Ann. Anim. Sci., Vol. 24, No. 1 (2024) 151–160 *DOI: 10.2478/aoas-2023-0059*

sciendo

DIETARY EFFECT OF S-METHYLMETHIONINE SULFONIUM CHLORIDE ON GROWTH, SERUM BIOCHEMICAL PARAMETERS, BODY COMPOSITION, AND EXPRESSION OF SOME RELATED GENES IN *OREOCHROMIS NILOTICUS*

Ahmed G.A. Gewida[1], Tarek Kamal Abouzed[2], Mohamed F. Abdelghany[1], Doaa K. Khames[3], Mohamed M. Zayed[4], Hanan B. Elsawy[5], Marwa F. AbdEl-Kader[6], Mohammed A.E. Naiel[7]♦

[1]Department of Fish Production, Faculty of Agriculture, Al-Azhar University, Cairo 11884, Egypt
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[3]Fish Nutrition Department, Central Lab for Aquaculture Research (CLAR), Abassa, Agriculture Research Center, Giza, Egypt
[4]Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh, Egypt
[5]Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[6]Department of Fish Health and Management, Sakha Aquaculture Research Unit, Central Laboratory for Aquaculture Research, A.R.C, Kafrelsheikh, Egypt
[7]Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt
♦Corresponding author: Mohammednaiel.1984@gmail.com; mnaiel@zu.edu.eg

Abstract

The main objective of the current trial was to investigate the impacts of tilapia diets supplemented with S-methylmethionine sulfonium chloride (MMSC) on the growth performance, feed efficiency, body analysis, blood biochemistry and regulation of myostatin (*MSTN*) and insulin-like growth factor-1 (*IGF-1*) genes of Nile tilapia (*Oreochromis niloticus*). The experimental fish (*n*=180) weighing 22.4±0.17 g were allocated randomly into three equal groups in triplicate as follows; the control group received an un-supplemented diet; the other two groups received two different levels of MMSC (0.2 and 0.4 g/kg) for eight weeks. The obtained findings demonstrated that tilapia diets enriched with small dosages of MMSC considerably (P<0.05) enhanced all assessed growth performance and feed efficiency indicators when compared with the control group. When compared to the control group, tilapia meals supplemented with 0.2 g MMSC significantly (P<0.05 or 0.01) raised blood protein profile, particularly total protein and globulin levels. Contrarily, blood creatinine levels were significantly (P<0.01) reduced in the group of tilapia fed diets containing MMSC (0.2 or 0.4 g/kg) compared to the group without receiving any supplementation. Whereas, fish body analysis showed higher significant increases in crude protein and ether extract levels (P<0.01) as well as reduced ash content (P<0.05) in the fish group that received lower MMSC doses in comparison to other treated and control groups. In addition, MMSC dietary supplementation significantly downregulated the expression of *MSTN* and upregulated IGF-1 mRNA expression compared with the control group. Additionally, both dosages of MMSC supplementation modestly enhanced the intestinal villus histomorphometric score indices with observed tall, thick, and various broad tips in comparison to the control group. In conclusion, it could be recommended that fish diets supplemented with 0.2 g MMSC per kg diet may promote the growth and general health status of Nile Tilapia.

Key words: vitamin U, growth, performance, tilapia, intestinal structure, blood biochemical, myostatin

Recently, the aquaculture sector has grown remarkably in comparison to other food production industries, mostly to meet the growing demand for fish as food, notably in a few underdeveloped countries where fish is regarded as the primary source of protein (FAO, 2018). Nile tilapia (*Oreochromis niloticus*) is regarded as one of the most important cultured species of tilapia because of its rapid growth rate, high survival rate in high-density crops, and high resistance to disease (El-Sayed, 2006; Sheikhzadeh et al., 2022). In recent years, higher production costs, primarily the feed, in addition to other production challenges, have led to decreased profit margins and account for approximately 70% of total costs (Macfadyen and Dawson, 2012; El-Sayed et al., 2015; Eltholth et al., 2015). Besides, stocking fish at high densities under intensive culture systems increases the risk of infectious diseases, leading to economic losses owing to higher chemotherapeutic costs and mortality rates (Naiel et al., 2021 a; Raza et al., 2022).

Furthermore, overuse of antibiotics in fish therapy causes antibiotic resistance in fish, which is considered to be a major hazard to human public health since antibiotic-resistant genes may transmit to animals, humans, and other aquatic species (Abdel-Latif et al., 2022; Naiel et al., 2023 b). Hence, it is intended to reduce the risk of pathogens in farmed fish by boosting their resistance to infections through the administration of better nutritional formulations, vaccinations, and immunostimulants (Naiel et al., 2021 c; Negm et al., 2021).

DL-methylmethionine sulfonium chloride (MMSC) is a naturally occurring amino acid derivative of methionine that exists in many plants, such as cabbage (Bourgis

et al., 1999; Kubińska et al., 2014). Although MMSC is not a true vitamin, it is categorized as vitamin U because of its role in preventing and curing gastrointestinal ulcers (Oztay et al., 2020). This property has led to the widespread use of MMSC as an effective therapy to prevent gastrointestinal ulcers (Kopinski et al., 2007). It has previously been reported that MMSC dietary supplementation increased weight gain and reduced feed conversion rate in various fish species (Rónyai et al., 2001).

Expression of the insulin-like growth factor-1 (IGF-1) gene provides an almost-instantaneous indicator of the growth status of Nile tilapia (Brown et al., 2012). Also, it is well reported that the correlation between IGF-1 mRNA levels in plasma or hepatic tissue and growth is remarkably strong and highly significant in tilapia fish (Zhong et al., 2022). Besides, alterations in blood IGF and hepatic IGF-1 mRNA levels may also be detected after two weeks of feeding fish under various dietary regime impacts (Cruz et al., 2006). In addition, MMSC is involved in producing various tissues, including muscles and bone (Bikle et al., 2015). The relative accuracy and efficiency of quantification of liver messenger RNA (mRNA) has been established for MMSC as a growth-regulating compound and its applicability as a growth indicator or marker in tilapia (Brown et al., 2012).

Therefore, this study sought to evaluate the effectiveness of an MMSC-supplemented diet in Nile tilapia on growth performance, feed efficiency, and serum biochemical indicators. The study also explored the possible involvement of specific genes that can promote weight gain, such as IGF-1 and MSTN.

Material and methods

Preparation of experimental diets

Three iso-nitrogenous diets (30% crude protein content) were formulated, containing three tested levels of MMSC (0.0, 0.2 and 0.4 g per kg diet), designated as T_0, $T_{0.2}$, and $T_{0.4}$, respectively. The MMSC product was purchased from Sigma-Aldrich Co. LLC., USA (MW, 199.70 g/mol; impurities, ≤0.002% heavy metals). The MMSC examined doses were chosen based on previous findings (Rónyai et al., 2001). In addition, the contents of Table 1 disclosed the ingredients and chemical analysis of the diets that were prepared. The feed ingredients were mechanically mixed well to ensure that the MMSC level harmonized well with the other feedstuffs, and then pelletized using a meat mincer with a 1.5-mm diameter, as described by Ibrahim et al. (2020). Then, the prepared diets were dried to a constant weight at 40°C and kept in a plastic bag at −4°C until further usage.

