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Research Article

Dietary Alpha-Monolaurin for Nile Tilapia (*Oreochromis niloticus*): Stimulatory Effects on Growth, Immunohematological Indices, and Immune-Related Gene Expressions

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Alpha-monolaurin is reported to exhibit strong antiviral and antibacterial effects. This paves the way for its use as a new generation of feed additives. The experiment was carried out to examine the effects of the inclusion of alpha-monolaurin as a feed additive for Nile tilapia (*Oreochromis niloticus*) growth and immune response. Four diets were formulated to include alpha-monolaurin at 0, 2, 4, and 6 g alpha-monolaurin kg^{-1} diets and fed to the Nile tilapia (initial weight, 3.19 ± 0.11 g) for 70 days. Compared to those of the control group, the final body weight, weight gain rate, specific growth rate, and efficiency of feed of fish fed 4 and 6 g alpha-monolaurin kg^{-1} diets were ($P \leq 0.05$) higher. The diet supplemented with a 2 g alpha-monolaurin kg^{-1} diet ($P \leq 0.05$) improved endogenous amylase and lipase more than other treatments. The intestinal villus length, width, and goblet cell number were increased ($P \leq 0.05$) in fish fed a 6 g alpha-monolaurin kg^{-1} diet. The same treatment displayed decreased ($P \leq 0.05$) AST and ALT and increased total protein, albumin, and globulin. The highest levels of IgG and IgM were also noted in fish fed a 6 g alpha-monolaurin kg^{-1} diet. The highest values of hematocrit, hemoglobin, and red blood cell and white blood cell counts were seen in fish fed either a 4 or 6 g alpha-monolaurin kg^{-1} diet. The highest levels of thyroxine, triiodothyronine, and growth hormone were recorded in fish fed a 6 g alpha-monolaurin kg^{-1} diet. Likewise, the same treatment recorded the highest levels of SOD, CAT, GSH, and GPx but the lowest MDA value. Diets supplemented with 4 g or 6 g alpha-monolaurin kg^{-1} displayed the highest gene expressions of IFN-γ and IL-1β; however, HP70 genes were downregulated. In summary, the study showed that monolaurin may exert immunostimulatory effects on the immune system of the Nile tilapia by modulation of the host immune response and through metabolite production.

1. Introduction

Aquaculture is one of the developing sectors with around 8% average annual growth rates [1]. It addresses the deficiency in meat production by farming of various aquatic animals. To achieve high production and sustainability of farmed aquatic animals, an intensive aquaculture system must be applied on a wide scale [2, 3]. Similarly, intensive tilapia culture aims to exploit production with trifling use of water [4]. However, challenges such as deteriorated environmental quality and outbreaks of diseases tremendously affect intensive aquaculture, resulting in significant financial losses.

There has been a growing interest in researching novel active biological substances to address these challenges and achieve sustainable intensive aquaculture [5, 6]. One of these novel substances is medium-chain fatty acids (MCFAs). MCFAs have received more attention recently due to their potential as an antibacterial substance and as an immune system and metabolic modulator [7–10]. Metabolically, MCFAs are directly absorbed into the liver after digestion and processed through β-oxidation in the mitochondria for energy supply use [11, 12]. Additionally, MCFAs maintain the optimum pH of the fish intestine [13], modulate the beneficial microbiota of the gut, and decrease pathogens [14]. MCFAs can also enhance the absorption of nutrients and stimulate the transport of nutrients into the enterocytes of the villi [9]. MCFAs can decrease amino acid oxidation, thereby, allowing increased protein utilization and reducing body fat deposition [15].

Families of MCFAs with 6 to 12 carbon atoms include important members, such as caproic acid (C6), caprylic acid (C8), capric acid (C10), and lauric acid (C12). Lauric acid (also known as dodecanoic acid) is conceded as the most potent MCFA [16, 17]. In the aquafeed industry, glycerol monolaurate was shown to significantly enhance the performance of different fish species, such as common carp (*Cyprinus carpio*), Atlantic salmon (*Salmo salar*), and Nile tilapia (*Oreochromis niloticus*) [18–22]. Although monolaurate has been commonly used in terrestrial animals, limited information is available for its use in the diets of fish, specifically for Nile tilapia. Hence, the current study aimed to examine the potential effects of alpha-monolaurin on growth, intestinal enzymes and morphology, biochemical parameters, antioxidant capacity, immune response, and related gene expressions of the Nile tilapia.

