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Productive Performance, Feed Utilization, Biochemical Parameters, and Economic Evaluation of the Nile Tilapia (*Oreochromis niloticus*) Fed Diets Containing Different Levels of Methionine

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ABSTRACT

This study aimed to explore the impact of adding methionine on growth performance, feed utilization, biochemical parameters, and economic assessment of *Oreochromis niloticus*. A total of 180 fries were acclimated and then randomly allocated into the experimental aquariums. Fish were distributed across 12 aquaria with 15 fry each, having an initial average weight of 83.50± 0.485g per aquarium. Methionine was incorporated at levels of 0, 5, 10, and 15g/ kg in experimental diets labeled D1, D2, D3, and D4, respectively for 56 days. Growth performance metrics such as total body weight gain (TBWG), daily gain (ADG) and specific growth rate (SGR) increased in the methionine-supplemented groups compared to the control. The mortality rate was 6.67% in the control group, while it was zero in the methionine-supplemented groups. The feed conversion ratio and protein efficiency ratio also improved. Total protein and albumin were higher in all methionine treatments compared to the control group. Levels of AST, ALT, and cholesterol were not significantly affected by methionine supplementation. The highest uric acid level was found in the group receiving 15g/ kg methionine. Fish body composition, including moisture, crude protein, and ash content, showed significant increases. Energy retention (ER%) improved by 6.90 and 21.29% in the groups receiving 10 and 15g/ kg methionine, respectively, compared to the control (D1). Protein productive value (PPV%) improved by 129.06, 142.41, and 151.32% for the 5, 10, and 15g/ kg methionine groups, respectively, compared to the control. The net improvement in feeding efficiency, reflected by a decrease in feeding costs, was 13.85, 25.44, and 31.54% compared to control. Our observations indicate that, including methionine in fish diets enhances performance metrics, increases total protein and globulin levels, improves energy retention and protein productive values, and boosts the net economic benefits of feeding.

INTRODUCTION

The tilapia is the world's second most widely cultured fish, following the carp. Its popularity in aquaculture is increasing due to its rapid growth, and tolerance to poor water quality **(Prabu *et al*., 2019; Tahoun, 2022)**. Methionine is an α-amino acid involved in the biosynthesis of proteins. It features a carboxyl group (deprotonated to $-COO^-$ at biological pH), an amino group (protonated to NH3+ at biological pH) in the α-position relative to the carboxyl group, and an S-methyl thioether side chain, categorizing it as a nonpolar, aliphatic amino acid **(Guedes *et al*., 2011)**.

Robinson and Wilson (1985) and **Lovell (1989)** highlighted that methionine is considered one of the most limiting amino acids in fish rations. According to **Webster *et al*. (1995)** and **Billah *et al*. (2022)**, supplementing with methionine has been shown to enhance growth and feed efficiency in various fish species, including the blue catfish (*Ictalurus furcatu*) and the stinging catfish fry. Similarly, **Kaushik *et al*. (1995)** and **Cheng *et al*. (2003)** observed improvements in the rainbow trout. While, **El-Saidy and Gaber (2002)** noted benefits in the Nile tilapia (*Oreochromis niloticus*). **Khan *et al*. (2003)** reported similar findings in the Indian major carp and *Cirrhinus mrigala*. Whereas, **Andrews and Page (1974)** did not observe these effects in the catfish. **Dabrowski and Kozak (1979)** found no such improvements in the grass carp, and **Reigh and Ellis (1992)** made similar observations in the red drum (*Sciaenops ocellatus*).

Moreover, **Abidi and Khan (2011)** and **Khan and Abidi (2011)** elucidated that methionine is essential for normal growth and development in fish. They also found that methionine deficiency led to a reduction in the final weight gain, protein content and feed conversion, while increasing fat deposition. **Espe *et al*. (2014)**, **Kwasek *et al*. (2014)**, **Wang *et al*. (2014)** and **Watson *et al*. (2014)** noted that amino acids can influence gene expression, particularly in marine fish species, regarding the adaptation to sulfur amino acid availability.

