

Optimizing red tilapia (*Oreochromis* sp.) aquaculture in peatlands: evaluating dietary methionine and lysine for growth performance and feed utilization

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Abstract. This study aimed to optimize the effects of dietary supplements of lysine (L) and/or methionine (M) on the feed utilization and growth performance of red tilapia (*Oreochromis* sp.) fingerlings cultivated in a peatland pond. For that purpose, an 8-week growth trial was conducted to evaluate four isolipidic (9.52%) and isonitrogenous (30.1%) practical diets, which comprise nearly 70% protein derived from plant sources, 25% from fish meal, and 5% from head shrimp meal. The methionine and lysine supplement varied as follows: 0% methionine and 0% lysine (ML1), 0.2% methionine and 0% lysine (ML2), 0.2% methionine and 0.3% lysine (ML3) and 0.2% methionine and 0.5% lysine (ML4) kg⁻¹ feed diet. The study systematically evaluated initial body weight (IBW), final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), efficiency of feed utilization (EFU), protein efficiency rate (PER), and survival rate (SR) across four treatment groups (ML1, ML2, ML3, ML4). The findings indicate significant differences in FBW, WG, SGR, and PER according to the methionine and lysine levels, but IBW, FCR, EFU, and SR did not change statistically between treatments (p < 0.05). The ML3 group exhibited the highest performance metrics, recording 16.85±0.42 g (FBW), 153.73±10.25% (WG), 1.66±0.07% day⁻¹ (SGR), and 34.01±1.57 g (PER), indicating the significance of optimizing amino acid concentrations in aquaculture diets to improve growth and feed efficiency in red tilapia.

Key Words: amino acids, feed utilization, growth performance, lysine, methionine, red tilapia.

Introduction. Aquaculture in peatland areas presents distinct challenges and opportunities due to the unique environmental conditions associated with peat soils, such as low pH, high organic matter content, and fluctuating nutrient dynamics. The interaction between aquaculture and the surrounding peatland environment necessitates careful consideration of species selection and management practices to ensure ecological balance and sustainability (Anamulai et al 2019). Peatland aquaculture, particularly the cultivation of red tilapia (*Oreochromis* sp.), has emerged as a significant sector due to the species resilience and adaptability to various environmental conditions (Sugiani et al 2019). Red tilapia is recognized for its rapid growth, high production yield, and robust disease resistance, making it one of the most farmed fish species worldwide (Taparhudee et al 2024). However, the nutrient-poor nature of peatland soils presents significant challenges to fish growth and productivity (Miettinen et al 2012). Another consideration is that the

aquaculture industry has been under increasing pressure to reduce its reliance on fishmeal (FM) due to rising costs and sustainability concerns (Orisasona & Ajani 2015). The transition to plant-based feedstuffs is viewed as a viable solution, providing more consistent availability and lower costs than FM (Villasante et al 2019), but it frequently presents challenges such as amino acid imbalances, lower protein content, and the presence of anti-nutritional factors (Chaikaew & Chavanich 2017; Wang et al 2019).

Despite these issues, research indicates that a considerable amount of dietary FM can be substituted with plant protein sources without negatively affecting fish growth and feed efficiency, as long as the diets include the required limiting amino acids (Yıldız et al 2014; Nácher-Mestre et al 2015). A potential strategy to address these challenges involves the addition of essential amino acids, specifically methionine and lysine, to fish feed (Tran-Ngoc et al 2019). The significance of these amino acids lies in their essential role for optimal growth and development, particularly as they frequently represent the limiting amino acids (LAAs) in various plant-based diets utilized in aquaculture (Zeng et al 2015; Portolés et al 2017).

Methionine and lysine are particularly important in these plant-based diets. Methionine is frequently the first limiting amino acid in plant-based diets, as it is required for protein synthesis, immune function and other metabolic functions (Limwachirakhom et al 2022). Similarly, lysine is necessary for tissue growth and repair but is frequently lacking in plant protein sources (Portolés et al 2017). Research has shown that adding these amino acids can improve growth performance, feed efficiency, and overall protein utilization in a variety of aquaculture species (Naylor et al 2000). However, the optimal levels of supplementation can differ significantly depending on the fish species, life stage, and culture environment (Zeng et al 2015). Excessive methionine, however, can lead to negative effects, including poor growth and survival, highlighting the importance of precise dosage (He et al 2015). The interaction between these amino acids and other dietary components can also affect the gastrointestinal microbiome, which is essential for nutrient absorption and overall health in aquaculture systems (Wang et al 2019). The results of the study above are the basis for conducting development research to fill the understanding of the specific dietary requirements of red tilapia in peat pond environments, particularly the optimal ratios and sources of these amino acids.

The cultivation of red tilapia in peat ponds presents unique challenges and opportunities, particularly in terms of fish feed nutrition. Essential amino acids, particularly methionine and lysine, are critical in improving growth performance and feed efficiency in aquaculture. This study aims to improve aquaculture practices for red tilapia in peatland ecosystems by systematically evaluating the effects of dietary supplementation with methionine, lysine, and their combination on growth performance and feed utilization.

Material and Method

Study time and location. The study was conducted from October to December 2021 at the Center of Freshwater Aquaculture Development in Jambi, Indonesia.

Experimental design. Four experimental dietary treatments were formulated with varying levels of DL-methionine and L-lysine. The control treatment (ML1) consisted of a feed diet without methionine and lysine supplementation. Treatment 2 (ML2) included a feed diet with 0.2% methionine and no lysine supplementation. Treatment 3 (ML3) featured a feed diet with 0.2% methionine and 0.3% lysine supplementation, while treatment 4 (ML4) comprised a feed diet with 0.2% methionine and 0.3% lysine supplementation. The feeding trial was executed using a completely randomized design over a duration of 8 weeks.

