20 days, the catfish seed stage were offered the test feed at satiation levels, distributed at 07:00, 12:00, and 17:00 WIB (Western Indonesian Time). Commencing on the seventh day after the initiation of test feed consumption, the collection of fish feces was commenced. The feed was supplemented with Cr_2O_3, serving as a digestibility indicator. Fish feces were diligently collected one-hour post-feeding, employing a pipette and sipon hose. These collected fecal samples were then securely deposited within appropriately labeled film bottles and refrigerated to ensure preservation. Subsequent analysis encompassed proximate composition and Cr_2O_3 content assessment.

Experimental culture conditions. Test fish, initially weighing 18.36±0.18 g, were carefully acclimatized, and subsequently introduced into fiber tanks, following a randomized stocking approach, with each tank accommodating 20 fish. The feeding regimen involved providing the test feed to the catfish seed stage fry at satiation levels, administered at 08:00, 13:00, and 18:00 WIB (Western Indonesian Time), consistently maintained over a duration of 63 days. Regular maintenance encompassed the practice of sprinkling the tanks and renewing water, accounting for approximately 30% of the rearing medium's volume. The morning renewal of water preceded the feeding sessions.

The water quality parameters adhered to Boyd's standards (2003), stipulating temperature levels within the range of 25-30°C, pH maintained at 6.5-8.6, dissolved oxygen (DO) levels exceeding 3 mg L^{-1}, and ammonia levels below 1 mg L^{-1}. Progress of the test fish was meticulously monitored through weekly weighing, wherein the test fish were assessed for weight gain, and their biomass within each experimental unit was calculated. Any occurrences of mortality were duly documented, detailing both the number and weight of affected fish. The cumulative feed consumption throughout the rearing period was determined by measuring the weight of feed before and after each feeding event.

Proximate, amino acid analysis procedures. The proximate analysis procedure for both the test feed and fish carcasses was conducted in accordance with the AOAC (2005) method. Protein content was determined using a semi-automatic Kjeldahl system (FOSS Kjeltec 2300). Fat content was measured employing an ether extraction method based on the Soxhlet technique (FOSS Soxtec 2043). Ash content was determined by incinerating the test feed and fish samples in a furnace maintained at 550°C for 24 hours.

Amino acid analysis was executed employing an Amino Acid Analyzer (LA8080, Hitachi High-Tech-Japan), following a precise protocol. The procedure entailed weighing the sample, approximating ±1 mg, and subjecting it to hydrolysis with 6N HCl at 110ºC over a span of 22 hours. The resulting hydrolysate was then filtered through a 0.2 mm filter and introduced into a High-Speed Amino Acid Analyzer equipped with an ion exchange resin column measuring 4.6 x 150 mm, operated at a temperature of 53°C. Amino acid separation was achieved using a gradient system employing sodium citrate buffer solutions at pH 3.3, pH 4.3, and pH 4.9, at a flow rate of 0.225 mL min^{-1}. Post-column derivatization was performed with a ninhydrin solution at a flow rate of 0.3 mL min^{-1}, enabling the identification of individual amino acids at wavelengths of 570 nm and 440 nm, following the methodology described by Ju et al (2008). The comprehensive amino acid profile of the test feed is meticulously presented in Table 2.

Table 2

Amino acid composition of test feed (g/100 g feed) employed throughout the study

Amino acid	*Test feed*				*P. hypophthalmus**
	A	*B*	*C*	*D*	
Lysine	1.86	2.10	2.28	3.17	2.268
Methionine	0.59	0.61	0.79	1.25	0.755
Arginine	1.02	1.16	1.49	1.82	1.447
Isoleucine	1.37	1.52	2.03	2.47	2.019
Threonine	1.18	1.22	1.36	1.73	1.358
Tryptophan	0.14	0.17	0.27	0.48	0.283
Valine	1.26	1.35	1.79	2.16	1.805
Histidine	0.57	0.63	0.85	0.93	0.841
Leucine	3.12	3.50	4.15	4.62	4.128
Phenylalanine	1.15	1.30	1.38	1.54	1.398

Note: * NRC (2012).