2. Materials and Methods

2.1. Experimental Culture Technique. The experiment was approved by the authority of the National Institute of Oceanography and Fisheries (NIOF) Egypt Committee for Institutional Care of Aquatic Organisms and Experiment Animals (NIOF-AQ4-F-23-R-016). Nile tilapia (*Oreochromis niloticus*) were collected from the aquaculture station at the Faculty of Agriculture, Benha University, Egypt. During the acclimation period, the tilapia were fed a control diet which consisted of 30.67% crude protein (CP) and 6.53% crude lipid for 2 weeks. Nile tilapia fingerlings (3.19 ± 0.11 g) were randomly distributed into 12 fiberglass tanks with 20 fish for each tank in triplicate. For 70 days, the fish were hand-fed daily (8:00 AM, 12:00 AM, and 4:00 PM). A third of the freshwater content in each tank was changed daily to collect the feces and uneaten feeds according to the methods of Hassaan et al. [23]. Water temperature (28°C), pH (8.2–8.3), dissolved oxygen (5.9 mg L^{-1}), and total ammonia were checked daily.

2.2. Experimental Diets. Alpha-monolaurin (RAC12) was provided by NUTRIVETmisr Feed Additives Inc., October City, Giza, Egypt. Isoproteic and isolipidic (32% and 19.0 kJ g^{-1}, respectively) diets [24] were formulated (Table 1). The diets prepared were a basal diet (no inclusion) and three diets that contained 2, 4, and 6 g alpha-monolaurin kg^{-1} diets. The ingredients were assorted thoroughly after adding various doses of alpha-monolaurin and converted to pellets (2 mm diameter size). The pellets were dried for 12 hours and stored in a deep freezer at −3°C prior to usage.

2.3. Collection of Samples and Analysis. At the end of feeding trials, fish were made to starve for 24 hours and sedated with MS-222 (50 mg L^{-1}). From each tank, five fish were sampled for blood, the liver, and the intestine. Chymotrypsin and trypsin activities were determined according to Deguara et al. [26], and lipase activities were estimated according to Zamani et al. [27]. At the end of the experiment, six fish were collected from each treatment for blood sample collection. Hematocrit was determined as described by Reitman and Frankel [28]. The hemoglobin count was estimated using hemoglobin kits, and the totalcount of WBC was carried out by the indirect method of Martins et al. [29]. The second part of the blood sample was centrifuged at 3000 rpm for 10 min to obtain the nonhemolyzed serum for determination of blood biochemical properties. Serum aspartate and alanine aminotransferase (AST) and (ALT) were measured according to the method described by Reitman and Frankel [28]. Plasma lysozyme activity was measured according to Schäperclaus et al. [30]. Amylase activity was estimated according to Bernfeld [31] at 540 nm, and starch was used as the substrate. 1 ml of the diluted sample was incubated for 3 min with 1 ml 1% starch (1 g soluble starch and 0.035 g NaCl in 100 ml 0.02 M Na3PO4, pH 6.9). Lipase activity was determined as described by Zamani et al. [27]; the titration method was detailed by using olive oil-gum. Oxidative enzymes activity as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA) were measured using diagnostic kits (Biodiagnostics, Giza, Egypt) following the manufacturer instructions based on the methodology which is described by Hassaan et al. [32] and Mohammady et al. [33]. Total serum protein and albumin were determined according to methods of Henry [34] and Wotton and Freeman [35], respectively. Globulin was calculated by subtracting total albumin from protein according to Coles [36]. Growth hormone was estimated according to the method described by Hassaan et al. [37]. Radioimmunoassay was performed to determine the thyroxine (T3) and triiodothyronine (T4) levels in the serum, as previously described by Van der Geyten et al. [38].

Table 1: Formulation and proximate composition of the basal diet (g kg diet^{-1}, dry matter) containing different levels of alpha-monolaurin.

Ingredients	g kg^{-1}
Fish meal (58% crude protein, CP)	50
Soybean meal (44% CP)	425
Yellow corn (8% CP)	280
Wheat bran	100
Corn gluten (60% CP)	80
Soy oil	40
Vitamin and minerals[1]	25
Proximate analysis (g kg^{-1})	
Crude protein	306.7
Crude lipid	65.3
Ash	10.7
Fiber	42.3
NFE[b]	557.4
Gross energy (MJ kg $^{-1}$)[3]	19.43