Experimental diet preparation. Following the selection of ingredients, nutritional composition analyses were performed to ensure a balanced basal diet in terms of protein and energy. Each diet was supplemented with DL-methionine feed grade minimum 99% (Sumitomo Shemical Campany, Limited) and L-lysine monohydrochloride feed grade minimum 99% (Cheiljedang Indonesia, Limited). The experimental diet showed a crude

protein content of 30.1% and an average digestible energy (DE) value of 440.17 kcal g⁻¹. The basal diet consisted of FM, shrimp head meal, soybean meal, polished bran, coconut meal, palm kernel meal, tapioca, fish vitamin premix, phytase enzyme, multi-enzyme, potassium diformate, antifungal agents, vitamin E, and molasses, as outlined in Table 1. The feed ingredients were accurately weighed, homogenously mixed, and formed into pellets using a feed pelleting machine capable of producing 200 kg per hour. The resulting crumbled feed, designed to sink slowly, was stored in a cool, dry place in sealed containers and fed to the experimental fish.

Table 1

	Treatment					
Ingredients	ML1	ML2	ML3	ML4		
Fish meal	25	25	25	25		
Shrimp head meal	5	5	5	5		
Soybean meal	20	20	20	20		
Polished bran	15	15	15	15		
Fine bran	5	5	5	5		
Coconut meal	7	7	7	7		
Palm kernel meal	20	20	20	20		
Tapioca	1.65	1.45	1.15	0.95		
Vitamin premix*	0.5	0.5	0.5	0.5		
Phytase enzyme	0.03	0.03	0.03	0.03		
DL-methionine	0	0.20	0.20	0.20		
L-lysine	0	0	0.30	0.50		
Multi enzyme	0.05	0.05	0.05	0.05		
Potassium diformate(KDF)	0.2	0.2	0.2	0.2		
Molasses	0.5	0.5	0.5	0.5		
Anti-fungal	0.05	0.05	0.05	0.05		
Vitamin E	0.02	0.02	0.02	0.02		
Total	100.00	100.00	100.00	100.00		
Chemical composition (%)						
Ingradiants	Treatment					
Ingredients —	ML1	ML2	ML3	ML4		
Crude protein	30.14	30.14	30.14	30.14		
Crude lipids	9.53	9.53	9.52	9.52		
Ash	10.57	10.56	10.56	10.56		
Crude fiber	5.39	5.39	5.39	5.39		
NFE	51.96	51.96	51.96	51.96		
Digestible Energy (kcal g ⁻¹)**	473.51	473.50	473.49	473.48		
E/P value ***	15.71	15.71	15.71	15.71		

Ingredients, chemical composition (% of dry matter - DM) and energy (kcal g⁻¹) by treatment

Note: *Each kg: vit A 170,000.00 IU, vit D3 50,000.00 IU, vit E 3,000.00 IU, vit K3 135.00 mg, vit B1 200.00 mg, vit B2 330.00 mg, vit B6 335.00 mg, vit B12 0.45 mg, biotin 4 .00 mg, folic acid 65.00 mg, calpan 1,000.00 mg, nicotinic acid 2,000.00 mg, iron 1,335.00 mg, copper 100.00 mg, zinc 3,350.00 mg. **Calculated based on digestible energy according to Watanabe (1988); 1 g of protein contains 5.6 kcal g⁻¹, 1 g of nitrogen-free extract contains 4.1 kcal g⁻¹, and 1 g of lipid contains 9.4 kcal g⁻¹; ***According to Steffens (1989), the E/P value for optimal growth of fish ranges from 8 to 12 kcal g⁻¹.

Proximate analysis. Proximate analysis following AOAC (2012) was used to determine the protein, lipid, ash, and fiber content of samples.

Experimental fish and facility. The experimental setup for studying red tilapia fingerlings included a systematic acclimatization and weaning process, essential for promoting optimal growth and health in aquaculture environments. The fingerlings, averaging 6.67 g in body weight and 8.83 cm in total length, were obtained from the Center of Freshwater Aquaculture Development in Jambi. They were then transported to a peatland pond for a two-week acclimatization period in a floating net system (hapa) measuring 4 m x 4 m x

1.5 m. Throughout the acclimation period, the fish received a commercial diet, a standard procedure aimed at stabilizing their health prior to the introduction of experimental diets (Ljunggren et al 2003).

The weaning process was carefully planned, starting with a two-week mix of commercial feed and the experimental diet and gradually increasing the proportion of the test feed until it made up 100% of the diet. This gradual transition is essential because it reduces stress and promotes acceptance of the new feed, which is especially important in juvenile fish (Chang et al 2006). Following the weaning phase, the fingerlings were randomly assigned to hapas (2 m x 2 m x 1.5 m) with a density of 66 fish per hapa. They were fed twice daily at a rate of 5% of their body weight. The experimental design included 12 hapas with a water depth of one meter, equipped with aerators and water pumps to maintain water quality, which is critical for the health and growth of fish in aquaculture systems ($k_aczyńska$ et al 2016).

Water quality. Monitoring water quality parameters, including temperature, pH and dissolved oxygen were monitored twice daily, while ammonia (NH_3) assessments were conducted bi-weekly. The assessment of water condition was conducted in accordance with the APHA (2012) method.