Experimental fish and feeding regime

A healthy Nile tilapia (*O. niloticus*) was obtained from the breeding pond of a commercial hatchery. The experimental fishes were maintained for three weeks to adjust from the fishery to farm conditions, and then the fish (22.4±0.17 g) were randomly distributed to floating cages (1 × 1 × 1 m^3). The fish were randomized into three cages as a control group and two treatment groups, with 20 fish in each group and three replications of each group (180 fish total: 60 controls and 120 treated fish; 60 for each diet group of 0.2 g/kg or 0.4 g/kg). For the feed test, three cement tanks were used, each with a water volume of 8 cubic meters. Each cement tank consists of three floating cages (one for each treatment).

Table 1. Ingredients and proximate chemical composition (% on dry matter basis) of diets containing different levels of vitamin U (S-methyl methionine)

Ingredients	Experimental diets (g/kg)		
	$T_{0.0}$	$T_{0.2}$	$T_{0.4}$
Fish meal	100	100	100
Soybean meal	432	432	432
Ground corn	205	205	205
Wheat bran	160	160	160
Starch	35	34.8	34.6
Cod fish oil	23	23	23
Corn oil	15	15	15
Mineral premix[1]	20	20	20
Vitamin premix[2]	10	10	10
Vitamin U	00	0.2	0.4
Total	1000	1000	1000
Proximate chemical analysis (%)			
dry matter	91.72	91.77	91.75
crude protein	30.15	30.11	30.02
ether extract	8.10	8.13	8.20
crude fiber	4.83	4.71	4.86
total ash	8.22	8.40	8.45
NFE[3]	48.7	48.65	48.47
GE (Kcal/100 g)[4]	446.67	446.52	445.93

[1]Mineral premix (per kg of premix): $CaHPO_4 \cdot 2H_2O$, 727.2 g; $MgCO_3 \cdot 7H_2O$, 127.5 g; KCl 50.0 g; NaCl, 60.0 g; $FeC_6H_5O_7 \cdot 3H_2O$, 25.0 g; $ZnCO_3$, 5.5 g; $MnCl_2 \cdot 4H_2O$, 2.5 g; $CuCl_2$, 0.785 g; $CoCl_3 \cdot 6H_2O$, 0.477 g; $CaIO_3 \cdot 6H_2O$, 0.295 g; $CrCl_3 \cdot 6H_2O$, 0.128 g; $AlCl_3 \cdot 6H_2O$, 0.54 g; Na_2SeO_3, 0.3 g.

[2]Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[3]Nitrogen free extract (NFE) = 100 – (protein % + lipid % + total ash % + crude fiber %).

[4]Gross energy (GE) was calculated according to NRC (1993) as 5.64, 9.44, and 4.11 kcal/g for protein, lipid, and NFE, respectively. (T_0, $T_{0.2}$ and $T_{0.4}$ are 0.0, 0.2 and 0.4 S-methylmethionine g/kg diet) respectively.

The water in each cement tank was exchanged at a rate of 10% per day. Throughout the experiment, a 5-HP blower (Everest Industries, New Delhi, India) was used to continuously provide aeration.

Fish sampling was conducted every two weeks by collecting all the fish stocked in each cage to measure body weight. During the feeding trial, the water quality parameters were controlled bi-weekly, with no significant

difference caused by the addition of MMSC. The average water temperature was 28.61±1.04°C, dissolved oxygen was 5.64±0.46 mg/L, pH was 7.10±0.23, and total ammonia concentration was 0.22±0.02 mg/L.

Growth performance, feed utilization, and survival rate

During 56 days feeding trial period, examined diets were offered twice daily (9:00 and 17:00 h) until apparent satiation. The amount of satiation was validated by apparent visual satiety. Diets were carefully handed out, ensuring no pellets remained on the bottom of the cage. While, the surviving fish were weighed every two weeks to determine consumed feeds and estimate biomass weight. Fish growth indicators, utilization of consumed feed, and survival rate were calculated as follows:

$$\textit{Weight gain (WG, g)} = FW - IW$$

$$\textit{Specific growth rate (SGR; \% g per day)} = 100 \times ((\ln FW - \ln IW) \div T)$$

Where *IW* is the initial weight (g), *FW* is the final weight (g), and *T* is the experimental period (days).

Feed intake (g feed/fish) = total feed consumed throughout the experiment ÷ fish number per cage.

Feed conversion ratio (FCR, g/g) = feed intake (g) ÷ weight gain (g).

Protein efficiency ratio (PER, g/g) = weight gain (g) ÷ protein intake (g).

Apparent protein utilization (APU, %) = 100 x (protein gain ÷ protein intake).

Energy utilization (EU, %) = 100 x (energy gain ÷ energy intake).

Survival rate (SR, %) = 100 x (final fish number ÷ initial fish number).

Proximate analyses

For proximate fish flesh and diet analysis, total moisture content was estimated applying the hot air oven by drying the sample at 105±2°C until a fixed weight was reported (AOAC, 1990). While, total lipid was assessed using Bligh and Dyer procedure applying chloroform/methanol (1/1, v/v) (Bligh and Dyer, 1959). Crude protein concentrations were calculated by converting the nitrogen content obtained using Kjeldahl's procedure (N×6.25) (AOAC, 1990). Finally, ash content was estimated after combustion in muffle for 20 h at 550°C (AOAC, 1990). All analyses were carried out on three different fish selected from each cage. The proximate composition of fish flesh and diet samples was determined in triplicate following Ayanda et al. (2019).

Histopathological and morphometric assessment of intestine

At the experiment's end, three fish from each treated group were anesthetized with buffered tricaine mesylate (30 mg/L), and the interior part of the intestines was dissected and extracted to measure the intestinal morphology. The intestine samples were fixed in 15% neutral formalin buffer for 48 hours, dehydrated in ethanol (70−100%), cleared in xylene, and embedded in paraffin. A microtome (Leica RM 2155, UK) was used to cut a paraffin thickness of 5 microns. Next, the slices were prepared by routine staining with hematoxylin and eosin (H&E) dyes for histopathological examination (Suvarna et al., 2018). All cross-sectional photos were photographed with a Leica microscope and an AmScope digital microscope camera. The lesion scoring system was designed as follows: 0, no lesions (0%); 1, minimal (0−25%); 2, mild (30−50%); and 3, moderate to severe (>50%) lesions detectable on histopathology.