[1]Vitamin and mineral mixture kg^{-1} contains 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B12, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, pantothenic acid, 8.0 g nicotinic acid, 400 mg folic acid, 20 mg biotin, 200 g choline, 4 g copper, 0.4 g iodine, 12 g iron, 22 g manganese, 22 g zinc, 0.04 g selenium, folic acid, 1.2 mg niacin, 12 mg d-calcium pantothenate, 26 mg pyridoxine, 6 mg HCl, 7.2 mg riboflavin, thiamin, 1.2 mg HCl, sodium chloride (NaCl: 39% Na, 61% Cl), 3077 mg ferrous sulfate (FeSO4.7H2O: 20% Fe), 65 mg manganese sulfate (MnSO4: 36% Mn), 89 mg zinc sulfate (ZnSO4.7H2O: 40% Zn), 150 mg copper sulfate (CuSO4.5H2O: 25% Cu), and 28 mg potassium iodide (KI: 24% K, 76% I). [2]NFE (nitrogen-free extract) = 100 − (crude protein + crude lipid + ash + fiber content). [3]Gross energy calculated using gross calorific values of 23.63, 39.52, and 17.15 kJ g^{-1} for protein, fat, and carbohydrate, respectively, according to Brett [25].

2.4. Intestinal Microscopic Examination. At the end of the trial, five fish were randomly selected to collect intestine and liver samples. The full protocol by Hassaan et al. [39] was used for the intestinal and liver morphological evaluation. The results are expressed as mean values ± standard error of the means.

2.5. Gene Expression. To measure the gene expression, the livers were collected from three samples following the methods of Ahmadifar et al. [40] and Hassaan et al. [41]. The primers of the target genes (IL-1β, IFN-γ, and HSP 70) and 18s rRNA as a housekeeping gene are shown in Table 2. The changes in the relative gene expression relative to the controls were measured in the hepatic tissue as described by Livak and Schmittgen [42].

2.6. Calculation and Statistical Analysis. The initial body weight (IBW) and the final body weight (FBW) (g) were measured at the start and the end of the feeding trial. Performance and feed efficiency parameters were calculated as follows:

Weight gain (WG) = FBW (g) − IBW (g)

Specific growth rate (SGR) = ln FBW − ln IBW/period (days)

Feed conversion ratio (FCR) = feed intake (g)/WG (g)

Protein efficiency ratio (PER) = weight gain (g)/consumed of protein (g)

Fish survival (%) = [(total fish number − dead fish number)/total fish number] × 100

2.7. Statistical Analysis. Before statistical analysis, the collected data were tested for normality and homoscedasticity. To differentiate data, one-way ANOVA was used followed by Tukey's test (SAS Software). The significance level was set at $P \leq 0.05$. The results are shown as means ± SEM.

3. Results

3.1. Growth Performance and Feed Utilization. The application of dietary alpha-monolaurin enhanced ($P \leq 0.05$) the growth and feed efficiency of Nile tilapia (Table 3). Fish fed a 6 g alpha-monolaurin kg-1 diet noted the highest performance versus fish fed a control diet. Furthermore, the better FCR values ($P \leq 0.05$) were obtained by fish fed a 6 g alpha-monolaurin kg^{-1} diet versus fish fed a control diet; however, it was not significantly different ($P \geq 0.05$) from fish fed 2 and 4 g alpha-monolaurin kg^{-1} diets. Broken-line regression analysis of the feed conversion ratio of Nile tilapia (*O. niloticus*) fed four experimental diets supplemented with α-monolaurin g kg^{-1} diet resulted in a value of 3.2 g of an alpha-monolaurin kg^{-1} diet. The survival rates of fish fed dietary alpha-monolaurin were higher than those of fish fed a control diet ($P < 0.05$, Table 3), while the best fish survival was recorded in fish fed 4 and 6 g alpha-monolaurin kg^{-1} diets.

3.2. Endogenous Enzymes Activities. The intestinal amylase and lipase activities are shown in Table 4. The inclusion of 2 g alpha-monolaurin kg^{-1} diet enhanced the activity of amylase and lipase compared to other treatments.

3.3. Morphometry of the Liver and Intestinal Tracts. The liver tissue of all treatments showed normal and healthy structures but with the occurrence of intrahepatic pancreases enfolding a branch from the portal vein (Figure1). The length of the villus, intervilli distance, and number of goblet cells in the middle intestines were affected ($P \leq 0.05$) by the inclusion of alpha-monolaurin (Table 5; Figure 2). The intestinal villus length and width were enhanced ($P \leq 0.05$) by inclusion of either a 4 or 6 g alpha-monolaurin kg^{-1} diet. However, fish fed a 6 g alpha-monolaurin kg^{-1} diet recorded the highest values for the intervilli distance and number of goblet cells.

3.4. Hematological Parameters. Dietary alpha-monolaurin supplementation had no effect ($P > 0.05$) on the red blood cell (RBC) count (Table 6). Diets enriched with alpha-monolaurin significantly ($P < 0.05$) improved hemoglobin and hematocrit compared to the control diet; the highest values were noted in fish fed a 6 g alpha-monolaurin kg^{-1} diet (Table 6). The highest values of the white blood cell (WBC) count, neutrophil, and lymphocyte levels were detected in fish fed 4 and 6 g

Table 2: Oligonucleotide name and sequence of qRT-PCR primers used in the experiment.