Parameters observed. The parameters observed in this study were the weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), efficiency of feed utilization (EFU), protein efficiency ratio (PER), survival rate (SR) and water parameters. The parameters of the growth performance were observed by sampling once every two weeks. The following formulas are used to measure the parameters for growth performance and feed utilization (National Research Council 2011):

1. Weight gain, WG (%) = $100 \times \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}}$

2.	Specific growth rate	, SGR (%) :	= 10	100	0 ×	$\frac{\text{Ln (final body weight (g))} - \text{Ln (initial body weight (g))}}{\text{Time period (in days)}}$	
۷.	Specific growth rate,			100		Time period (in days)	

3. Feed conversion ratio (FCR) = $\frac{\text{Feed consumed (g)}}{\text{Final body weight (g)}-\text{Initial body weight (g)}}$

4. Efficiency of feed utilization, EFU (%) = $100 \times \frac{\text{Final body weight-Initial biomass (g)}}{\text{Diets fed (g)}}$

5. Protein efficiency ratio, PER (%) = $100 \times \frac{\text{Final body weight-Initial body weight (g)}}{\text{Diets fed x Protein content (g)}}$

6. Survival rate, SR (%) = $100 \times \frac{\text{Final number of fish}}{\text{Initial number of fish}}$

Statistical analysis. All experiments were performed in triplicate. The data are expressed as the mean±standard deviation (n = 3). The samples were analyzed for differences using a one-way ANOVA followed by a Tukey HSD post hoc test, utilizing SPSS Statistical Software, Version 27.0. Statistical significance was assessed using a p-value threshold of less than 0.05 to evaluate differences in mean growth parameters and feed utilization among fish across different experiments. The study's water condition measurements were analyzed descriptively and compared to the required conditions outlined in the literature.

Results. The growth performance and feed utilization of red tilapia after 8 weeks of experiment were presented in Table 2. Table 2 indicates that the inclusion of methionine and lysine in the diet significantly influenced the FBW, WG, SGR, and PER, while having no significant impact on IBW, FCR, EFU, and SR. No significant differences were observed in IBW, FCR, EFU, and SR among the treatments (ML1-ML4), as all values were comparable with no statistical significance (p > 0.05). FBW, WG, SGR, and PER exhibit a significant increase across treatments, with ML3 demonstrating the highest FBW (16.85±0.42 g), WG (153.73±10.25%), SGR (1.66±0.07% day⁻¹), and PER (34.01±1.57%). This is followed by

ML4, ML2, and ML1, indicating a clear influence of methionine and lysine levels on FBW, WG, SGR, and PER.

Factors	Treatments					
Factors	ML1	ML2	ML3	ML4		
IBW (g)	6.63±0.47 ^a	6.61±0.22 ^a	6.64±0.20 ^a	6.81±0.05ª		
FBW (g)	14.65±0.08ª	15.15 ± 0.09^{ab}	16.85±0.42 ^c	15.86 ± 0.44^{ab}		
WG (%)	121.87±17.31ª	129.24±8.70 ^{ab}	153.73±10.25 ^b	132.96±7.65 ^{ab}		
SGR (% day ⁻¹)	1.42 ± 0.13^{a}	1.48 ± 0.07^{ab}	1.66±0.07 ^b	1.51 ± 0.06^{ab}		
FCR	2.06±0.70 ^a	2.01 ± 0.58^{a}	2.05 ± 0.14^{a}	1.90±0.36ª		
EFU (%)	42.25±12.73 ^a	43.33±13.08ª	43.22±2.81 ^a	44.75±7.89 ^a		
PER (%)	26.74±1.82ª	28.46±1.01ª	34.01±1.57 ^b	30.17±1.57 ^{ab}		
SR (%)	91.33±2.52ª	94.00±3.00 ^a	96.00±1.00ª	91.66±3.05ª		

The growth performance and feed utilization of red tilapia after 8 weeks of experiment

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

Red tilapia had the highest WG on the ML3 diet ($153.73\pm10.25\%$) and the lowest on the ML1 diet ($121.87\pm17.31\%$). The ML1 diet showed the lowest SGR ($1.42\pm0.13\%$ day⁻¹). The value increased in the ML2 diet group ($1.48\pm0.07\%$ day⁻¹), peaked in the ML3 diet ($1.66\pm0.07\%$ day⁻¹), and then decreased in the ML4 diet group ($1.51\pm0.06\%$ day⁻¹), as shown in Table 2. The ML3 group diet had the highest PER of $34.01\pm1.57\%$, while the ML1 group diet had the lowest value of $26.74\pm1.82\%$.

The ML4 diet had the lowest FCR (1.90 ± 0.36), which was not significantly different from other treatments (p < 0.05). The ML1 diet had the highest FCR (2.06 ± 0.70). Similarly, there were no significant differences in EFU between fish fed different diets (p < 0.05). The ML1 diet had the lowest EFU value ($42.25\pm12.73\%$) and the highest in the ML4 diet group ($44.75\pm7.89\%$) (Table 2).

After conducting an analysis, it was determined that the diet in treatment ML3 had the best proximate composition, consisting of 33.61% protein, 9.85% lipid, and 11.77% fiber. It was found that treatment ML1 had the lowest levels of both protein and lipid content, with 32.46% protein and 9.02% lipid respectively. The approximate composition of diets that contain methionine and lysine supplements is presented in Table 3, which is based on red tilapia that are fed diets.

Table 3

Table 2

Provimate composition (%)	Treatments				
Proximate composition (%)	ML1	ML2	ML3	ML4	
Crude protein (%)	32.46	33.02	33.61	32.46	
Crude lipid (%)	9.02	9.30	9.85	9.39	
Crude fiber (%)	10.87	11.57	11.77	11.73	
Ash (%)	12.62	12.48	12.51	12.93	
NFE	35.03	33.63	32.26	33.49	
Digestible Energy (kcal g ⁻¹)*	410.19	410.22	413.07	407.35	
E/P value**	12.64	12.42	12.29	12.55	

Proximate composition of diets supplemented with methionine and lysine for red tilapia after an 8-week experiment

Note: *Calculated based on digestible energy according to Watanabe (1988); 1 g of protein contains 5.6 kcal g^{-1} , 1 g of nitrogen-free extract contains 4.1 kcal g^{-1} , and 1 g of lipid contains 9.4 kcal g^{-1} ; **According to Steffens (1989), the E/P value for optimal growth of fish ranges from 8 to 12 kcal g^{-1} .