According to **NRC (2001)**, methionine is crucial for fish and, along with lysine, is often one of the main limiting amino acids in unbalanced diets or those high in plant-based protein sources. **Yossa *et al*. (2023)** demonstrated that, to prevent methionine deficiency symptoms like reduced growth and feed efficiency, commercial forms of methionine (crystalline or intact) are effectively used in fish feeds to meet known requirements. **Santiago and Lovell (1988)** determined that the methionine requirement for the Nile tilapia with a body weight (BW) of 0.06g was 0.8/ kg diet of the diet. Later, **Nguyen and Allen Davis (2009)** estimated this requirement at 0.49% of the diet (with a cysteine level of 0.45% of diets) for the tilapia with an initial body weight of 1.3g. Therefore, our investigation aimed to evaluate the effects of incorporating methionine at 0, 5, 10 and 15g/ kg diets on the growth productivities of the tilapia.

MATERIALS AND METHODS

This research was conducted at the Fish Laboratory of the Biological Agriculture Research Institute in collaboration with the Hydrobiology Department of the Veterinary Research Institute, both part of the National Research Center.

Experimental setup

A total of 180 fish individuals with an initial body weight of 5.56± 0.03g were acclimated and then randomly assigned to experimental aquariums. The fish were divided into 12 aquariums, each housing 15 fish (average total initial weight of 83.50± 0.485g), with aquariums measuring 80×40×30cm and having a capacity of 60 liters.

Experimental diets

Methionine was incorporated into the diets at four levels: 0, 0.50, 1.00, and 1.50%, corresponding to 0, 5, 10, and 15g/ kg diet for D1, D2, D3, and D4, respectively (Table 1).

Table 1. Composition of different experimental diets

Ingredient	Experimental diets				Price of tone LE
	Control zero methionine	5 g methionine /kg diet	10 g methionine /kg diet	15 g methionine /kg diet	
	D_1	D_2	D_3	D_4	
Composition of tested diets					
Methionine	0.00	0.50	1.00	1.50	90000
Concentration (56% CP)	17.00	17.00	17.00	17.00	25000
Soybean meal (44% CP)	40.00	40.00	40.00	40.00	33000
Ground yellow corn (8% CP)	28.00	28.00	28.00	28.00	12500
Wheat bran (13% CP)	10.00	9.50	9.00	8.50	14500
Vegetable oil	3.00	3.00	3.00	3.00	50000
Vitamins and minerals mixture**	2.00	2.00	2.00	2.00	40000
Price of ton fed (LE)	24700	25075	25450	25800	---
Price of kg fed (LE)	24.700	25.075	25.450	25.800	---

** Vit. A (E672) (IU) 876.19, Vit. D3 (IU) 1141.39, Vit. E 114.30, Vit. K3 7.55, Vit. B1 13.71, Vit. B2 11.44, Vit. B6 15.33, Vit. B12 0.03, Niacin 60.96, Calpan 30.48, Folic Acid 3.04, Biotin 0.37, Vit. C 11.44, Selenium 0.27, Manganese 19.04, Iron 9.15, Iodine 0.77, Zinc 76.19, Raanan Aqua Feed Px, Maridav Ghana Limited.

The experimental diets were continuous for 56 days, and tested diets were hand-fed for 56 days extended approximately from the middle of November 2023 to the middle of January 2024.

Parameters of growth performance

Body weight gain (BWG) = Final weight - Initial weight.

Survival rate (SR%) = Number of fish at final / Number of fish at start x100.

Specific growth rate (SGR) =

[In final weight (g) - In initial weight (g)] / Experimental days *100

Calculation of feed conversion ratio (FCR)

FCR = total dry matter intake, (TDMI), g / total body weight gain (TBWG), g.

Calculation of crude protein efficiency ratio (CPER)

(PER) = total body weight gain (TBWG), g / total crude protein intake (TCPI), g.