Gene	Primer sequence 5′–3′	Accession no.
18s	F:5′-GGTTGCAAAGCTGAAACTTAAAGG-3′ R:5′TTCCCGTGTTGAGTCAAATTAAGC-3′	AF497908.1
INF-γ	F:5′-AAGAATCGCAGCTCTGCACCAT-3′ R:5′-GTGTCGTATTGCTGTGGCTTCC-3	XM_005448319.1
IL-1β	F:5′-CAAGGATGACGACAAGCCAACC-3′ R:5′-AGCGGACAGACATGAGAGTGC-3′	XM_003460625.2
HSP70	F:5′-CATCGCCTACGGTCTGGACAA-3′ R:5′-TGCCGTCTTCAATGGTCAGGAT-3′	′FJ207463.1

INF-γ = interferon gamma; IL-1β = interleukin 1β; HSP70 = heat shock protein 70; 18s rRNA = internal reference gene (house-keeping gene).

Table 3: Growth performance and feed utilization of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g·kg^{-1}	4 g·kg^{-1}	6 g·kg^{-1}		
Initial body weight (g fish^{-1})	3.10	3.31	3.18	3.17	0.11	0.647
Final body weight (g fish^{-1})	15.05^{c}	15.65^{bc}	17.2^{ab}	18.6^{a}	0.44	0.015
Weight gain (g fish^{-1})	11.95^{c}	12.34^{bc}	14.01^{ab}	15.43^{a}	0.49	0.022
Specific growth rate (%day^{-1})	2.63^{ab}	2.59^{b}	2.81^{ab}	2.94^{a}	0.07	0.099
Feed conversion ratio	1.650^{a}	1.45^{b}	1.35^{b}	1.25^{c}	0.05	0.019
Protein efficiency ratio	1.950^{b}	2.075^{b}	2.395^{a}	2.590^{a}	0.06	0.009
Fish survival (%)	97.50^{c}	98.30^{b}	99.00^{a}	100.00^{a}	1.26	0.006

Means followed by different superscripts in the same row are significantly different ($P < 0.05$). WG = final weight (g) – initial weight (g); specific growth rate (SGR) = lnW2 – lnW1/t (days); FCR = feed intake (g)/weight gain (g); protein efficiency ratio (PER) = weight gain (g)/protein ingested (g).

Table 4: Intestinal tract morphology of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g·kg^{-1}	4 g·kg^{-1}	6 g·kg^{-1}		
Villus length (μm)	237.12^{b}	330.10 ^{ab}	381.16^{a}	389.17^{a}	5.14	0.047
Villus width (μm)	95.18^{d}	130.57^{c}	162.30^{a}	171.12^{a}	5.32	0.025
Intervilli distance (μm)	61.52^{c}	75.98^{c}	89.25^{b}	101.15^{a}	1.12	0.032
Goblet cells (mm^{2})	15.13^{c}	18.27^{b}	21.87^{a}	22.23^{a}	0.56	0.049

Means followed by different superscripts in the same row are significantly different ($P < 0.05$).