The percentage of red tilapia that survived in diets that contained varying amounts of methionine and lysine is depicted in Figure 1. Fingerlings of red tilapia that were fed with different levels of methionine and lysine did not exhibit statistically significant differences

among treatments (p < 0.05). The rates ranged from 91.33% to 96%, with the ML3 group exhibiting the highest rate (96%), and the ML1 group exhibited the lowest rate (91.33%). Therefore, the amount of methionine and lysine that was added to the feed did not have any impact on the survival rate of the fish that were used in the experiment, which were red tilapia.

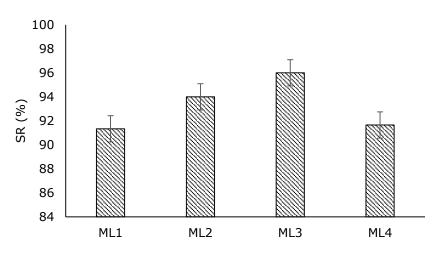


Figure 1. Effects of methionine and lysine supplementation on the survival rate of red tilapia fingerlings.

Water quality determined in this study are temperature, pH, dissolved oxygen, and ammonia (NH₃). Table 4 presents the average and minimum-maximum values of water quality parameters observed during the experiment. Water temperature (°C) and pH showed no significant fluctuations, while dissolved oxygen (DO) and ammonia (NH₃) levels fluctuated. The water temperature remained within the recommended range for red tilapia. However, pH and dissolved oxygen were below the recommended range, and NH₃ exceeded the recommended levels. The low pH, low dissolved oxygen, and high ammonia concentrations are typical of peat ponds, which are characterized by high organic matter content, as described by Notodarmojo et al (2017) and Sutapa et al (2020).

Table 4

Fluctuation of environmental factors during experimental red tilapia cultivation

Variable		Morning	Evening	Optimum value
Temperature (°C)	Min-max	26.20-27.41	27.15-29.03	25-30*
	Average	26.81	28.09	
рН	Min-max	6.12-6.69	6.63-6.98	6.5-8.5*
	Average	6.39	6.81	
Dissolved oxygen (mg L ⁻¹)	Min-max	2.38-3.18	2.78-3.22	> 5**
	Average	2.75	3.03	
Ammonia (mg L ⁻¹)	Min-max	0.013-0.562	0.014-0.529	< 0.5***
	Average	0.365	0.346	

Note: *Makori et al (2017); **Öz & Aral (2023); ***Ahmed et al (2020).

The pH levels in this study ranged from 6.12 to 6.98, with averages of 6.39 in the morning and 6.81 in the evening. These values reflect the acidic nature of peat pond environments, which can be harmful to fish health if not managed correctly. The optimal pH range for red tilapia is 6.5 to 8.5 (Makori et al 2017), implying that the observed values are at the lower end of the acceptable range. Dissolved oxygen levels varied from 2.38 to 3.22 mg L⁻¹, with averages of 2.75 mg L⁻¹ in the morning and 3.03 mg L⁻¹ in the evening. These values are significantly lower than the recommended level of > 5 mg L⁻¹ for red tilapia (Öz & Aral 2023), indicating a possible risk of hypoxia. Low dissolved oxygen levels can cause stress and reduced growth rates in fish (Sidoruk & Cymes 2018). Aeration and water circulation are two strategies for increasing oxygen levels that should be considered in peat pond aquaculture. Ammonia levels varied from 0.013 to 0.562 mg L⁻¹, with averages of 0.365 mg L⁻¹ in the morning and 0.346 mg L⁻¹ in the evening. These values are within an acceptable range, as levels less than 0.5 mg L⁻¹ are considered safe for red tilapia (Zeitoun et al 2016; Matondang et al 2022). However, continuous monitoring is required, as ammonia can accumulate and become toxic if not properly managed.

Discussion. Methionine and lysine are essential amino acids that are critical for protein synthesis, growth, and overall health in fish. Methionine, a sulfur-containing amino acid, plays a critical role in numerous metabolic processes, whereas lysine is essential for growth and tissue repair. Insufficient levels of these amino acids may result in impaired growth, suboptimal feed conversion, and elevated mortality rates in fish (Oyedokun et al 2022; Ruby et al 2022). Understanding the optimal levels of these amino acids in fish diets is crucial for maximizing aquaculture productivity.

The IBW of fish among treatment groups is a vital parameter in assessing growth performance. The consistency in IBW is crucial as it provides a benchmark for evaluating growth performance and reduces confounding variables that may influence the experimental results. The IBW of the fish among all treatment groups exhibited no significant differences (p > 0.05). The consistency in IBW is crucial as it provides a standard for assessing growth performance. Prior research has demonstrated that an imbalance of amino acids may result in diminished feed consumption, potentially impacting growth parameters (de Almeida et al 2010). However, the absence of substantial differences in IBW in this study implies that the fish were of similar size and health at the beginning of the experiment, thereby reducing the potential confounding effects on growth results.