Serum biochemical parameters analysis

After the termination of the feeding trial, three fish were randomly collected from each treatment, maintained in a fasting condition for 24 hours before blood collection, and anesthetized by adding a few drops of clove oil to the water. A sterile syringe placed in a centrifuge tube was used to collect a blood sample from the tail vein. The sample was allowed to clot at room temperature and then centrifuged at 2000×g for 10 minutes to collect serum, which was then stored at −20°C until analysis.

A specific kit (Biodiagnostic Company, Dokki, Giza, Egypt) was used to measure total protein albumin (Lowry et al., 1951). Globulin was determined by subtracting albumin from total proteins, and the albumin/globulin ratio was determined by dividing the albumin value by the globulin value.

RNA extraction and real time-polymerase chain reaction

Qiazol reagent was used to extract total RNA from liver and muscle samples. The concentration and purity of RNA were determined by spectrophotometry at 260/280 nm. A Quanti Tect Reverse Transcription Kit (Qiagen, Valencia, California, USA) was used to reverse transcribe 1 µg of total RNA into complementary single-stranded DNA in a two-step real-time-polymerase chain reaction (RT-PCR) using random hexamer primers. The first step was to use a Wipeout Buffer to remove any genomic DNA contamination. The housekeeping genes *β-Actin* and *GAPDH* were applied as an internal reference control. Table 2 shows the sequences of all used PCR primers. The thermal cycling conditions were as follows: incubate at 95°C for 5 minutes; then perform 40 cycles: 94°C for 20 s and 60°C for 30 s, and 72°C for 30 s. The identity and specificity of PCR products was confirmed by applying melting curve analysis. A Rotor-Gene Q (Qiagen) was used to collect data automatically and then analyze the threshold cycle value (C^t). The $2^{-\Delta\Delta Ct}$ protocol was applied to obtain the relative expression of both the *MSTN* and *IGF-1* mRNA.

Table 2. The PCR primers sequences

Gene	Primer sequence	Accession number	Size (pb)
IGF-1-F	5′ AGTTTGTCTGTGGAGAGCGA3′	NM_001279503.1	549
IGF-1-R	5′ CACAGTACATCTCAAGGCGC3′		
MSTN-F	5′ CACTGTGGACTTCGAGGACT3′	FJ972683.1	2413
MSTN-R	5′ CTCTGGGGTTGGCTTTGTTC3′		
β-actin-F	5′ ACCTTCAACACCCCCGCCAT 3′	EU887951.1	543
β-actin-R	5′ ACAGGGACAGCACAGCCTGGAT 3′		
GAPDH-F	5′ GGAGAAAGCTGCTAA 3′	NM_008084	1257
GAPDH-R	5′ ACGACCTGGTCCTCGGTGTA 3′		

IGF-1, insulin-like growth factor-1; MSTN, myostatin gene; ***β***-actin, beta-actin.

Statistical analysis

All results were expressed as the mean ± standard error of the mean of the three replicates of each measurement variable. All data analysis was performed by one-way analysis of variance (ANOVA) to evaluate the effect of MMSC supplementation; the difference between the means was tested at a probability level of 5%, with the use of Duncan's test as a post-test. Data and the uniformity of variance were determined by the Kolmogorov-Smirnov test. All statistical analyses were performed using the statistical software package SPSS version 20 (SPSS, Richmond, Virginia, USA).

Results

Growth performance and feeding efficiency

After 8 weeks of feeding with MMSC, both the growth performance (defined by final weight, weight gain, and specific growth rate) and the feed utilization efficiency parameters (defined by feed conversion ratio, protein efficiency ratio, and apparent protein utilization) were significantly improved ($P<0.05$) for the treatment groups at both dietary levels (0.2 g/kg and 0.4 g/kg) compared with the control group (Table 3).

Proximate analysis

The whole-body composition of Nile tilapia was significantly affected ($P<0.05$) by dietary MMSC supplementation, except for moisture content (Table 4). Body protein and lipid content significantly increased ($P<0.05$), whereas ash content decreased, increasing with supplementation up to 0.4 g/kg.

Serum biochemical parameters analysis

The results showed no significant differences between the control group and groups that received 0.2 g/kg and 0.4 g/kg MMSC in the serum level of ALT enzyme activity, calcium, magnesium and phosphorus (Table 5). In comparison to the control group, supplemented tilapia diet with 0.2 g/kg or 0.4 g/kg MMSC considerably increases total protein and globulin levels, while low dose of MMSC significantly increases albumin and A/G ratio**.**

Histological examination

The histological examination of H&E-stained fish intestine (Figure 1) revealed partial villi development, with tall and broad tip formations, as well as intact villus tips with microvilli, and mildly intraepithelial lymphocytic infiltrations in the fish group that received 0.2 g MMSC supplemented diets. While fish fed diets supplemented with 0.4 g MMSC/kg showed the enhanced formation of intestinal villi, tall and thick with notable wide tips, and increased absorptive surface characterizations, as well as prominent inter-epithelium lymphocytic infiltrations and spindle cells in the lamina propria. Furthermore, nutritional treatment with MMSC increased the villus height and crypt depth of the jejunum mucosa (Table 6).

***IGF-1* and MSTN mRNA expression**

The group with 0.2 g MMSC supplementation showed significantly ($P\leq0.05$) upregulated *IGF-1* mRNA expression compared with the control group. In contrast, the 0.4% MMSC supplementation group showed non-significantly decreased *IGF-1* mRNA expression compared with the control group and highly significant downregulation compared with the 0.2% group (Figure 2).

Regarding *MSTN*, the 0.2% MMSC supplementation group showed downregulated MSTN mRNA expression significantly ($P\leq0.05$) by 1.5-fold compared with controls. In contrast, the 0.4% MMSC supplementation group showed no significant decrease in *MSTN* mRNA expression compared with controls and highly significant downregulation compared with the 0.2% group (Figure 2). In addition, MMSC supplementation significantly ($P\leq0.05$) downregulated *MSTN* mRNA expression compared with the control group (Figure 2).

Table 3. Growth performance parameters, survival rate, and feed efficiency of Nile tilapia fish fed diets supplemented with graded amounts of vitamin U (S-methylmethionine) for 56 days

Parameters	Experimental diets (g/kg)			P value
	$T_{0.0}$	$T_{0.2}$	$T_{0.4}$	
IW (g)	22.48±0.17	22.28±0.04	22.36±0.08	0.124
FW (g)	49.41±0.37 c	54.37±0.35 a	52.50±0.18 b	0.045
WG (g)	26.93±0.36 c	32.09±0.38 a	30.14±0.25 b	0.001
SGR (% g/day)	1.41±0.02 c	1.59±0.09 a	1.52±0.03 b	0.041
TFI (g/fish)	41.88±0.84 a	45.04±0.55 b	44.95±0.21 b	0.014
FCR (g/g)	1.56±0.19 a	1.40±0.19 c	1.49±0.09 b	0.010
PER	1.74±0.28 c	1.95±0.01 a	1.84±0.08 b	0.042
APU (%)	3.16±0.08 c	7.84±0.17 a	6.34±0.12 b	0.021
EU (%)	0.58±0.03 a	0.51±0.01 c	0.54±0.03 b	0.003
SR (%)	100	100	100	0.894

IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; SR, survival rate; TFI, total feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; APU, apparent protein utilization; EU, energy utilization. T_0, $T_{0.2}$ and $T_{0.4}$ are 0.0, 0.2 and 0.4 S-methylmethionine g/kg diet, respectively. Values in the same row with different letters are significantly different at $P<0.05$. SEM: standard error of the mean.