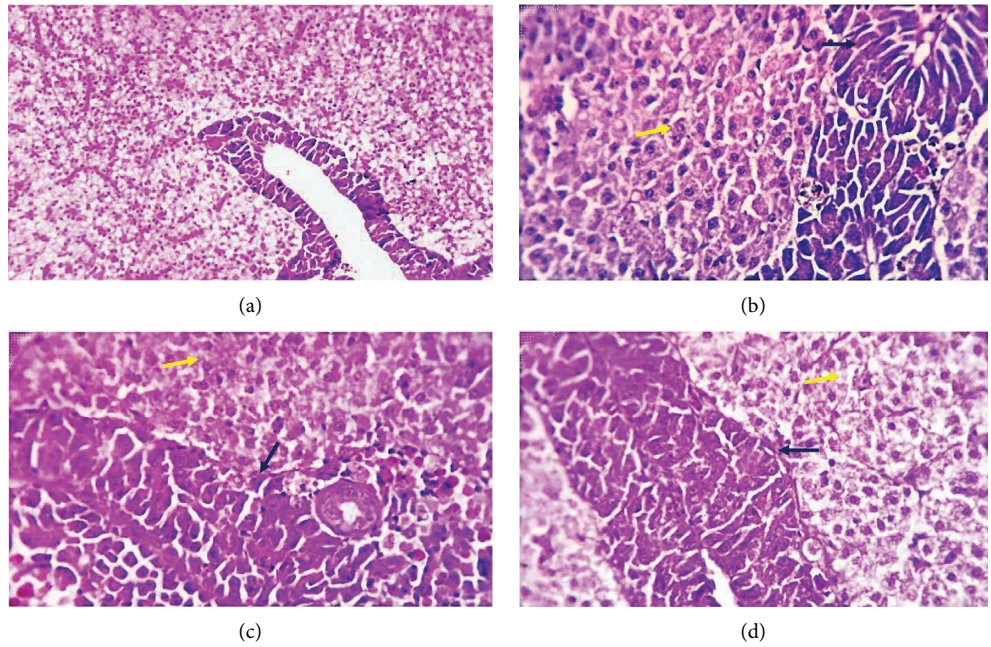
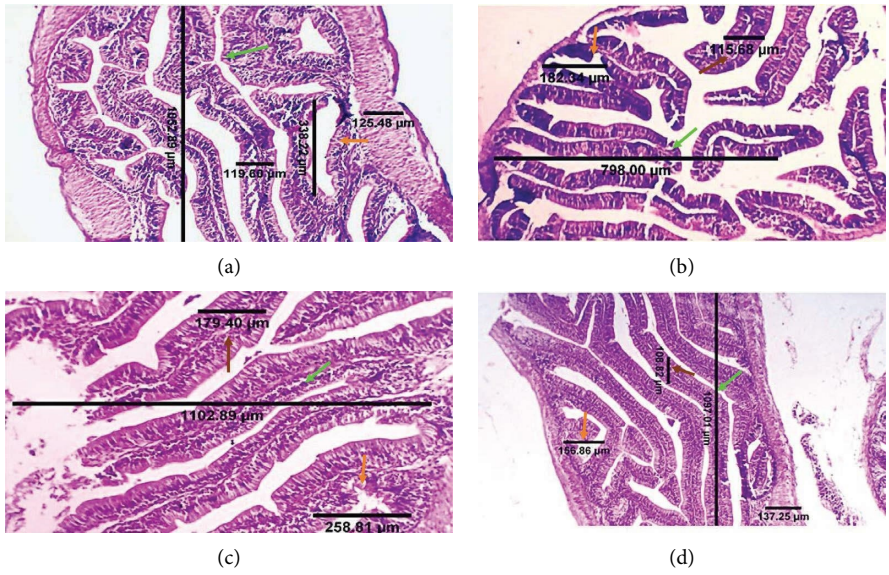


Figure 1: Photomicrographs of the liver tissue sections of the Nile tilapia stained with hematoxylin and eosin (HE) showing normal lobular arrangement, central venules, and portal area structures: (a) control group; (b) 2 g diet α-monolaurin kg^{-1}; (c) 4 g α-monolaurin kg^{-1} diet; (d) 6 g α-monolaurin kg^{-1} diet ((HE ×400): scale bar = 50 μm).

Table 5: Intestinal digestive enzyme (U/g tissue) of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g·kg^{-1}	4 g·kg^{-1}	6 g·kg^{-1}		
Amylase	801.23^{c}	870.56^{b}	960.20^{b}	1120.20^{a}	59.80	0.0001
Lipase	906.10^{ab}	997.22^{a}	1105.54^{ab}	1320.54^{a}	50.23	0.0491

Means followed by different superscripts in the same row are significantly different ($P < 0.05$).



(a) (b) (c) (d)

Figure 2: Photomicrographs of the small intestine sections of the Nile tilapia stained with HE showing the branching anastomosing mucosal villi and with goblet cells: (a) control group; (b) 2 g diet α-monolaurin kg^{-1}; (c) 4 g α-monolaurin kg^{-1} diet; (d) 6 g α-monolaurin kg^{-1} diet ((HE ×400): scale bar = 50 μm).

alpha-monolaurin kg^{-1} diets; however, dietary alpha-monolaurin supplementation had no effect ($P \geq 0.05$) on monocyte and eosinophils levels.

3.5. Serum Biochemical Parameters. No differences ($P > 0.05$) were found in the values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) among the treatments (Table 7). However, an increase ($P < 0.05$) in serum hematology parameters was observed with dietary inclusion of alpha-monolaurin (Table 7). The addition of 4 or 6 g alpha-monolaurin kg^{-1} diets recorded the highest values of total protein and albumin, while the highest level of globulin was detected in fish fed a 6 g alpha-monolaurin kg^{-1} diet. Furthermore, immunoglobulin G (IgG) and immunoglobulin M (IgM) ($P < 0.05$) improved in fish fed dietary alpha-monolaurin (Table 7). The highest levels of IgG and IgM were noted with the addition of a 6 g alpha-monolaurin kg^{-1}.