Feed efficiency

Feed efficiency (FE%) = [weight gain (g) / feed intake (g)]

Protein productive value (PPV%) = [PR_1– PR_0 / PI] 100.

PR_1 = body protein at the nd.

PR_0 = body protein at start.

PI = Protein intake.

Energy retention percentages (ER%)

Energy retention (ER%) = E-E_0 / E_F X 100

E= Carcass energy (kcal) at the end

E_0= Carcass Energy in fish (kcal) at the start.

E_F = Feed intake energy (kcal).

Blood sampling

Blood samples were collected from nine fish in each group using a 3ml syringe after anesthetizing with clove oil (0.5ml/ L). The samples were then centrifuged at 3000rpm for 15 minutes, and the serum was stored at -20°C.

Body composition

Initially, 15 fish were analyzed, and at the end of the study, with nine fish from each treatment group were randomly selected to assess whole body composition.

Analytical procedures

The analysis of the experimental diets and fish body composition was conducted following **AOAC (2016)** methods. Total protein and albumin were measured colorimetrically according to **Cannon *et al*. (1974)** and **Tietz (1990)**. Globulin levels were calculated by subtracting albumin from total protein. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured following **Reitman and Frankel (1957)**, cholesterol as per **Ellefson and Caraway (1976)**, glucose following **Caraway and Watts (1987)**, and uric acid as described by **Tietz (1990)**. Each biochemical parameter was colorimetrically analyzed using kits from Spectrum diagnostics, Egypt, and an Agilent Cary UV-Vis spectrophotometer (100/300 Series).

Statistical analysis

The collected data were analyzed using the one-way analysis of variance (ANOVA) **SPSS (2020)**. Duncan’s multiple range test **(Duncan, 1955)** was used to separate the means.

RESULTS

Chemical analysis of the experimental diets

Table (2) shows that the crude protein content ranged from 29.57 to 29.64% across the four diets. GE ranged from 4512 to 4542kcal/ kg, and metabolizable energy (ME) ranged from 349.38 to 353.94kcal/ kg. Additionally, the protein energy ratio varied from 83.69 to 84.84mg CP/ kcal ME among the four diets. These values are considered sufficient to meet the nutritional requirements of the Nile tilapia, and all tested diets were found to be isocaloric and isonitrogenous.

Table 2. Chemical analysis of the different experimental diets

Ingredient	Tested diets			
	Control zero methionine	5g methionine /kg diet	10g methionine /kg diet	15g methionine /kg diet
	D_1	D_2	D_3	D_4
Moisture	7.60	7.78	7.52	7.57
Dry matter (DM)	92.40	92.22	92.48	92.43
Organic matter (OM)	92.19	93.17	92.59	92.65
Crude protein (CP)	29.64	29.62	29.57	29.58
Crude fiber (CF)	6.80	6.08	6.55	6.61
Ether extract (EE)	4.60	4.31	4.75	4.83
Nitrogen free extract (NFE)	51.15	53.16	51.72	51.63
Ash	7.81	6.83	7.41	7.35
Gross energy kcal/ kg DM	4512	4537	4535	4542
Gross energy cal/ g DM	4.512	4.537	4.535	4.542
Metabolizable energy kcal/ kg DM	349.38	353.94	352.28	352.66
Protein energy ratio (mg CP/ Kcal ME)	84.84	83.69	83.94	83.88

Gross energy (kcal/ kg DM) was calculated according to (Blaxter1968; MacRae and Lobley 1982). Where, each g CP = 5.65 Kcal, g. EE = 9.40 kcal and g CF and NFE = 4.15 Kcal, Metabolizable energy (ME): calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively. Calculated according to (NRC 2011), Protein energy ratio (mg CP/ Kcal ME): Calculated according to **NRC (2011)**.