Table 4. Body chemical analysis (% on dry matter basis) of Nile tilapia fish fed diets supplemented with graded amounts of vitamin U (S-methyl methionine) for 56 days

Parameters	Experimental diets (g/kg)			P value
	$T_{0.0}$	$T_{0.2}$	$T_{0.4}$	
DM	24.12±0.06	24.72±0.01	24.61±0.04	0.652
CP	59.37±0.32 c	61.17±0.9 a	60.42±0.03 ab	0.005
EE	18.24±0.40 b	19.64±0.13 a	18.72±0.05 ab	0.012
Ash	22.21±0.23 a	18.19±0.42 b	19.92±0.47 b	0.022

DM, dry matter; CP, crude protein; EE, ether extract. Values in the same row with different letters are significantly different at $P<0.05$. T_0, $T_{0.2}$ and $T_{0.4}$ are 0.0, 0.2 and 0.4 S-methylmethionine g/kg diet, respectively.

Table 5. Blood biochemical constituents of Nile tilapia fish fed diets supplemented with graded amounts of vitamin U (S-methylmethionine) for eight weeks

Parameters	Experimental diets (g/kg)			P value
	$T_{0.0}$	$T_{0.2}$	$T_{0.4}$	
TP (g/dl)	5.3±0.45 b	5.9±0.24 a	5.02±0.31 b	0.011
Albumin (g/dl)	1.1±0.04 b	1.0±0.01 b	1.50±0.02 a	0.034
Globulin (g/dl)	4.2±0.14 b	4.9±0.31 a	3.52±0.21 ab	0.021
A/G ratio	0.26±0.01 b	0.20±0.01 b	0.43±0.02 a	0.042
Creatinine (g/dl)	1.09±0.01 a	0.75±0.01 b	0.80±0.001 b	0.005
ALT (U/L)	25.01±1.5	24.12±1.4	24.05±1.6	0.067
Calcium (mg/dl)	8.8±0.45	9.1±0.23	9.3±0.78	0.321
Magnesium (mg/dl)	3.01±0.21	3.02±0.15	3.11±0.16	0.087
Phosphorus (mg/dl)	10.1±0.41	9.96±0.31	9.53±0.52	0.164

TP, total protein; ALB, albumin; GLOB, globulin; A/G ratio, albumin/globulin ration; ALT, alanine aminotransferase. Values in the same row with different letters are significantly different at $P<0.05$. T_0, $T_{0.2}$ and $T_{0.4}$ are 0.0, 0.2 and 0.4 S-methylmethionine g/kg diet, respectively.

Table 6. Score of enhancing of the intestine limit alteration in 0.0, 0.2 and 0.4 S-methylmethionine supplemented groups

Organ	Alterations	$T_{0.0}$	$T_{0.2}$	$T_{0.4}$
Intestine	Destructed tips	3	0	0
	Short villi	3	2	0
	Goblet cell hyperplasia	1	2	1
	Intraepithelial lymphocytic infiltrations	1	2	3
	Broad tips	0	2	3

(0 = no detectable histopathological lesion, 1 = minimal, 2 = mild, 3 = moderate to severe).

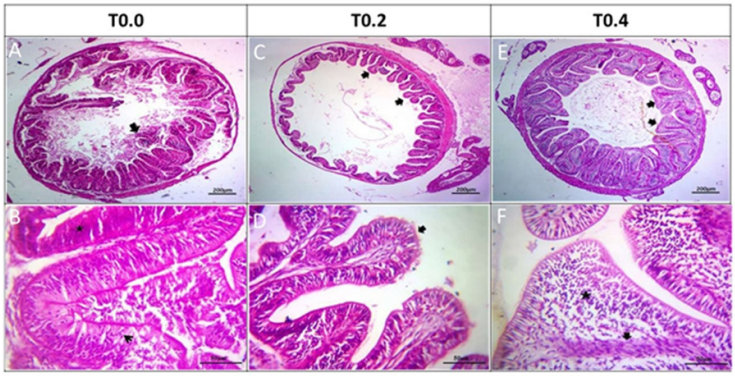

Figure 1. Representative photomicrographs of H&E-stained fish intestine in: A) control group, showing apparent villi (arrows); C) 0.2 g/kg group, showing partial development of the villi, with tall and broad tips formations (arrows); D) 0.2 g/kg group, showing intact villus tips with microvilli (arrow) in addition to mildly intraepithelial lymphocytic infiltrations; E) 0.4 g/kg group showing more development of intestinal villi, with tall and thick with marked broad tips (arrows), and increased absorptive surface characterizations; and F) 0.4 g/kg group showing marked inter-epithelium lymphocytic infiltrations (star), in addition to spindle cells in the lamina propria (arrow)

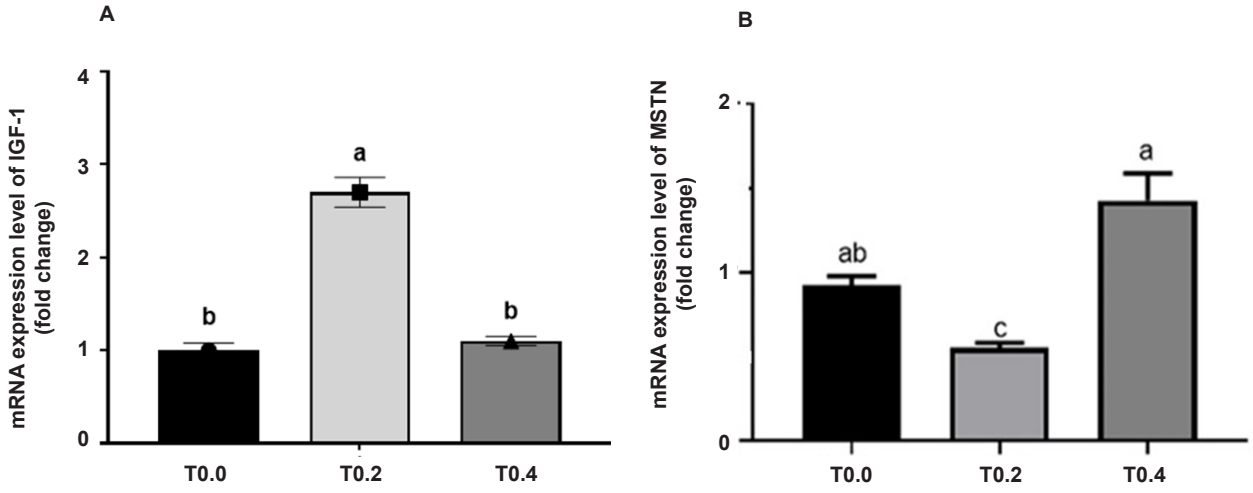

Figure 2. mRNA expression of *IGF-1* and *MSTN* of juvenile Nile tilapia fed on diets supplemented with vitamin U (S-methylmethionine) for eight weeks. Values with different superscripts are significantly different at $P<0.05$. (T_0, $T_{0.2}$ and $T_{0.4}$ are 0.0, 0.2 and 0.4 S-methylmethionine g/kg diet, respectively)