The FBW demonstrated significant variations among the treatment groups, with ML3 attaining the highest FBW at 16.85±0.42 g. This discovery corroborates prior studies suggesting that dietary supplementation of lysine and methionine can improve growth performance in fish (Rachmawati et al 2022; Ruby et al 2022). The positive correlation between increased levels of these amino acids and growth performance is evidenced by the substantial increase in FBW from ML1 to ML4. The statistical significance of these findings (p < 0.05) suggests that dietary modifications can effectively improve the growth of metrics in aquaculture species.

The WG in the ML3 group was significantly elevated, reaching an impressive 153.73±10.25% increase relative to the initial weight. This outcome aligns with findings from other studies indicating enhanced weight gain in fish provided diets enriched with methionine and lysine (Oyedokun et al 2022; Teodósio et al 2022). The significant weight gain observed in ML3 compared to ML1, ML2 and ML4 highlights the importance of optimizing amino acid levels in aquaculture diets for enhanced growth performance.

The SGR followed a similar trend to weight gain, with ML3 exhibiting the highest SGR at $1.66\%\pm0.07\%$ day⁻¹. This metric illustrates the fish's growth dynamics by measuring the efficiency of growth in relation to time. The notion that methionine and lysine levels are advantageous for improving growth rates in red tilapia is further supported by the substantial increase in SGR in ML3 when contrasted with ML1, ML2, and ML4 (de Souza Reis et al 2011; Lee et al 2020). In a study on pearlspot (*Etroplus suratensis*) fingerlings, the best SGR was achieved with dietary methionine at 2.2% and lysine at 0.9%, resulting in SGR values of 3.35 ± 0.01 and 3.3 ± 0.01 , respectively (Ruby et al 2022). This indicates that both amino acids significantly enhance growth performance when included at these levels.

The FCR was statistically consistent across all treatment groups, with values ranging from 1.90 to 2.06, despite the substantial differences observed in growth parameters. This discovery implies that the fish's ability to convert feed into body mass did not vary significantly, even though their growth performance was improved by increased amino acid levels. Hansen et al (2011) and Kamiya et al (2021) have previously reported comparable results, suggesting that FCR may not always be directly correlated with dietary amino acid supplementation.

The treatment groups did not exhibit any significant differences in the EFU, suggesting that all groups had similar feed efficiency. Despite the varying levels of

methionine and lysine, the fish were able to effectively utilize the feed provided, as evidenced by the consistency in EFU. The significance of growth metrics in the assessment of the effects of dietary amino acids is further underscored by the absence of statistical significance in EFU (Shazali et al 2019).

Unlike FCR and EFU, the PER exhibited substantial increases with elevated concentrations of methionine and lysine. The ML3 group exhibited the highest PER at $34.01\pm1.57\%$, signifying that fish in this treatment group converted dietary protein into body mass more efficiently than those in the lower methionine and lysine groups. This discovery underscores the essential function of amino acids in enhancing protein synthesis and overall growth performance in red tilapia (Niu et al 2015).

The SR in the ML3 group was 96.00%, showing no significant improvement relative to ML1, ML2, and ML4. The elevated SR reflects the beneficial effect of optimized dietary amino acid levels on the health and resilience of red tilapia. The notable disparities in SR among the treatment groups indicate that sufficient nutrition is crucial for improving the overall health of Mandarin fish (*Siniperca chuatsi*), an aquaculture species (Zou et al 2022).

The findings have significant implications for aquaculture. Optimizing essential amino acid levels, particularly methionine and lysine, can enhance growth performance and survival rates in red tilapia, thereby increasing productivity and profitability for aquaculturists. These findings support further research on red tilapia and other aquaculture species' diets. A diet with 1 g kg⁻¹ lysine and 0.4 g kg⁻¹ methionine outperformed the control in common carp (*Cyprinus carpio*) fingerling growth and feed utilization (Kawa Jameel & Sofy Omar 2023). These findings suggest that lysine and methionine dosages can significantly improve growth metrics. Juvenile Nile tilapia (*Oreochromis niloticus*) need methionine supplementation to grow, with optimal levels around 1.5% (He et al 2015). Conversely, a study on juvenile golden pompano (*Trachinotus ovatus*) indicated that diets supplemented with DL-methionine significantly improved growth and nutrient utilization, particularly when fishmeal was replaced with soybean meal (Niu et al 2015).

In another study, juvenile red sea bream (*Pagrus major*) fed low fishmeal diets with methionine and lysine showed improved growth, but the study focused on taurine (Takagi et al 2011). This suggests that methionine and lysine can boost growth in fish-fed plant-based diets, which often lack them. Supplementing grass carp (*Ctenopharyngodon idella*) with lysine and methionine improved growth and feed conversion ratios (Yang et al 2010). The study found that a diet with reduced protein and these amino acids produced better growth metrics than unsupplemented diets. When combined with lysine, methionine improved growth rates and feed efficiency in juvenile common carp diets (Deng et al 2011). Growth rates and feed conversion ratios improved with methionine supplementation at 0.4% and lysine at 1%, which led to enhanced growth rates and feed conversion ratios.

Red tilapias thrive in warmer waters, with an optimal temperature range of 25 to 30°C. Temperatures below 20°C can reduce growth rates and increase disease susceptibility, whereas temperatures above 30°C can cause stress and death (Makori et al 2017). High pH levels can increase the proportion of toxic unionized ammonia (NH₃), whereas low pH levels can impair fish metabolism and ability to absorb nutrients (Ahmed et al 2020). The ideal pH range for optimal growth is 6.5-8.5 (Makori et al 2017). Within this range, tilapia have higher feed conversion rates and overall health. Maintaining a stable pH is critical for reducing stress and promoting healthy growth conditions.