Growth and survival ratio

As indicated in Table (3), the findings reveal that dietary treatments led to body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and survival rate (SR) in fish groups fed diets containing 5, 10, and 15g of methionine per Kg of diet (D2, D3, and D4, respectively), compared to the control group (D1). Additionally, the mortality rate was 6.67% in the control group (D1), whereas it was zero in the other three groups (D2, D3, and D4). Overall, an increase in the level of methionine inclusion in the diet resulted in significant improvements in the mentioned parameters.

Table 3. Growth performance, specific growth rate and survival ratio of *O. niloticus* feeding on different concentrations of methionine

Item	Tested diets				SEM	Sign. *P*<0.05
	Control zero methionine	5g methionine /kg diet	10g methionine /kg diet	15g methionine /kg diet		
	D_1	D_2	D_3	D_4		
Number of fish	45	45	45	45	-	-
Initial weight, g (IW)	84	83	85	82	0.485	NS
Final weight, g (FW)	223^d	250^c	276^b	292^a	8.155	*
Total body weight gain, g (TBWG)	139^d	167^c	191^b	210^a	8.209	*
Duration experimental period			56 days			
Average daily gain, g (ADG)	2.48^d	2.98^c	3.41b	3.75^a	0.146	*
Specific growth rate (SGR)	0.76^d	0.85^c	0.92^b	0.98^a	0.025	*
Number of fish at the starter	45	45	45	45	-	-
Number of fish at the end	42	45	45	45	0.131	NS
Survival ratio (SR)	93.33^b	100^a	100^a	100^a	0.871	*
Number of dead fish	3	Zero	Zero	Zero	0.131	NS
Mortality rate percentages	6.67	Zero	Zero	Zero	0.871	NS

a, b, c and d: Means in the same row having different superscripts differ significantly ($P< 0.05$).
SEM: Standard error of mean NS: Not significant *: Significant at ($P< 0.05$).

Feed utilization of experimental groups

The results of feed utilization, presented in Table (4), indicate that the values of feed intake (FI), feed conversion ratio (FCR), protein intake (CPI), and protein efficiency ratio (PER) increased with the incorporation of methionine in the diets of the Nile tilapia fish. Both FCR and PER values showed an increase with 5, 10, and 15g of methionine/kg diet.

Table 4. Feed utilization of *O. niloticus* feeding on different concentrations of methionine

Item	Tested diets				SEM	Sign. *P*<0.05
	Control zero methionine	5 g methionine /kg diet	10 g methionine /kg diet	15 g methionine /kg diet		
	D_1	D_2	D_3	D_4		
Total body weight gain, g (TBWG)	139^d	167^c	191^b	210^a	8.209	*
Feed intake (FI), g	428^c	445^{ab}	443^b	452^a	2.841	*
Feed conversion ratio (FCR)	3.08^b	2.66^b	2.32^a	2.15^a	0.110	*
Feed crude protein %	29.64	29.62	29.57	29.58	-	-
Crude protein intake (CPI), g	126.86^c	131.81^{ab}	131.00^b	133.70^a	0.816	*
Protein efficiency ratio (PER)	1.096^d	1.267^c	1.458^b	1.571^a	0.056	*

a, b, c and d: Means in the same row having different superscripts differ significantly ($P< 0.05$). SEM: Standard error of mean *: Significant at $P< 0.05$. FCR: Expressed as g of DM intake / g gain. PER: Expressed as g of g gain / g CP intake.

Biochemical parameters of the different experimental groups

Data from Table (5) indicate that total protein and albumin levels increased in all methionine groups compared to the control. The 0.5% methionine (D2) group exhibited the highest significant protein concentration, followed by the 1.5% methionine (D4) group, then the 1.0% methionine (D3) group. Similarly, albumin levels were significantly increased in D4, followed by D3, and then D2. Conversely, globulin level significantly increased in D2 but decreased in both D3 and D4 compared to the control.

Regarding liver enzymes, AST and ALT showed non-significantly in all methionine-treated groups compared to the control group. Similarly, cholesterol concentration in the serum did not significantly change ($P > 0.05$) in all methionine-treated groups compared to the control group. However, glucose levels significantly decreased in the D3 and D4 groups with high methionine concentrations. Uric acid concentration exhibited its highest significant value in D4, followed by D3, then D2, compared to the control.