Discussion

Vitamin U is widely known as S-methylmethionine, or methylmethionine sulfonium (El-Saway et al., 2022). It is produced biologically from L-methionine and S-adenosylmethionine by the enzyme methionine S-methyltransferase in hepatocytes (Bradbury et al., 2014). Moreover, methionine or its derivatives were shown to be an important amino acid in most of plant ingredients applied into fish feed (Ceccotti et al., 2022). Therefore, the main purpose of this feeding investigation was to evaluate the effects of methionine-derived MMSC supplementation on performance, feed efficiency, blood constituents, body chemical composition and intestinal morphometric characteristics of Nile tilapia, as well as to investigate the key molecular mechanisms of the observed alterations.

Results show that a low dose of MMSC supplementation can effectively promote weight gain, and efficiency of consumed feed, and diminish the feed conversion ratio compared with an un-supplemented fish group. Also, the addition of MMSC to the tilapia diet enhances the protein efficiency ratio, apparent protein consumption, and energy utilization. Despite the fact that fish experiment trial data are scarce, it has previously been shown that including 25 mg MMSC/kg into broiler feed significantly increased weight gain by 3.6% and reduced feed conversion ratio by approximately 2.0% when compared to the control group (Shaw et al., 2009). Also, Kovaleva (1986) demonstrated that supplementing broiler diets with MMSC improved body weight by 11% while decreasing food consumption by 4.8% compared with an un-supplemented group. Moreover, the inclusion of naturally occurring sulfonium compounds (such as vitamin U) in fish

diet ingredients enhanced the growth and development of various teleost species, including rainbow trout, African catfish, red sea bream, carp, and goldfish (Nakajima, 1991; Rónyai et al., 2001). These findings suggest that the methyl group transferring pathway stimulates the release of both di-methylthetin-homocysteine (DMT-Hcys) and betaine-homocysteine (Bet-Hcys) S-methyltransferases enzymes from livers, which contribute significantly to the stimulation of growth and swimming movements of both red sea bream and yellowtail fish (Nakatsuru et al., 1987; Nakajima, 1993).

There is a substantial relationship between the physical anatomy of the gastrointestinal tract and the dietary practices of fish (Naiel et al., 2023 a). Also, it is possible to develop a fish immune defense system against infectious diseases by providing a balanced diet that supports gut health and digestion (Dawood, 2021; Abdelnour et al., 2023). In the present investigation, dietary MMSC supplementation resulted in increased villus height and crypt depth of the intestine. According to Hu et al. (2023), rice field eel (*Monopterus albus*) offered diets enriched with methionine significantly increased lipid digestion and absorption while also improving gut microbial balance. Furthermore, Gao et al. (2019) demonstrated that inadequate levels of methionine reduced the height of intestinal villus and microvilli, as well as the quantity of goblet cells in turbot (*Scophthalmus maximus* L.). This beneficial effect on the intestinal structure was shown to be strongly associated with higher levels of digestibility and absorption of essential nutrients, and it could boost growth performance (Negm et al., 2021). Besides, this impact might be attributed to MMSC's ability to heal and protect the gut from ulcer erosion (Elbers et al., 1995). Hence, because of its ability to improve gut health and physical structure, the MMSC might be used as a dietary growth stimulant for Nile tilapia.

Changes in total plasma or serum protein levels have been widely employed as a broad clinical indication of terrestrial and aquatic creatures' health status, stress, and welfare (Coeurdacier et al., 2011; Naiel et al., 2021 b). For instance, globulins, particularly gamma globulins, are critical for fish immune response and general health status (Naiel et al., 2022 a). Consequently, increasing blood globulin levels is regarded to be an important innate reaction in fish (Kumar et al., 2013; El-Bab et al., 2022). On the other hand, because fish can excrete creatinine via the kidney, creatinine blood levels might be employed to assess renal filtration performance (Gharib et al., 2022; Naiel et al., 2022 b). According to our findings, a small dosage of dietary MMSC supplementation increased total protein and globulin levels as well as decreased creatinine concentration compared with control group. The observed findings were found to be consistent with Azeredo et al. (2017) findings that treating European seabass cells with methionine improved immune responses via increased innate immune defenses such as nitric oxide and superoxide anion generation, as well as increased ATP yields.

It is generally understood that the flavor of fish flesh is directly connected to its protein and fat content, and that differences in these elements are key determinants of both customer choice and the quality of the processed products (Doğan and Ertan, 2017; Naiel et al., 2022 c). Early evidence revealed that methionine is a unique sulfur-based amino acid that can be utilized to build proteins and has a direct association with the cause or prevention of fatty liver (Peres and Oliva-Teles, 2001; Toohey, 2014; Ayanda et al., 2019). These prior findings supported our results that dietary MMSC supplementation at low doses significantly improved the crude protein and crude fat content of fish flesh. Besides, recent research indicates that sulfur-based substances, at appropriate low concentrations, may prevent fatty liver (Toohey, 2014). These benefits may be connected to the effectiveness of MMSC in boosting protein synthesis and enhancing meat quality, including fat content via stimulating *IGF-1* gene pathways (Espe et al., 2016).

The process of muscle growth is called myogenesis, which is a complex regulatory process that includes the interaction of several proteins. Myogenic regulatory factors and myocyte-enhancing factor 2 (*MEF2*) are two important transcriptional proteins that are positively related to skeletal muscle development (Xiao et al., 2017). Both *MSTN* and *IGF-1* are considered myogenic regulatory factors (Naya and Olson, 1999; Zanou and Gailly, 2013). The *IGF-1* downstream signaling cascade includes genes that play an important role in cellular proliferation and protein synthesis (Adams, 2002). As a powerful negative myogenic modulator, MSTN can cause muscle mass loss. While changing the composition of muscle fiber types, *MSTN* modulates the expression of myogenic differentiation factor 1 and MEF2 (Hennebry et al., 2009). Therefore, inhibition of its expression leads to increased muscle mass (Wang and McPherron, 2012). In addition, downregulation of *MSTN* expression can lead to muscle and muscle fiber hypertrophy (Wang and McPherron, 2012), whereas its overexpression decreases the quality and size of skeletal muscle fibers (Reisz-Porszasz et al., 2003).