Dissolved oxygen is essential for the respiration of aquatic organisms, such as red tilapia. The optimal dissolved oxygen levels for tilapia culture must be sustained above 5 mg L⁻¹ (Öz & Aral 2023). Low dissolved oxygen levels can result in hypoxia, adversely affecting fish growth, immune function, and overall health. Aeration is essential for boosting dissolved oxygen levels and addressing hypoxia in aquatic environments, including peatland ponds. Mechanical aerators, such as paddlewheel systems, enhance oxygen diffusion, especially at night when DO levels typically decrease due to respiration exceeding photosynthesis (Boyd et al 2018). Aeration and water circulation are essential for maintaining sufficient oxygen levels, particularly in high-density aquaculture systems (Zain et al 2019).

The acceptable concentration of NH_3 for red tilapia is generally below 0.5 mg L⁻¹, as higher concentrations can be toxic and lead to impaired growth and increased mortality

(Ahmed et al 2020). The toxicity of NH₃ is affected by pH and temperature; elevated pH and temperature levels enhance the concentration of NH₃ in water, intensifying its toxic effects (Ariffin et al 2019; Maulini et al 2022). In the peatland cultivation, the dynamics of ammonia concentration can be particularly pronounced due to the unique properties of peat, which can influence nutrient cycling and water chemistry. Peatlands are known to release organic acids and other compounds that can alter the pH and, consequently, the speciation of ammonia in the water column. This can lead to increased concentrations of toxic NH₃, especially in conditions where the water is poorly aerated or where organic matter decomposition is high (Wu et al 2014). To mitigate these risks, it is essential to implement effective management strategies, including regular monitoring of ammonia levels, optimizing aeration to enhance dissolved oxygen, and employing biological filtration systems that can help convert toxic ammonia into less harmful forms (Wood et al 2019). Additionally, understanding the specific interactions between peatland substrates and aquaculture practices can lead to improved management practices that maintain water quality and fish health.

Conclusions. The study demonstrated that supplementing the diet with 0.2% methionine and 0.3% lysine resulted in the highest growth rates for red tilapia in peatland ponds, highlighting the importance of amino acid balance in aquaculture diets. While plant-based feed is more sustainable and cost-effective, it may lead to amino acid imbalances and lower protein content, requiring careful management. Optimizing red tilapia aquaculture in peatland environments requires addressing both water quality and dietary formulation, with continuous monitoring of dissolved oxygen and ammonia levels to prevent toxicity and ensure fish health and growth.

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References

- Ahmed K. M., Ibrahim M. A., Mounes H. A. M., 2020 Effect of using low molecular weight chitosan on water quality, quality indices and stress reduction of Nile tilapia. Egyptian Journal for Aquaculture 10(2):47-65.
- Anamulai S., Sanusi R., Zubaid A., Lechner A. M., Ashton-Butt A., Azhar B., 2019 Land use conversion from peat swamp forest to oil palm agriculture greatly modifies microclimate and soil conditions. PeerJ 7:e7656.
- American Public Health Association (APHA), 2012 Standard methods for the examination of water and wastewater. 22nd edition. American Public Health Association, Water Environment Federation, Washington DC, USA, 724 pp.
- Ariffin F. D., Halim A. A., Hanafiah M. M., Awang N., Othman M. S., Azman S. A. A., Bakri N. S. M., 2019 The effects of african catfish, *Clarias gariepinus* pond farm's effluent on water quality of Kesang River in Malacca, Malaysia. Applied Ecology and Environmental Research 17(2):1531-1545.
- Association of Official Analytical Chemists (AOAC) International, 2012 Official methods of analysis of AOAC international. 17th edition. Arlington, pp. 25-42.
- Boyd C. E., Torrans E. L., Tucker C. S., 2018 Dissolved oxygen and aeration in ictalurid catfish aquaculture. Journal of the World Aquaculture Society 49(1):7-70.
- Chaikaew P., Chavanich S., 2017 Spatial variability and relationship of mangrove soil organic matter to organic carbon. Applied and Environmental Soil Science 2017: 4010381.
- Chang Q., Liang M. Q., Wang J. L., Chen S. Q., Zhang X. M., Liu X. D., 2006 Influence of larval co-feeding with live and inert diets on weaning the tongue sole *Cynoglossus semilaevis*. Aquaculture Nutrition 12(2):135-139.