3.6. Hormonal and Immunological Assay. Inclusions of alpha-monolaurin had a positive effect ($P < 0.05$) on thyroxine, triiodothyronine, and growth hormone levels (Table 8). The highest levels of thyroxine and triiodothyronine recorded were with the inclusion of alpha-monolaurin at 4 and 6 g kg^{-1} diets, while the highest level of growth hormone was detected in fish fed a 6 g alpha-monolaurin kg^{-1} diet.

3.7. Oxidative Stress Responses. The SOD, CAT, GSH, GPx, and MDA activities are presented in Table 9. The application of dietary alpha-monolaurin significantly ($P < 0.05$) improved oxidative response enzymes. Inclusion of a 6 g alpha-monolaurin kg^{-1} diet recorded the highest levels of SOD, CAT, GSH, and GPx but the lowest value of MDA.

3.8. Gene Expression. The INF-γ, IL-1β, and HP70 genes expressions are presented in Figures 3–5. Fish fed dietary alpha-monolaurin significantly ($P < 0.05$) upregulated INF-γ and IL-1β compared with fish fed a control diet, but the HP70 gene was downregulated. Fish fed 4 and 6 g alpha–monolaurin kg^{-1} diets displayed the highest gene expressions of INF-γ and IL-1β.

4. Discussion

Monolaurin is a medium-chain fatty acid (MCFA) that has been found to exhibit growth-promoting and immunomodulatory functions in broiler chicken, mice, and white shrimp. Only few studies have used and reported the effects of lauric acid as feed additives on aquatic animals [22, 43]. In the present work, dietary alpha-monolaurin supplementation resulted in higher growth, demonstrating that alpha-monolaurin improved growth and nutrient utilization,

Table 6: Hematological parameters of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g·kg^{-1}	4 g·kg^{-1}	6 g·kg^{-1}		
Hemoglobin (g dl^{-1})	8.50^{c}	10.50^{b}	11.8^{ab}	13.55^{a}	0.444	0.006
Hematocrit (%)	27.00^{b}	30.50^{ab}	31.45^{ab}	32.70^{a}	1.249	0.113
RBC × 10^{6} mm^{3}	2.75	3.08	3.10	3.55	0.404	0.617
WBC × 10^{4} mm^{3}	26.50^{c}	41.00^{b}	52.00^{a}	54.00^{a}	2.562	0.005
Neutrophil %	6.50^{b}	10.00^{ab}	12.50^{a}	12.50^{a}	0.968	0.032
Lymphocyte %	74.00^{b}	76.50^{b}	80.00^{a}	82.50^{a}	0.791	0.005
Monocyte %	3.5.00	5.00	4.50	6.00	1.369	0.665
Eosinophil %	1.50	3.50	3.50	3.50	0.500	0.107

Means followed by different superscripts in the same row are significantly different ($P < 0.05$).

Table 7: Serum biochemical indices of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g·kg^{-1}	4 g·kg^{-1}	6 g·kg^{-1}		
Alanine aminotransferase (UL^{-1})	37.50	37.50	36.50	36.00	2.106	0.939
Aspartate aminotransferase (UL^{-1})	16.10	15.5	13.5	14.50	1.392	0.630
Total protein (g dL^{-1})	2.05^{c}	2.75^{b}	2.95^{b}	3.25^{a}	0.066	0.009
Albumin (g dL^{-1})	0.90^{c}	1.25^{b}	1.35^{b}	1.8^{a}	0.079	0.006
Globulin (g dL^{-1})	1.15^{b}	1.45^{a}	1.60^{a}	1.45^{a}	0.043	0.008
IgG immunoglobulin G (mg dL^{-1})	112.5^{c}	119.75^{b}	124.7^{ab}	130.75^{a}	1.700	0.007
IgM immunoglobulin M (mg dL^{-1})	7.95^{c}	8.4^{bc}	8.95^{b}	10.4^{a}	0.226	0.006

Means followed by different superscripts in the same row are significantly different ($P < 0.05$).

Table 8: Hormonal parameters of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g kg^{-1}	4 g kg^{-1}	6 g kg^{-1}		
T_4 thyroxine (ng dL^{-1})	0.80^{b}	0.94^{b}	1.355^{a}	1.42^{a}	0.0853	0.0161
T_3 triiodothyronine (ng dL^{-1})	7.90^{b}	10.8^{ab}	12.95^{a}	13.45^{a}	0.8477	0.0305
GH growth hormone (ng dL^{-1})	1.58^{c}	2.04^{bc}	2.465^{ab}	2.65^{a}	0.1312	0.0153

Means followed by different superscripts in the same row are significantly different ($P < 0.05$).