Compared with the control group, the MMSC-supplemented groups at lower doses significantly increased the expression of *IGF-1* mRNA in liver, and this increased expression was accompanied by an increase in body weight. These findings are consistent with those for chickens as reported by Zhai et al. (2012) and Wen et al. (2017). They observed that the methionine supplementation upregulates *IGF-1* expression and thus can improve breast muscle weight and growth performance. Moreover, another study documented that the MMSC administration (1.4 g/kg diet) in chicks with a low level of choline or methionine can improve growth performance compared with controls (Augspurger et al., 2005). Furthermore, other research has shown that the addition of encapsulated methionine to the pelleted feed and the extruded feed significantly increases the expression of *IGF-1* in the muscle, whereas the addition of crystalline methionine only numerically increases the expression of

IGF-1 (Shan et al., 2017; Nazeemashahul et al., 2020). These results can be attributed to the stimulating effect of methionine on the gene expression of *IGF-1* encoding growth-related hormones, as well as its inhibitory effect on the expression of genes related to protein degradation and negative muscle regulators, such as myostatin (Del Vesco et al., 2015).

In addition, the results of the current trial indicate that supplemented tilapia diets with low levels of MMSC negatively regulates the expression of *MSTN* mRNA compared with control and fish group fed diets containing high level of MMSC. In support of these results, other studies note that methionine supplementation decreases *MSTN* mRNA expression in chickens (Wen et al., 2014). This effect is due to the methylation of the MSTN gene in the presence of methionine, which reduces its mRNA expression (Liu et al., 2010). Furthermore, the impact of lowering *MSTN* mRNA expression was discovered to be associated with body weight alteration, with the group supplemented with low MMSC levels achieving significant weight gain. Finally, it is possible that dietary methionine reduces *MSTN* mRNA expression via increasing methylation of an *MSTN* exon region (Liu et al., 2010).

Conclusions

This study suggests that incorporating a low dose of MMSC (0.2 g/kg) into tilapia diet significantly improves final weight gain, feed conversion ratio, and *IGF-1* expression when compared to the control group. This improvement was linked to elevated *IGF-1* mRNA expression, enhanced intestinal morphological structure, and diminished *MSTN* mRNA expression. When MMSC-treated groups were compared to the control group, serum biochemical measures were not altered. Thus, further research is required to study the molecular signals involved in the impact of MMSC supplementation. In addition, research is desired to identify the exact optimal dosage of MMSC concentrations for enhancing tilapia production.

Ethics approval and consent to participate

All experimental procedures used are approved from Institutional Animal Care and Use Committee − Zagazig University (Approval No: ZU-IACUC/2/F/472/2022), and follow the animal care guidelines and the National Science Council's Guide for the Care and Use of Laboratory Animals.

Data availability

Data are available upon request.

Competing interests

The authors announce that there is no conflict of interest in the publication of this article.

References

Abdel-Latif H.M., El-Ashram S., Yilmaz S., Naiel M.A., Kari Z.A., Hamid N.K.A., Dawood M.A., Nowosad J., Kucharczyk D. (2022). The effectiveness of *Arthrospira platensis* and microalgae in relieving stressful conditions affecting finfish and shellfish species: an overview. Aquacult. Rep., 24: 101135.

Abdelnour S.A., Ghazanfar S., Abdel-Hamid M., Abdel-Latif H.M., Zhang Z., Naiel M.A. (2023). Therapeutic uses and applications of bovine lactoferrin in aquatic animal medicine: an overview. Vet. Res. Communic., 1–15.

Adams G.R. (2002). Invited Review: Autocrine/paracrine *IGF-I* and skeletal muscle adaptation. J. Appl. Physiol., 93: 1159–1167.

AOAC (1990). Official Methods of Analysis of the Association of the Official Analysis Chemists. Association of Official Analytical Chemists (15th ed.). AOAC International, Washington, DC.

Augspurger N.R., Scherer C.S., Garrow T.A., Baker D.H. (2005). Dietary S-methylmethionine, a component of foods, has choline-sparing activity in chickens. J. Nutr., 135: 1712–1717.

Ayanda I.O., Ekhator U.I., Bello O.A. (2019). Determination of selected heavy metal and analysis of proximate composition in some fish species from Ogun River, Southwestern Nigeria. Heliyon, 5: e02512.

Azeredo R., Serra C.R., Oliva-Teles A., Costas B. (2017). Amino acids as modulators of the European seabass, *Dicentrarchus labrax*, innate immune response: an *in vitro* approach. Sci. Rep., 7: 18009.

Bikle D.D., Tahimic C., Chang W., Wang Y., Philippou A., Barton E.R. (2015). Role of *IGF-I* signaling in muscle bone interactions. Bone, 80: 79–88.

Bligh E.G., Dyer W.J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911–917.

Bourgis F., Roje S., Nuccio M.L., Fisher D.B., Tarczynski M.C., Li C., Herschbach C., Rennenberg H., Pimenta M.J., Shen T.L. (1999). S-methylmethionine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. Plant Cell., 11: 1485–1497.

Bradbury L.M., Ziemak M.J., El-Badawi-Sidhu M., Fiehn O., Hanson A.D. (2014). Plant-driven repurposing of the ancient S-adenosylmethionine repair enzyme homocysteine S-methyltransferase. Biochem. J., 463: 279–286.

Brown C.L., Cruz E.M.V., Bolivar R.B., Borski R.J. (2012). Production, growth, and insulin-like growth factor-I (IGF-I) gene expression as an instantaneous growth indicator in Nile tilapia *Oreochromis niloticus*. Funct. Genom. Aquacult., 79.

Ceccotti C., Biasato I., Gasco L., Caimi C., Bellezza Oddon S., Rimoldi S., Brambilla F., Terova G. (2022). How different dietary methionine sources could modulate the hepatic metabolism in rainbow trout? Curr. Issues Molec. Biol., 44: 3238–3252.

Coeurdacier J.L., Dutto G., Gasset E., Blancheton J.P. (2011). Is total serum protein a good indicator for welfare in reared sea bass (*Dicentrarchus labrax*)? Aquat. Liv. Res., 24: 121–127.

Cruz E.M.V., Brown C.L., Luckenbach J.A., Picha M.E., Bolivar R.B., Borski R.J. (2006). Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, *Oreochromis niloticus*. Aquaculture, 251: 585–595.

Dawood M.A. (2021). Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. Rev. Aquacult., 13: 642–663.

Del Vesco A.P., Gasparino E., De Oliveira Grieser D., Zancanela V., Soares M.A.M., De Oliveira Neto A.R. (2015). Effects of methionine supplementation on the expression of oxidative stress-related genes in acute heat stress-exposed broilers. Brit. J. Nutr., 113: 549–559.

Doğan G., Ertan Ö.O. (2017). Determination of amino acid and fatty acid composition of goldband goatfish [*Upeneus moluccensis* (Bleeker, 1855)] fishing from the Gulf of Antalya (Turkey). Int. Aquatic Res., 9: 313–327.