- de Almeida E. C., Fialho E. T., Rodrigues P. B., Zangeronimo M. G., de Freitas Lima J. A., de Oliveira Fontes D., 2010 Ractopamine and lysine levels on performance and carcass characteristics of finishing pigs. Revista Brasileira De Zootecnia 39(9):1961-1968.
- de Souza Reis R., de Toledo Barreto S. L., Gomes P. C., Lima H. J. D., Medina P. M., Ferreira F., 2011 Relationship of methionine plus cystine with lysine in diets for laying Japanese quails. Revista Brasileira de Zootecnia 40(5):1031-1037.
- Deng J., Kong L., An Q., Bi B., Tao L., Zhang X., 2011 Effect of dietary pH adjustment on the utilization of supplemental methionine and lysine by juvenile common carp, *Cyprinus carpio*. Journal of the World Aquaculture Society 42(5):696-704.
- Hansen A. C., Hemre G. I., Karlsen Ø., Koppe W., Rosenlund G., 2011 Do plant-based diets for atlantic cod (*Gadus morhua* L.) need additions of crystalline lysine or methionine? Aquaculture Nutrition 17(2):362-371.
- He J. Y., Long W. Q., Han B., Tian L. X., Yang H. J., Zeng S. L., Liu Y. J., 2015 Effect of dietary L-methionine concentrations on growth performance, serum immune and antioxidative responses of juvenile Nile tilapia, *Oreochromis niloticus*. Aquaculture Research 48(2): 665-674.
- Kamiya M., Yamada T., Higuchi M., 2021 Effects of low-crude protein diets supplemented with rumen-protected lysine and methionine on fattening performance and nitrogen excretion of Holstein steers. Animal Science Journal 92(1):e13562.
- Kawa Jameel L., Sofy Omar S., 2023 Effects of dietary supplementation of lysine and methionine on growth performance, blood parameters and body composition of common carp (*Cyprinus carpio*) fingerling. ZANCO Journal of Pure and Applied Sciences 35(3):159-170.
- Łączyńska B., Palińska-Żarska K., Nowosad J., Biłas M., Krejszeff S., Müller T., Kucharczyk D., Żarski D., 2016 Effect of age, size and digestive tract development on weaning effectiveness in crucian carp, *Carassius carassius* (Linnaeus, 1758). Journal of Applied Ichthyology 32(5):866-872.
- Lee C. Y., Song A. A. L., Loh T. C., Rahim R. A., 2020 Effects of lysine and methionine in a low crude protein diet on the growth performance and gene expression of immunity genes in broilers. Poultry Science 99(6):2916-2925.
- Limwachirakhom R., Triwutanon S., Chumkam S., Jintasataporn O., 2022 Effects of chromium-L-methionine in combination with a zinc amino acid complex or selenomethionine on growth performance, intestinal morphology, and antioxidative enzymes in red tilapia *Oreochromis* spp. Animals 12(17):2182.
- Ljunggren L., Staffan F., Falk S., Linden B. V. D., Mendes J., 2003 Weaning of juvenile pikeperch, *Stizostedion lucioperca* L., and perch, *Perca fluviatilis* L., to formulated feed. Aquaculture Research 34(4):281-287.
- Makori A. J., Abuom P. O., Kapiyo R., Anyona D. N., Dida G. O., 2017 Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North sub-county, Busia County. Fisheries and Aquatic Sciences 20(1): 30.
- Matondang P. A. S., Taparhudee W., Yoonpundh R., Jongjaraunsuk R., 2022 Water quality management guidelines to reduce mortality rate of red tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*) fingerlings raised in outdoor earthen ponds with a recirculating aquaculture system using machine learning techniques. Asean Journal of Scientific and Technological Reports 25(4):30-41.
- Maulini R., Sahlinal D., Arifin O., 2022 Monitoring of pH, amonia (NH₃) and temperature parameters aquaponic water in the 4.0 revolution era. IOP Conference Series: Earth and Environmental Science 1012(1):012087.
- Miettinen J., Hooijer A., Shi C., Tollenaar D., Vernimmen R., Liew S. C., Malins C., Page S.
 E., 2012 Extent of industrial plantations on Southeast Asian peatlands in 2010 with analysis of historical expansion and future projections. GCB Bioenergy 4(6):908-918.
- Nácher-Mestre J., Serrano R., Beltrán E., Pérez-Sánchez J., Silva J., Karalazos V., Hernández F., Berntssen M. H. G., 2015 Occurrence and potential transfer of mycotoxins in gilthead sea bream and Atlantic salmon by use of novel alternative feed ingredients. Chemosphere 128:314-320.

- National Research Council (NRC), 2011 Nutrient requirements of fish and shrimp. The National Academies Press, Washington DC, 392 pp.
- Naylor R. L., Goldburg R. J., Primavera J. H., Kautsky N., Beveridge M. C. M., Clay J., Folke C., Lubchenco J., Mooney H., Troell M., 2000 Effect of aquaculture on world fish supplies. Nature 405(6790):1017-1024.
- Niu J., Figueiredo-Silva C., Dong Y., Yue Y. R., Lin H. Z., Wang J., Wang Y., Huang Z., Xia D. M., Lü X., 2015 Effect of replacing fish meal with soybean meal and of DL-methionine or lysine supplementation in pelleted diets on growth and nutrient utilization of juvenile golden pompano (*Trachinotus ovatus*). Aquaculture Nutrition 22(3):606-614.
- Notodarmojo S., Mahmud, Larasati A., 2017 Adsorption of natural organic matter (nom) in peat water by local Indonesia tropical clay soils. International Journal of GEOMATE 13(38):111-119.
- Orisasona O., Ajani E., 2015 The growth and mineral utilization of *Clarias gariepinus* fingerlings fed phytase-supplemented toasted lima bean (*Phaseolus lunatus*) diets. Journal of Aquaculture Research and Development 6(9):1000361.
- Oyedokun J. O., Ogunwole O. A., Oyelese O. A., 2022 Amino acid digestibility of catfish (*Clarias gariepinus*) fed soyabean meal supplemented with lysine and DL-methionine. Asian Journal of Biological Sciences 15(3):152-163.
- Öz M., Aral O., 2023 The effect of zeolite (clinoptilolite) as a feed additive and filter material for freshwater aquariums. Journal of Agricultural Production 4(1):39-46.
- Portolés T., Ibáñez M., Garlito B., Nácher-Mestre J., Karalazos V., Silva J., Alm M., Serrano R., Pérez-Sánchez J., Hernández F., Berntssen M. H. G., 2017 Comprehensive strategy for pesticide residue analysis through the production cycle of gilthead sea bream and Atlantic salmon. Chemosphere 179:242-253.
- Rachmawati D., Elfitasari T., Samidjan I., Nurhayati D., Riyadi P. H., 2022 Influence of dietary lysine level on growth performance, feed efficiency, and body composition of sangkuriang catfish (*Clarias gariepinus* var. sangkuriang) fingerlings. Pertanika Journal of Tropical Agricultural Science 45(4):1053-1067.
- Ruby P., Ahilan B., Antony C., Manikandavelu D., Moses Samuel T. L. S., 2022 Effect of dietary lysine and methionine supplementation on the growth and physiological responses of pearlspot fingerlings, *Etroplus suratensis*. Indian Journal of Animal Research 56(8):945-958.
- Shazali N., Loh T. C., Foo H. L., Samsudin A. A., 2019 Gut microflora and intestinal morphology changes of broiler chickens fed reducing dietary protein supplemented with lysine, methionine, and threonine in tropical environment. Revista Brasileira de Zootecnia 48:e20170265.
- Sidoruk M., Cymes I., 2018 Effect of water management technology used in trout culture on water quality in fish ponds. Water 10(9):1264.
- Steffens W., 1989 Principles of fish nutrition. Ellis Horwood Limited, Chicester, UK, 384 pp.
- Sugiani D., Urwaningsih U., Andriyanto S., Lusiastuti A. M., 2019 [Bacteria in snakehead fish *Channa striata*, mahseer *Tor* spp., and Asian redtail catfish *Hemibagrus* sp.: identification, virulence, and susceptibility to several antibiotics]. Jurnal Riset Akuakultur 13(4):347-356. [in Indonesian]
- Sutapa I. D. A., Prihatinningtyas E., Daryanta, 2020 IPAG60 as alternative solution to provide clean water in peatland areas. IOP Conference Series: Earth and Environmental Science 477:012030.
- Takagi S., Murata H., Goto T., Hatate H., Endo M., Yamashita H., Miyatake H., Ukawa M., 2011 Role of taurine deficiency in inducing green liver symptom and effect of dietary taurine supplementation in improving growth in juvenile red sea bream *Pagrus major* fed non-fishmeal diets based on soy protein concentrate. Fisheries Science 77(2): 235-244.
- Taparhudee W., Jongjaraunsuk R., Nimitkul S., Suwannasing P., Mathurossuwan W., 2024 Optimizing convolutional neural networks, XGBoost, and hybrid CNN-XGBoost for precise red tilapia (*Oreochromis niloticus* Linn.) weight estimation in river cage culture with aerial imagery. AgriEngineering 6(2):1235-1251.