Table 9: Hepatic oxidative response (U/g protein) of Nile tilapia fed different levels of alpha-monolaurin.

Items	Experimental treatments				±SE	P value
	Control	2 g·kg^{-1}	4 g·kg^{-1}	6 g·kg^{-1}		
MDA	27.78^{a}	24.45^{ab}	20.50^{bc}	19.30^{c}	1.0764	0.0159
CAT	47.045^{c}	66.65^{b}	75.45^{b}	103.320^{a}	4.6987	0.0048
GSH	263.320^{b}	277.52^{b}	305.00^{b}	379.015^{a}	14.3550	0.0160
GPX	189.00^{c}	215.00^{bc}	241.00^{ab}	269.00^{a}	7.7338	0.0073
SOD	10.365^{d}	26.615^{c}	36.35^{b}	41.55^{a}	1.1441	0.0002

Means followed by different superscripts in the same row are significantly different ($P < 0.05$). ^{a}MDA, malondialdehyde. ^{b}CAT, catalase. ^{c}GSH, glutathione. ^{d}GPx, glutathione peroxidase. ^{e}SOD, superoxide dismutase.

which could then enhance enzymatic digestibility, immunomodulatory functions, and gut microbial community [22, 44–46].

The feed conversion ratio is used as one of the main tools by aquafeed companies as well as farmers to monitor not only feed performance but fish statues health as well [39, 47, 48]. The best feed conversion ratio and protein efficiency ratio values were noted for fish fed diets complemented with alpha-monolaurin compared to those fed the control diet. Our findings agree with those of previous studies which showed the potential of using glycerol monolaurate as a feed additive or growth promoter for aquatic animals [13, 46]. Recently, Wang et al. [22] observed that dietary 750 mg kg^{-1} of glycerol monolaurate improved weight gain and specific growth rates and reduced FCR of the zebrafish (*Danio rerio*). In a prior study, Nordrum et al. [15] found that medium-chain triglycerides improved the digestibility of starch and crude protein as well as increased the retention of nitrogen for the Atlantic salmon (Salmo salar). Inclusion of MCFA in the diet was effective in

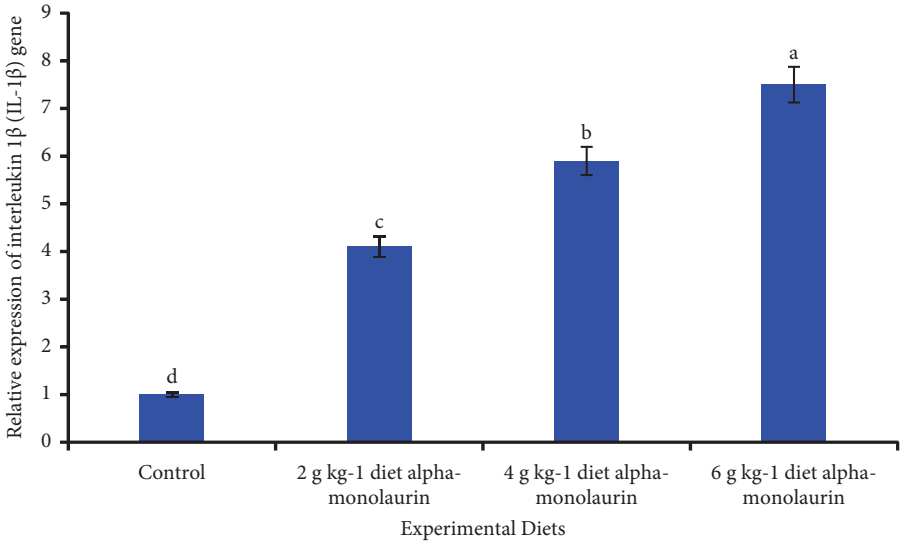


FIGURE 3: Relative expression of interleukin 1β (IL-1β) gene/18s rRNA of the Nile tilapia after feeding diets supplemented with 2, 4, and 6 g alpha-monolaurin kg^{-1} for 70 days. Bars having different letters are significantly different at $P < 0.05$.