Methodology and analytical techniques. The analysis and assessment of various research parameters were carried out employing established analytical methodologies. The methodologies utilized were as follows: proximate analysis encompassed the determination of water content, protein, fat, crude fiber, ash, and extract material without nitrogen. These determinations were conducted in accordance with the guidelines outlined in AOAC (2005). Quantitative analysis of Cr_2O_3 content within both the feed and fecal samples followed the procedures delineated by Takeuchi (1988). The measurement of digestive enzyme activity involved distinct methodologies for individual enzymes: protease activity assessment adhered to the method presented by Bergmeyer et al (1983). Amylase activity was determined following the protocol established by Worthington (1993). Lipase activity measurement was performed in accordance with the methodology detailed by Borlongan (1990). The concentration of soluble protein within the samples was quantified utilizing the Bradford method, as described by Bradford (1976). Relative growth rate (RGR), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR) were computed employing the methodology prescribed by NRC (2012). Total digestibility (TD), protein digestibility (PD), and energy digestibility (ED) were evaluated in accordance with the Watanabe (1995) method. Each parameter was calculated using the following formulae:

WG (g) = final body weight (g) - initial body weight (g)
FUE (%) = final weight - initial weight / the weight of feed consumed x 100
FCR = feed intake (g) / body weight gain (g)
PER = 100 × (final weight - initial weight) / the amount of diet consumed x protein content of diet
RGR (%) = 100 × (final weight - initial weight) / (times of experiment x initial weight)
SR (%) = 100 x (final count / initial count)
TD = 100 x (1 - Cr_2O_3 indicator level in feed / Cr_2O_3 indicator level in feces)
PD = 100 x [1-(protein content in feed / protein content in feces)]
ED = 100 x [1-(carbohydrate content in feed/carbohydrate content in feces]

Results. The digestive enzyme activities within the gastrointestinal tract of catfish seed stage, nourished with varying methionine dosages, are outlined in Table 3. The activities of trypsin and amylase demonstrated a noteworthy escalation ($p < 0.05$) upon the inclusion of methionine within the diet. However, there was no noticeable difference among the methionine doses of 0.45, 0.90, and 1.35 g/100 g feed. In contrast, the activities of chymotrypsin and lipase displayed a substantial enhancement ($p < 0.05$) corresponding to the increasing dose of methionine in the test diet.

Table 3
Digestive enzyme activity in the intestinal tract of catfish seed stage fed diets with varied methionine dosages

Digestive enzymes (U/g tissue)	*Test feed*			
	A	*B*	*C*	*D*
Trypsin	$0.82±0.14^{b}$	$1.43±0.16^{a}$	$1.49±0.12^{a}$	$1.45±0.16^{a}$
Chymotrypsin	$4.65±0.13^{d}$	$7.08±0.14^{b}$	$7.62±0.16^{a}$	$6.57±0.14^{c}$
Lipase	$569±43^{d}$	$1502±14^{b}$	$1583±27^{a}$	$1402±16^{c}$
Amylase	$1423±10^{b}$	$1748±22^{a}$	$1752±20^{a}$	$1739±10^{a}$

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

The addition of methionine into catfish feed has been observed to enhance feed digestibility, as evidenced by the outcomes depicted in Table 4. Among the experimental groups, the most elevated values for total digestibility, energy digestibility, and protein digestibility were recorded in the seed stadia catfish that were nourished with test diet C (0.90 g/100 g feed), signifying a significant differentiation ($p < 0.05$) from the other treatments.

Table 4
Feed digestibility values of seed stadia catfish nourished with the test diet over a 63-day period

Parameter (%)	*Test feed*			
	A	*B*	*C*	*D*
Total digestibility	$59.14±0.25^{d}$	$68.32±0.22^{b}$	$74.18±0.24^{a}$	$63.21±0.22^{c}$
Energy digestibility	$50.27±0.18^{d}$	$64.47±0.12^{b}$	$70.78±0.12^{c}$	$58.62±0.13^{c}$
Protein digestibility	$64.31±0.15^{d}$	$70.72±0.16^{b}$	$76.52±0.14^{a}$	$69.86±0.16^{c}$

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

Furthermore, an enhancement in protein digestibility exhibits a substantial augmentation in amino acid digestibility (Table 5). The data presented in Table 5 elucidates that both essential and non-essential amino acids demonstrated a significant increase in the test feed containing methionine, in comparison to the feed without this addition.