Table 5. Biochemical parameters of *O. niloticus* feeding on different concentrations of methionine

Item	Tested diets				SEM	Sign. $P < 0.05$
	Control zero methionine	5g methionine /kg diet	10g methionine /kg diet	15g methionine /kg diet		
	D_1	D_2	D_3	D_4		
Total protein (g/dl)	2.49^{b}	3.36^{a}	2.53^{b}	2.55^{b}	0.111	*
Albumin (g/dl)	0.71^{c}	1.02^{b}	1.19^{a}	1.21^{a}	0.061	*
Globulin (g/dl)	1.78^{b}	2.34^{a}	1.34^{c}	1.34^{c}	0.125	*
Albumin: Globulin ratio	0.40^{c}	0.44^{b}	0.89^{a}	0.90^{a}	0.072	*
AST (Unit/l)	217.2^{a}	215.7^{a}	156.8^{a}	222.6^{a}	8.098	NS
ALT (Unit/l)	64.48^{a}	72.88^{a}	59.25^{a}	60.52^{a}	1.619	NS
Glucose (mg/dl)	103.1^{a}	101.1^{a}	81.83^{b}	91.45^{ab}	2.573	*
Cholesterol (mg/dl)	120.0^{a}	122.0^{a}	109.5^{a}	117.5^{a}	1.475	NS
Uric acid (mg/dl)	17.97^{b}	22.31^{ab}	23.18^{ab}	26.36^{a}	0.917	*

a, b, c and d: Means in the same row having different superscripts differ significantly ($P < 0.05$). SEM: Standard error of mean NS: Not significant *: Significant at $P < 0.05$. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Fish body composition of different experimental groups

Feeding the Nile tilapia diets containing methionine resulted in a significant increase in body composition, including moisture, crude protein, and ash content percentages. Conversely, values for dry matter, organic matter, ether extract, and gross energy decreased as shown in Table (6).

Energy retention and protein productive value percentages

Data from Table (7) indicate that incorporating methionine at a level of 0.50% in the Nile tilapia fish diet had no significant effect ($P > 0.05$) on energy retention (ER) % compared to the control (D1). However, fish groups that received 1.00 or 1.50% methionine recorded significantly higher ER% compared to D1 or D2. The corresponding improvements in ER% were recorded at 6.90 and 21.29% when fish received 10 or 15g of methionine per kg of diet (D3 and D4, respectively) compared to the control (D1). Additionally, protein productive value (PPV) % significantly increased when fish groups received diets containing methionine at different levels (5, 10, and 15g of methionine per kg of diet). The corresponding improvements in PPV% were recorded at 129.06, 142.41, and 151.32% when fish received 5, 10, and 15g of methionine per kg of diet (D2, D3, and D4, respectively) compared to the control, which is considered 100%.

Table 6. Fish body composition of initial and different experimental groups that fed tested diets

Item	Body composition of initial fish	Tested diets				SEM	Sign. *P*< 0.05
		Control zero methionine	5g methionine /kg diet	10g methionine /kg diet	15g methionine /kg diet		
		D_1	D_2	D_3	D_4		
Moisture	80.41	68.76^d	78.77^a	78.29^b	74.75^c	1.207	*
Dry matter (DM)	19.59	31.24^a	21.23^d	21.71^c	25.25^b	1.207	*
			Chemical analysis on DM basis				
Organic matter (OM)	82.04	88.44^a	84.47^b	81.05d	82.95^c	0.823	*
Crude protein (CP)	65.56	60.23^c	64.10^a	62.25^b	61.52^b	0.437	*
Ether extract (EE)	16.48	28.21^a	20.37^c	18.80^d	21.43^b	1.090	*
Ash	17.96	11.56^d	15.53^c	18.95^a	17.05^b	0.823	*
Gross energy kcal/ 100g	525.33	605.47^a	553.64^b	528.43^c	549.03^b	8.593	*
Gross energy cal/ g DM	5.2533	6.0547^a	5.5364^b	5.2843^c	5.4903^b	0.086	*

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05), SEM: Standard error of mean
*: Significant at *P*< 0.05, Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968) and MacRae and Lobley (1982)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.