El-Bab A.F.F., Saghir S.A., El-Naser I.A.A., El-Kheir S.M.A., Abdel-Kader M.F., Alruhaimi R.S., Alqhtani H.A., Mahmoud A.M., Naiel M.A., El-Raghi A.A. (2022). The effect of dietary *Saccharomyces cerevisiae* on growth performance, oxidative status, and immune response of sea bream (*Sparus aurata*). Life, 12: 1013.

El-Saway H.B., Soliman M.M., Sadek K.M., Nassef E., Abouzed T.K. (2022). Beneficial impact of dietary methyl methionine sulfonium chloride and/or L-carnitine supplementation on growth performance, feed efficiency, and serum biochemical parameters

in broiler chicken: role of *IGF-1* and *MSTN* genes. Trop. Anim. Health Prod., 54: 98.

El-Sayed A.F.M. (2006). Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. Adv. Aquacult. Nutr., VIII Int. Symposium on Aquacult. Nutr., 15−17 November, Autonomous University of Nuevo Leon, Monterrey, Nuevo Leon, Mexico.

El-Sayed S.F., Hassan H.A., El-Mogy M.M. (2015). Impact of bio- and organic fertilizers on potato yield, quality and tuber weight loss after harvest. Potato Res., 58: 67–81.

Elbers A., Vos J., Hemke G., Hunneman W. (1995). Effect of hammer mill screen size and addition of fibre or S-methylmethionine-sulphonium chloride to the diet on the occurrence of oesophagogastric lesions in fattening pigs. Vet. Record., 137: 290–293.

Eltholth M., Fornace K., Grace D., Rushton J., Häsler B. (2015). Characterisation of production, marketing and consumption patterns of farmed tilapia in the Nile Delta of Egypt. Food Policy, 51: 131–143.

Espe M., Veiseth-Kent E., Zerrahn J.E., Rønnestad I., Aksnes A. (2016). Juvenile Atlantic salmon decrease white trunk muscle *IGF-1* expression and reduce muscle and plasma free sulphur amino acids when methionine availability is low while liver sulphur metabolites mostly is unaffected by treatment. Aquacult. Nutr., 22: 801–812.

FAO (2018). The State of World Fisheries and Aquaculture 2018 − Meeting the sustainable development goals. CC BY-NC-SA 3.0 IGO, Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome.

Gao Z., Wang X., Tan C., Zhou H., Mai K., He G. (2019). Effect of dietary methionine levels on growth performance, amino acid metabolism and intestinal homeostasis in turbot (*Scophthalmus maximus* L.). Aquaculture, 498: 335–342.

Gharib A.A., Abdel-Hamid E.A., Mousa M.A., Naiel M.A. (2022). Improving water quality, growth performance, and modulating some stress physiological biomarkers in *Cyprinus carpio* using raw date nuclei as a zinc adsorbent agent. Appl. Water Sci., 12: 159.

Hennebry A., Berry C., Siriett V., O'Callaghan P., Chau L., Watson T., Sharma M., Kambadur R. (2009). Myostatin regulates fiber-type composition of skeletal muscle by regulating *MEF2* and *MyoD* gene expression. American J. Physiol. Cell Physiol., 296: C525–C534.

Hu Y., Zhang J., Cai M., Chu W., Hu Y. (2023). Methionine-mediated regulation of intestinal lipid transportation induced by high-fat diet in rice field eel (*Monopterus albus*). Aquacult. Nutr., 2023: 5533414.

Ibrahim R.E., Ahmed S.A., Amer S.A., Al-Gabri N.A., Ahmed A.I., Abdel-Warith A.W.A., Younis E.S.M., Metwally A.E. (2020). Influence of vitamin C feed supplementation on the growth, antioxidant activity, immune status, tissue histomorphology, and disease resistance in Nile tilapia, *Oreochromis niloticus*. Aquacult. Rep., 18: 100545.

Kopinski J., Fogarty R., McVeigh J. (2007). Effect of s-methylmethionine sulphonium chloride on oesophagogastric ulcers in pigs. Australian Vet. J., 85: 362–367.

Kovaleva G. (1986). Efficient utilization of vitamins U and Bc in feed mixtures by broiler chickens. Ref. J., 9: 76–78.

Kubińska M., Tykałowski B., Jankowski J., Koncicki A. (2014). Immunological and biochemical indicators in turkeys fed diets with a different methionine content. Polish J. Vet. Sci., 17: 687–695.

Kumar S., Raman R., Kumar K., Pandey P., Kumar N., Mallesh B., Mohanty S., Kumar A. (2013). Effect of azadirachtin on haematological and biochemical parameters of *Argulus*-infested goldfish *Carassius auratus* (Linn. 1758). Fish Physiol. Biochem., 39: 733–747.

Liu G.Q., Kai Z., Zhang L.L, Cao S.Q. (2010). Dietary methionine affect meat quality and myostatin gene exon 1 region methylation in skeletal muscle tissues of broilers. Agric. Sci. China., 9: 1338–1346.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265–275.

Macfadyen L.P., Dawson S. (2012). Numbers are not enough. Why e-learning analytics failed to inform an institutional strategic plan. J. Educ. Technol. Soc., 15: 149–163.

Naiel M.A., Negm S.S., Abd El-hameed S.A., Abdel-Latif H.M. (2021 a). Dietary organic selenium improves growth, serum biochemical indices, immune responses, antioxidative capacity, and modulates transcription of stress-related genes in Nile tilapia reared under sub-optimal temperature. J. Thermal Biol., 99: 102999.

Naiel M.A., Khames M.K., Abdel-Razek N., Gharib A.A., El-Tarabily K.A. (2021 b). The dietary administration of miswak leaf powder promotes performance, antioxidant, immune activity, and resistance against infectious diseases on Nile tilapia (*Oreochromis niloticus*). Aquacult. Rep., 20: 100707.

Naiel M.A., Farag M.R., Gewida A.G., Elnakeeb M.A., Amer M.S., Alagawany M. (2021 c). Using lactic acid bacteria as an immunostimulants in cultured shrimp with special reference to *Lactobacillus* spp. Aquacult. Int., 29: 219–231.

Naiel M.A., Abd El-hameed S.A., Arisha A.H., Negm S.S. (2022 a). Gum arabic-enriched diet modulates growth, antioxidant defenses, innate immune response, intestinal microbiota and immune related genes expression in tilapia fish. Aquaculture, 556: 738249.

Naiel M.A., Gewida A.G., Merwad A.R.M., Abdel-Hamid E.A., Negm S.S., Alagawany M., Farag M.R. (2022 b). The effects of various organic fertilizers with or without adsorbents on the productivity, antioxidant status and immune responses of Nile tilapia raised in cement ponds. Aquaculture, 548: 737593.