Table 5

Amino acid profile of the seed stage catfish body following nourishment with the test diet throughout the study

Amino acid	*Treatment*			
	A	*B*	*C*	*D*
	Essential amino acid (EAA)			
Lysine	3.64±0.07^{b}	4.32±0.04^{a}	4.42±0.06^{a}	4.40±0.05^{a}
Methionine	1.38±0.08^{b}	2.74±0.06^{a}	2.84±0.07^{a}	2.70±0.07^{a}
Arginine	2.12±0.12^{b}	3.47±0.14^{a}	3.48±0.14^{a}	3.48±0.13^{a}
Isoleucine	1.42±0.06^{b}	2.63±0.05^{a}	2.64±0.03^{a}	2.65±0.02^{a}
Threonine	0.93±0.12^{b}	1.74±0.15^{a}	1.77±0.13^{a}	1.77±0.14^{a}
Tryptophan	0.15±0.04^{b}	0.83±0.03^{a}	0.83±0.03^{a}	0.82±0.03^{a}
Valine	1.72±0.05^{b}	2.83±0.04^{a}	2.88±0.03^{a}	2.84±0.04^{a}
Histidine	0.42±0.03^{b}	1.42±0.02^{a}	1.44±0.03^{a}	1.40±0.02^{a}
Leucine	2.72±0.12^{b}	3.83±0.12^{a}	3.86±0.14^{a}	3.83±0.15^{a}
Phenylalanine	2.18±0.10^{b}	3.42±0.13^{a}	3.42±0.12^{a}	3.40±0.12^{a}
	Non-essential amino acid (NEAA)			
Tyrosine	0.22±0.03^{b}	0.76±0.04 ^{a}	0.77±0.03^{a}	0.77±0.03^{a}
Glycine	2.98±0.13^{b}	3.85±0.14 ^{a}	3.86±0.12^{a}	3.85±0.13^{a}
Proline	2.58±0.12^{b}	3.87±0.18 ^{a}	3.88±0.16^{a}	3.86±0.16^{a}
Alanine	2.94±0.14^{b}	3.67±0.12^{a}	3.69±0.14^{a}	3.67±0.14^{a}
Serine	2.76±0.06^{b}	3.97±0.03^{a}	3.97±0.05^{a}	3.98±0.05^{a}
Aspartate	3.85±0.12^{b}	4.88±0.12^{a}	4.88±0.13^{a}	4.87±0.12^{a}
Glutamate	6.78±0.18^{b}	7.54±0.15^{a}	7.55±0.14^{a}	7.54±0.13^{a}
Amino acid count	38.79±0.11^{b}	55.77±0.11^{a}	56.18±0.12^{a}	55.83±0.12^{a}
Average	2.28±0.06^{b}	3.28±0.06^{a}	3.25±0.07^{a}	3.28±0.07^{a}

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

The findings obtained from the investigation, as outlined in Table 6, demonstrate that the application of the experimental feed throughout the 63-day period significantly affects WG, FUE, RGR, FCR, and PER ($p < 0.05$). However, the influence on the SR of catfish in the seed stage shows no statistical significance ($p > 0.05$), as indicated by the consistent SR value of 100% across all treatments.

Table 6

Growth parameters (WG, RGR) and FUE, FCR, PER, alongside survival rate of seed stage catfish nourished with varied methionine contents in the test diets throughout the study

Parameters	*Test feed*			
	A	*B*	*C*	*D*
Initial body weight (g)	18.36±0.18^{a}	18.37±0.20^{a}	18.35±0.16 ^{a}	18.36±0.18^{a}
Final bodyweight (g)	56.74+0.14^{d}	65.38 +0.13^{b}	70.52+0.12^{a}	62.28+0.13^{c}
WG (g fish^{-1})	38.38±0.16^{d}	47.01+0.16^{b}	52.17+0.14^{a}	43.92+0.11^{c}
FUE (%)	58.26±0.18^{d}	74.52±0.21^{b}	82.23±0.18^{a}	70.67±0.16^{c}
RGR (% day^{-1})	3.31±0.21^{d}	4.05±0.22^{b}	4.49±0.21^{a}	3.79±0.23^{c}
FCR	1.85±0.23^{d}	1.48±0.20^{a}	1.12±0.20^{a}	1.63±0.22^{c}
PER	2.67±0.15^{d}	3.86±0.14^{b}	4.28±0.10^{a}	3.47±0.12^{c}
SR (%)	100^{a}	100^{a}	100^{a}	100 ^{a}

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

The experimental fish fed with feed containing 0.90 g/100 g of methionine (test feed C) displayed significantly higher values of WG, FUE, RGR, PER, and concurrently lower FCR compared to fish fed with alternative treatments ($p < 0.05$). Conversely, fish fed with feed lacking methionine (test feed A) demonstrated notably lower WG, FUE, PER, and higher FCR values compared to other experimental feed groups, indicating a significant difference ($p < 0.05$) throughout the study period.