- Teodósio R., Aragão C., Conceição L. E. C., Dias J., Engrola S., 2022 Metabolic fate is defined by amino acid nature in gilthead seabream fed different diet formulations. Animals 12(13):1713.
- Tran-Ngoc K. T., Haidar M. N., Roem A. J., Sendão J., Verreth J. A. J., Schrama J. W., 2019 Effects of feed ingredients on nutrient digestibility, nitrogen/energy balance and morphology changes in the intestine of Nile tilapia (*Oreochromis niloticus*). Aquaculture Research 50(9):2577-2590.
- Villasante A., Ramirez C., Catalán N., Opazo R., Dantagnan P., Romero J., 2019 Effect of dietary carbohydrate-to-protein ratio on gut microbiota in Atlantic salmon (*Salmo salar*). Animals 9(3):89.
- Wang Y., Liu M., Wang B., Jiang K., Wang M., Wang L., 2019 A global view of hepatopancreas and intestinal reveals the potential influencing mechanism of aflatoxin B1 on nutrition and metabolism in *Litopenaeus vannamei*. Aquaculture Nutrition 25(6):1354-1366.
- Watanabe T., 1988 Fish nutrition and mariculture. Department of Aquatic Bioscience, Tokyo University of Fisheries, JICA, 223 pp.
- Wood A. T., Andrewartha S. J., Elliott N. G., Frappell P. B., 2019 Hypoxia during incubation does not affect aerobic performance or haematology of Atlantic salmon (*Salmo salar*) when re-exposed in later life. Conservation Physiology 7(1):coz088.
- Wu Z., You F., Wen A., Ma D., Zhang P., 2014 Physiological and morphological effects of severe hypoxia, hypoxia and hyperoxia in juvenile turbot (*Scophthalmus maximus* L.). Aquaculture Research 47(1):219-227.
- Yang H. J., Liu Y. J., Tian L. X., Liang G. Y., Lin H. R., 2010 Effects of supplemental lysine and methionine on growth performance and body composition for grass carp (*Ctenopharyngodon idella*). American Journal of Agricultural and Biological Sciences 5(2):222-227.
- Yıldız M., Köse İ., Issa G., Kahraman T., 2014 Effect of different plant oils on growth performance, fatty acid composition and flesh quality of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 46(12):2885-2896.
- Zain R. A. M. M., Shaari N. F. I., Amin M. F. M., Jani M., 2019 Effect of zeolite on the water quality and growth performance of red hybrid tilapia (*Oreochromis niloticus*).
 Proceedings of the 2nd International Conference on Advance and Scientific Innovation, ICASI 2019, 18 July, Banda Aceh, Indonesia, 8 pp.
- Zeitoun M. M., El-Azrak K. E. M., Zaki M. A., Nemat-Allah B. R., Mehana E. E., 2016 Effects of ammonia toxicity on growth performance, cortisol, glucose and hematological response of Nile tilapia (*Oreochromis niloticus*). Aceh Journal of Animal Science 1(1): 21-28.
- Zeng S. L., Long W. Q., Tian L. X., Xie S. W., Chen Y. J., Yang H. J., Liang G. Y., Liu Y. J., 2015 Effects of dietary aflatoxin B1 on growth performance, body composition, haematological parameters and histopathology of juvenile Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Nutrition 22(5):1152-1159.
- Zou J. M., Zhu Q. S., Liang H., Lu H. L., Liang X. F., He S., 2022 Lysine deprivation regulates Npy expression via GCN2 signaling pathway in mandarin fish (*Siniperca chuatsi*). International Journal of Molecular Sciences 23(12):6727.

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