Table 7. Energy retention (ER) and protein productive value (PPV)% of *O. niloticus* feeding on different concentrations of methionine

Item	Tested diets				SEM	Sign. *P*<0.05
	Control zero methionine	5g methionine /kg diet	10g methionine /kg diet	15g methionine /kg diet		
	D_1	D_2	D_3	D_4		
Initial weight (IW), g	84	83	85	82	0.485	NS
Final weight (FW), g	223^d	250^c	276^b	292^a	8.155	*
Calculation the energy retention						
Energy content in final body fish (cal / g)	6.0547^a	5.5364^b	5.2843^c	5.4903^b	0.086	*
Total energy at the end in body fish (E)	1350^c	1384^c	1458^b	1603^a	30.87	*
Energy content in initial body fish (cal / g)			5.2533			
Total energy at the start in body fish (E_0)	441	436	447	431	2.565	NS
Energy retained in body fish ($E-E_0$)	909^c	948^{bc}	1011^b	1172^a	31.37	*
Energy of the feed intake (Cal / g feed)	4.512	4.537	4.535	4.542	-	-
Quantity of feed intake	428^c	445^{ab}	443^b	452^a	2.841	*
Total energy of feed intake (EF)	1931^c	2019^c	2009^b	2053^a	14.28	*
Energy retention (ER)%	47.07^c	46.95^c	50.32^b	57.09^a	1.29	*
Calculation of the protein productive value (PPV)%						
Crude protein % in final body fish	60.23^c	64.10^a	62.25^b	61.52^b	0.437	*
Total protein at the end in body fish (PR_1)	134.31^c	160.25^b	171.81^{ab}	179.64^a	5.372	*
Crude protein % in initial body fish			65.56			
Total protein at the start in body fish (PR_2)	55.07	54.41	55.73	53.76	0.318	NS

Protein Energy retained in body fish (PR_3) = (PR_1 – PR_2)	78.93^{c}	105.84^{b}	116.08^{ab}	125.88^{a}	5.454	*
Crude protein in feed intake (CP%)	29.64	29.62	29.57	29.58	-	-
Total Protein intake (PI), g	126.86^{c}	131.81^{ab}	131.00^{b}	133.70^{a}	0.816	*
Protein productive value (PPV)%	62.22^{c}	80.30^{b}	88.61^{a}	94.15^{a}	3.758	*

a, b and c: Means in the same row having different superscripts differ significantly (P< 0.05).
SEM: Standard error of mean. NS: Not significant *: Significant at P< 0.05.

Economical evaluation of different experimental groups

As shown in Table (8), the results of economic analysis revealed that although incorporating methionine in feed formulation increased the cost of feed formulation from 24.700 LE in the control diet (D1) to 25.075, 25.450, and 25.800 LE per ton for the other diets (D2, D3, and D4, respectively), there was a net improvement realized by 13.85, 25.44, and 31.54% for D2, D3, and D4, respectively, compared to the control, which received a diet without methionine.

Table 8. Economical evaluation of *O. niloticus* feeding on different concentrations of methionine

Item	Tested diets			
	Control zero methionine	5g methionine /kg diet	10g methionine /kg diet	15g methionine /kg diet
	D_1	D_2	D_3	D_4
Costing of kg feed (LE)	24.700	25.075	25.450	25.800
Relative to control (%)	100	101.52	103.04	104.45
Feed conversion ratio (FCR)	3.08	2.66	2.32	2.15
Feeding cost (LE) per (kg weight gain)	76.08	66.70	59.04	55.47
Relative to control (%)	100	87.67	77.60	72.91
Net improving in feeding cost (%)	Zero	13.85	25.44	31.54

LE.: Egyptian pound
Diet formulation calculated according to the local prices at year 2023, as presented in Table (1)
Feed cost (L.E) FCR×FI. Cost per diet.