The outcomes from the proximate analysis of the fish's body after being fed diets with varying methionine doses (Table 7) showed no significant impact ($p > 0.05$) on dry matter and ash content. However, a noticeable effect ($p < 0.05$) was observed on the protein and fat content in the body composition of the seed stage catfish.

Table 7

Body chemical composition (g kg^{-1}) of seed stage catfish following the study

Composition	*Test feed*				
	Initial	*A*	*B*	*C*	*D*
Dry matter	79.5 ± 0.15^{a}	79.6 ± 0.15^{a}	79.4 ± 0.16^{a}	79.6 ± 0.14^{a}	79.5 ± 0.15^{a}
Crude protein	42.4 ± 0.16^{a}	48.4 ± 0.10^{b}	48.5 ± 0.12^{b}	48.8 ± 0.11^{b}	48.6 ± 0.12^{b}
Crude lipid	10.6 ± 0.12^{a}	7.3 ± 0.10^{b}	7.4 ± 0.11^{b}	7.4 ± 0.12^{b}	7.3 ± 0.10^{b}
Ash	16.38 ± 0.10^{a}	16.39 ± 0.12^{a}	16.39 ± 0.11^{a}	16.38 ± 0.10^{a}	16.38 ± 0.10^{a}

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

Discussion. The introduction of distinct dosages of methionine into the feed exhibited a marked and statistically significant influence on the digestive enzyme activity within seed stage catfish ($p < 0.05$). This is in alignment with the assertion that the enzymatic activity in the fish's digestive tract serves as a direct indicator of its digestive ability (Wen et al 2009). The outcomes presented in Table 3 underscore a discernible elevation in trypsin, chymotrypsin, lipase, and amylase activities in the digestive tract of seed stadia catfish as the methionine dosages in the test feed increased. This observed phenomenon suggests a potential positive modulation by methionine, which could stimulate heightened secretion of digestive enzymes from the pancreas into the intestine. These findings concur with analogous studies conducted by Kuang et al (2012) on *Cyprinus carpio*, El-Wahab et al (2016) on *Oreochromis niloticus*, and Elesho et al (2021) on *Clarias gariepinus*.

The intricate relationship between enzyme presence, digestive enzyme activity levels within the fish's digestive tract, and feed digestibility is well-established (Liao et al 2015). Notably, in comparison to the test feed devoid of methionine supplementation (test feed A), the seed stage catfish fed with methionine-supplemented feed displayed higher total energy, and protein digestibility values (Table 4). Of particular significance, treatment C (0.90 g/100 g feed) exhibited the highest total digestibility value at 74.18±0.24 (Table 4), affirming that the seed stadia catfish could digest 70.78±0.12% of the ingested nutrients, while the energy digestibility value shows the amount of carbohydrate, fat and protein feed that can be digested by fish. Additionally, treatment C also yielded the highest protein digestibility value at 76.52±0.14%, signifying its positive impact on protein assimilation, thereby contributing to enhanced growth. These results align with research on other fish species (Zhou et al 2006; Fontagné-Dicharry et al 2017; Fang et al 2021).

Methionine supplementation positively impacted parameters such as WG, FUE, RGR, and PER, while decreasing FCR. The increased growth of the test fish in this study proves that the addition of methionine in feed increases feed intake and feed utilization efficiency. Comparatively, the test fish group exposed to feed without added methionine (test feed A) exhibited notably lower values for WG, ADCp, FUE, RGR, and PER. Interestingly, an excess of methionine within the feed (test feed D) did not engender further growth improvements. The highest values for WG, FUE, RGR, PER and the lowest FCR were consistently attained by the test fish fed with methionine-supplemented feed at the dosage of 0.90 g per 100 g feed (test feed C). The significant growth performance observed may be ascribed to the essential amino acid composition of the experimental feed closely resembling that of the tested fish species (as depicted in Table 2). This correlation is consistent with the findings posited by He et al (2019), who asserted that an optimally growth-stimulating diet should closely mimic the amino acid composition of the cultured fish.