Naiel M.A., Abdelghany M.F., Khames D.K., Abd El-hameed S.A., Mansour E.M., El-Nadi A.S., Shoukry A.A. (2022 c). Administration of some probiotic strains in the rearing water enhances the water quality, performance, body chemical analysis, antioxidant and immune responses of Nile tilapia, *Oreochromis niloticus*. Appl. Water Sci., 12: 209.

Naiel M.A., Ghazanfar S., Negm S.S., Shukry M., Abdel-Latif H.M. (2023 a). Applications of antimicrobial peptides (AMPs) as an alternative to antibiotic use in aquaculture: a mini-review. Ann. Anim. Sci., 23: 691–701.

Naiel M.A., El-Kholy A.I., Negm S.S., Ghazanfar S., Shukry M., Zhang Z., Ahmadifar E., Abdel-Latif H.M. (2023 b). A mini-review on plant-derived phenolic compounds with particular emphasis on their possible applications and beneficial uses in aquaculture. Ann. Anim. Sci., 23: 971–977.

Nakajima K. (1991). Effects of dimethyl-β-propiothetin on growth and thrust power of rainbow trout. J. Jap. Soc. Fish. Sci., 57.

Nakajima K. (1993). Dimethylthetin- and betaine-homocysteine methyltransferase activities from livers of fish, chicken, and mammals. J. Jap. Soc. Fish. Sci., 59: 1389–1393.

Nakatsuru Y., Nemoto N., Nakagawa K., Masahito P., Ishikawa T. (1987). O^6-methylguanine DNA methyltransferase activity in liver from various fish species. Carcinogenesis, 8: 1123–1127.

Naya F.J., Olson E. (1999). MEF2: a transcriptional target for signaling pathways controlling skeletal muscle growth and differentiation. Curr. Opin. Cell Biol., 11: 683–688.

Nazeemashahul S., Prasad Sahu N., Sardar P., Fawole F.J., Kumar S. (2020). Additional feeding of vitamin–mineral-based nutraceutical to stress-exposed rohu, *Labeo rohita*, enhances the *IGF-1* gene expression and growth. Aquacult. Res., 51: 2649–2666.

Negm S.S., Ismael N.E., Ahmed A.I., Asely A.M.E., Naiel M.A. (2021). The efficiency of dietary *Sargassum aquifolium* on the performance, innate immune responses, antioxidant activity, and intestinal microbiota of Nile tilapia (*Oreochromis niloticus*) raised at high stocking density. J. Appl. Phycol., 33: 4067–4082.

Oztay F., Tunali S., Kayalar O., Yanardag R. (2020). The protective effect of vitamin U on valproic acid-induced lung toxicity in rats via amelioration of oxidative stress. J. Biochem. Mol. Toxicol., 34: e22602.

Peres H., Oliva-Teles A. (2001). Effect of dietary protein and lipid level on metabolic utilization of diets by European sea bass (*Dicentrarchus labrax*) juveniles. Fish Physiol. Biochem., 25: 269.

Raza S.H.A., Abdelnour S.A., Alotaibi M.A., AlGabbani Q., Naiel M.A., Shokrollahi B., Noreldin A.E., Jahejo A.R., Shah M.A., Alagawany M.. (2022). MicroRNAs mediated environmental stress

responses and toxicity signs in teleost fish species. Aquaculture, 546: 737310.

Reisz-Porszasz S., Bhasin S., Artaza J.N., Shen R., Sinha-Hikim I., Hogue A., Fielder T.J., Gonzalez-Cadavid N.F. (2003). Lower skeletal muscle mass in male transgenic mice with muscle-specific overexpression of myostatin. Am. J. Physiol.-Endocrinol. Metab., 285: E876–E888.

Rónyai A., Csengeri I., Váradi L. (2001). Preliminary results of vitamin U supplementation on the production performance of Siberian sturgeon (*Acipenser baerii*) and African catfish (*Clarias lazera*), and that of placentin on sterlet (*Acipenser ruthenus*). In: 4th International Symposium on Sturgeon, Oshkosh, Wisconsin, USA, H. Rosenthal, R.M. Bruch, F.P. Binkowski, S.I. Doroshov (eds.). J. Appl. Ichthyol., pp. 1–480.

Shan L.L., Li X.Q., Zheng X.M., Gan T., Guo T., Leng X.J. (2017). Effects of feed processing and forms of dietary methionine on growth and *IGF-1* expression in Jian carp. Aquacult. Res., 48: 56–67.

Shaw A., Blake J., Narvaez-Solarte W., Gunawardana P. (2009). Effects of vitamin U on live performance and intestinal tract integrity in broilers. J. Appl. Ani. Res., 35: 1–7.

Sheikhzadeh N., Ahmadifar E., Soltani M., Tayefi-Nasrabadi H., Mousavi S., Naiel M.A. (2022). Brown seaweed (*Padina australis*) extract can promote performance, innate immune responses, digestive enzyme activities, intestinal gene expression and resistance against *Aeromonas hydrophila* in common carp (*Cyprinus carpio*). Animals, 12: 3389.

Suvarna K.S., Layton C., Bancroft J.D. (2018). Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sci., pp. 1–573.

Toohey J.I. (2014). Sulfur amino acids in diet-induced fatty liver: a new perspective based on recent findings. Molecules, 19: 8334–8349.

Wang Q., McPherron A.C. (2012). Myostatin inhibition induces muscle fibre hypertrophy prior to satellite cell activation. J. Physiol., 590: 2151–2165.

Wen C., Chen X., Chen G., Wu P., Chen Y., Zhou Y., Wang T. (2014). Methionine improves breast muscle growth and alters myogenic gene expression in broilers. J. Anim. Sci., 92: 1068–1073.

Wen C., Jiang X., Ding L., Wang T., Zhou Y. (2017). Effects of dietary methionine on breast muscle growth, myogenic gene expression and *IGF-I* signaling in fast-and slow-growing broilers. Sci. Rep., 7: 1–7.

Xiao Y., Wu C., Li K., Gui G., Zhang G., Yang H. (2017). Association of growth rate with hormone levels and myogenic gene expression profile in broilers. J. Anim. Sci. Biotechnol., 8: 1–7.

Zanou N., Gailly P. (2013). Skeletal muscle hypertrophy and regeneration: interplay between the myogenic regulatory factors (*MRFs*) and insulin-like growth factors (*IGFs*) pathways. Cell. Mol. Life Sci., 70: 4117–4130.

Zhai W., Araujo L., Burgess S., Cooksey A., Pendarvis K., Mercier Y., Corzo A. (2012). Protein expression in pectoral skeletal muscle of chickens as influenced by dietary methionine. Poultry Sci., 91: 2548–2555.

Zhong H., Lou C., Ren B., Zhou Y. (2022). Insulin-like growth factor 1 injection changes gene expression related to amino acid transporting, complement and coagulation cascades in the stomach of tilapia revealed by RNA-seq. Front. Immunol., 4290.

Received: 10 XII 2022
Accepted: 4 V 2023