DISCUSSION

The current results on growth performance and survival rates across different experimental groups demonstrated increases in final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and survival rate (SR) when fish were fed diets containing 5, 10, and 15g of methionine per kg of diet, compared to the control group. Furthermore, the mortality rate was zero in the methionine-treated groups, while it was 6.67% in the control group. Feed utilization parameters, including feed intake (FI), feed conversion ratio (FCR), crude protein intake (CPI), and protein efficiency ratio (PER), also increased with methionine inclusion. Specifically, both FCR and PER improved with 5, 10, and 15g of methionine per kg of diet.

These findings align with those of **El-Saidy and Gaber (1997)**, who studied the total replacement of fish meal with soybean meal supplemented with various levels of L-methionine (0.5, 1, 1.5, and 2%) in diets for the Nile tilapia (*Oreochromis niloticus*) fry. After 12 weeks, they observed significant differences ($P< 0.01$) in final individual weight, weight gain percentage, SGR, FCR, PER, and food intake among the fish groups. The best performance metrics were recorded in the group receiving a 1% L-methionine diet. Survival rates did not differ among the groups, and no morphological differences were observed between the control and methionine-supplemented diets.

Additionally, **El-Saidy and Gaber (1997)** noted that when the protein level was below 30%, fish-fed diets containing fish meal had higher weight gains compared to those without fish meal. They suggested that the essential amino acid profiles of the lower-protein diets might have been inadequate when soybean meal completely replaced fish meal. They also observed significant differences ($P< 0.01$) in FCR, PER, and feed intake between treatments.

In contrast, **Tacon *et al*. (1984)** found that adding 0.2% L-methionine to a methionine-deficient diet for the rainbow trout (*Oncorhynchus mykiss*) did not improve growth compared to the control diet. However, **Shiau *et al*. (1987)** reported that methionine supplementation improved growth performance in the tilapia. Furthermore, **Murai *et al*. (1986)** found that adding 0.4% crystalline L-methionine enhanced the nutritional value of soybean meal. **Thebault (1985)** and **Murai *et al*. (1986)** demonstrated that crystalline methionine is rapidly absorbed in fish and converted into methionine sulfoxide. Recently, **Yossa *et al*. (2023)** showed that dietary methionine significantly ($P< 0.05$) influenced final body weight (FBW), weight gain (WG), thermal-unit growth coefficient (TGC), PER, and hepatosomatic index (HSI).

Biochemical analyses are crucial for assessing the physiological status of fish, particularly when introducing new food additives or growth promoters, as they help identify potential adverse effects **(Rashidian *et al*., 2020; Authman *et al*., 2021)**. Parameters such as protein, albumin, and globulin provide insights into fish health, nutritional impacts, and immunological responses to external stimuli. In this study, both protein and albumin levels increased in the methionine-treated groups compared to the control. Methionine, an essential amino acid, enhances crude protein, total amino acids, and crude lipid levels in the whole body and liver **(Sui *et al*., 2023)**.

Liver enzymes, specifically ALT and AST, are biomarkers for liver function. Their unchanged levels in this study indicate methionine's positive effect on fish. Glucose and cholesterol levels are stress-sensitive indicators. The unchanged cholesterol levels in methionine-treated groups suggest no adverse effects from using methionine as a food additive to boost fish growth. However, the decreased glucose levels in fish receiving higher methionine doses (1 and 1.5%) may be attributed to methionine's metabolic effects, including reduced fasting insulin and blood glucose levels **(Stone *et al*., 2014)**.

Furthermore, the elevated uric acid levels in methionine-treated groups reflect increased protein and amino acid content. This finding aligns with that of **Tombarkiewicz *et al*. (2024)**, who observed higher uric acid levels in chickens following high methionine doses.