The introduction of methionine into the feed is believed to enhance fish appetite, consequently minimizing feed wastage. The amplified growth of fish observed in the present study serves as an indication that methionine supplementation within the feed enhances FUE. As elucidated by the outcomes presented in Table 6, the experimental group fed with test feed C (0.90 g per 100 g feed) demonstrated the highest FUE value, registering at 82.23±0.18%, concurrently with the most pronounced RGR value of 4.49±0.21% day^{-1}. This phenomenon can be attributed to the sulfur-containing nature of methionine, where sulfur acts as a methyl group donor, leading to its conversion by S-adenosylmethionine (SAM) into homocysteine. This resultant homocysteine is subsequently employed in protein synthesis, enabling fish to harness feed nutrients more effectively for growth, thereby accentuating FUE (Elesho et al 2021). Supporting these findings, Nwanna et al (2012) suggested a direct relationship between feed efficiency values and fish body weight gain, indicating that higher feed efficiency reflects optimal feed utilization for growth. Similar research outcomes have been documented in studies involving various fish species such as *S. maximus* (Gao et al 2019), *C. gariepinus* (Elesho et al 2021), *O. mykiss* (Fontagné-Dicharry et al 2017) and *P. hypophthalmus* fingerlings (Rachmawati et al 2023a).

The assessment of FUE reveals an inverse correlation with feed conversion, where a lower feed conversion value indicates more efficient feed utilization for growth, as emphasized by Aliu & Omenogor (2021). This perspective aligns with the outcomes of our study, wherein fish fed with test feed C (0.90 g per 100 g feed) exhibited the highest FUE value at 82.23±0.18%, alongside the lowest FCR value of 1.12±0.20 and the highest RGR value of 4.49±0.21% day^{-1} (Table 6). According to NRC (2012), an optimal FCR value for fish falls within the range of 0.8-1.8. Thus, the FCR value attained in this study remains favorable, as it resides within the recommended optimal range. Consistent research outcomes have been documented by Elesho et al (2021) regarding *C. gariepinus*, Gao et al (2019) pertaining to *S. maximus*, and He et al (2019) in the context of *R. canadum*, aligning with the results of the present investigation. Furthermore, this study's results underscore the enhancement of PER within seed stadia catfish due to the incorporation of methionine into the feed. Comparable findings have been reported in various fish species, including *O. niloticus* (El-Wahab et al 2016) and *C. carpio* (Zhou et al 2021).

The outcomes of the study unequivocally evidenced the favorable acceptance of all test feed treatments by the seed stage catfish, with a notable absence of mortalities throughout the 63-day duration. The data presented in Table 6 corroborate this finding by revealing a lack of statistically significant differences ($p > 0.05$) in fish SRs. This is further substantiated by the SR of 100% across all seed stage catfish cohorts subjected to the test diet, indicating that the incorporation of methionine within the diet exerts no discernible impact on fish survival. This outcome aligns with analogous research findings reported by Wu et al (2017), Wang et al (2016), Tang et al (2009).

Analysis of the seed stadia catfish's body composition following consumption of feed enriched with varying methionine doses (refer to Table 7) revealed a consistent decrease in whole-body fat levels corresponding to higher methionine dosage levels in the feed. Conversely, this reduction was inversely proportional to the increase in protein concentration in the fish's bodies, suggesting a positive association with methionine inclusion in the feed. Augmented protein intake, particularly methionine, can promote increased amino acid deposition, protein retention, and energy retention towards body protein. These cumulative effects lead to reduced body fat accumulation, thereby stimulating growth. Previous research by Wang et al (2023) has indicated that elevated methionine concentrations in fish bodies, resulting from increased protein intake, enhance energy retention as body protein while reducing energy retention as body fat, thereby facilitating accelerated growth. The indispensability of methionine as an essential amino acid for protein synthesis (Espe et al 2008) and lipid metabolism (Brosnan &

Brosnan 2006) in fish is also underscored by this study's findings. The elevation in body protein content observed among the test fish in this study correlated positively with an increased PER. The fish group fed with 0.90 g/100 g feed methionine supplementation (test feed C) demonstrated the most substantial increments in protein digestibility, closely followed by the highest enhancement in FUE in comparison to other test feeds. Similar outcomes have been documented in studies involving *O. niloticus* (El-Wahab et al 2016), *C. carpio* var. Jian (Xiao et al 2010), and *O. mykiss* (Fontagné-Dicharry et al 2017).

Conclusions. The study's findings unequivocally demonstrate the substantial impact ($p < 0.05$) of supplementing methionine into soybean-based feed, a prominent source of vegetable protein. This supplementation is shown to exert pronounced effects on a multitude of parameters, including growth rates, feed utilization efficiency, digestive enzyme activity, protein digestibility, and the overall nutrient composition of catfish during the seed stage. Remarkably, the best dose of methionine is established at 0.90 g per 100 g of feed in the seed stage of catfish.

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