Feeding the Nile tilapia diets with varying levels of methionine significantly ($P < 0.05$) increased fish body composition in terms of moisture, crude protein, and ash content. Conversely, values for dry matter, organic matter, ether extract, and growth energy decreased. According to **El-Saidy and Gaber (1997)**, fish flesh compositions did not differ significantly ($P < 0.05$) between treatments, averaging 75.87% moisture, 16.46% fat, and 9.69% ash. However, protein content differed significantly ($P < 0.01$), with the best results observed in fish fed a diet containing 1% methionine. This suggests that a diet supplemented with 1% L-methionine can completely replace fish meal in a diet for the Nile tilapia fry, without negatively affecting fish performances and feed utilization.

Agbo *et al*. (2014) found no significant differences in whole-body protein, moisture, lipid, and energy contents among diets, except in the group fed a diet with 1.5% methionine, which had lower protein content. These values are higher than the initial whole-body composition. Ash content in fish fed the control diet was significantly higher than in those fed plant-based diets. There was also a significant increase in protein and lipid, compared to initial carcass values in all dietary treatments, in *Tilapia zillii* as reported by **Polat (1999)**, in the rohu (**Mukhopadhyay & Ray, 2001)** and in the Nile tilapia **(El-Saidy & Gaber, 2002)**.

Including 0.50% methionine in the Nile tilapia diets had no significant effect (on energy retention (ER%) compared to control and two other groups. However, fish fed 10 or 1.5g methionine/ kg diet showed significant increases in ER% compared to D1 and D2, with improvements of 6.90 and 21.29%, respectively. Protein productive value (PPV%) significantly increased with different levels of methionine, showing improvements of 129.06, 142.41, and 151.32% for fish receiving 5, 10, and 15g methionine/kg diet (D2, D3, and D4, respectively) compared to control (D1).

The economic analysis indicated that, although incorporating methionine into feed formulations increases the cost, diets containing methionine (D2, D3, and D4) showed a net improvement over the control diet without methionine. **Agbo *et al*. (2014)** reported that the cost-effectiveness evaluation of their study showed that the basal diet with methionine additives has a slight decrease in kilogram cost production. They also noted that DL-methionine supplementation in diets did not enhance cost-effectiveness. Similarly, **Jauncey (1998)** observed that supplementing essential amino acids in the tilapia feeds led to growth improvements that justified the additional costs. Methionine might be more expensive than other commercial options since it was priced based on feed grade. Thus, finding cheaper sources of methionine could significantly enhance cost-effectiveness, especially for diets 2, 3, and 4, aligning with **Agbo *et al*. (2014)**.

Abozaid *et al*. (2024) reported that net revenue increased by 6.80, 9.47, and 19.03% when *Saccharomyces cerevisiae* was included at 4, 8, and 12g/ kg diet for D2, D3, and D4 of the Nile tilapia, respectively, compared to the control group without *Saccharomyces cerevisiae*. **Goda *et al*. (2012)** noted that rising feed prices are a significant factor limiting profitability in fish culture and recommended a diet containing 1g of *Saccharomyces cerevisiae* per 100g as the most cost-effective for culturing the Nile tilapia fingerlings. Economic efficiency encompasses both technical or productive efficiency and price efficiency. **Azevedo *et al*. (2015)** demonstrated that economic efficiency results from multiplying productive efficiency by price efficiency. **Abareethan and Amsath (2015)** highlighted that probiotics can reduce feed costs, which is crucial for evaluating the practicality of fish diets. Probiotics not only enhance aquatic animal performance but also improve feed utilization, growth performance, immunity, and stress tolerance while preventing intestinal disorders and pre-digesting anti-nutritional factors **(Suzer *et al*., 2008)**.

CONCLUSION

Based on our observations, incorporating methionine into fish diets improved growth performance and feed utilization, reduced glucose levels, increased crude protein content in fish body composition, enhanced both energy retention and protein productive values, and increased the net improvement in the feeding cost.

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