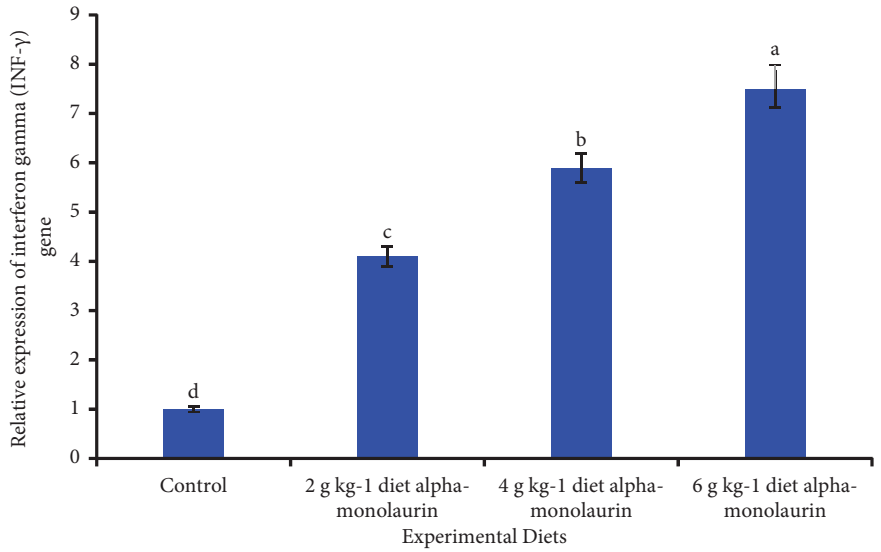


FIGURE 4: Relative expression of the interferon gamma (INF-γ) gene/18s rRNA of the Nile tilapia after feeding diets supplemented with 2, 4, and 6 g alpha-monolaurin kg^{-1} for 70 days. Bars having different letters are significantly different at $P < 0.05$.

boosting the growth rates and nutrient efficiency of gilthead sea bream (*Sparus aurata*) [43]. Moreover, lauric acid supplementation significantly improved weight gain, the specific growth rate, and the feed efficiency ratio of black sea bream (*Acanthopagrus schlegelii*) [3]. On the other hand, the dietary inclusion with high levels (15 or 30%) of medium-chain triglycerides decreased the feed intake of polka-dot grouper (*Cromileptes altivelis*) [49], while the feed intake of sunshine bass and the rainbow trout (*Oncorhynchus mykiss*) was not affected by supplementation of medium-chain triglycerides [50, 51]. Also, dietary supplementation with butyrate did not affect growth performance and feed utilization of the European seabass (*Dicentrarchus Labrax*) [52, 53], rainbow trout [54], or gilthead sea bream [55]. As recommended by Hassaan et al. [56] and Soltan et al. [57], there are many factors that explain the discrepancy of different results, such as organic acid type, age, fish species, and condition of the culture.

For a clear understanding of the capacity for nutritional absorption, regular monitoring of the morphometric parameters of the fish intestines is used [41]. The absorption of feed efficiency is dependent on the surface area of the villi [33, 58]. Goblet cells can play an important role in protecting the layer of the mucosal intestine via secreting mucus, subsequently enhancing digestion and preventing pathogenic bacterial growth [59, 60]. The length of the villus and the number of goblet cells in the fish intestines were positively affected by dietary alpha-monolaurin supplementation, especially the 6 g alpha-monolaurin kg^{-1} diet. According to the current findings, fish administered dietary

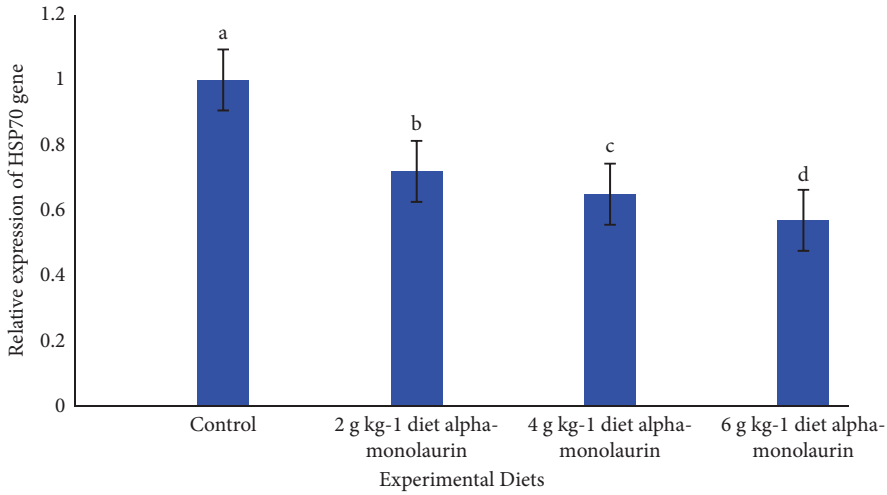


FIGURE 5: Relative expression of the HSP70 gene/18s rRNA of the Nile tilapia after feeding diets supplemented with 2, 4, and 6 g alpha-monolaurin kg^{-1} for 70 days. Bars having different letters are significantly different at $P < 0.05$.

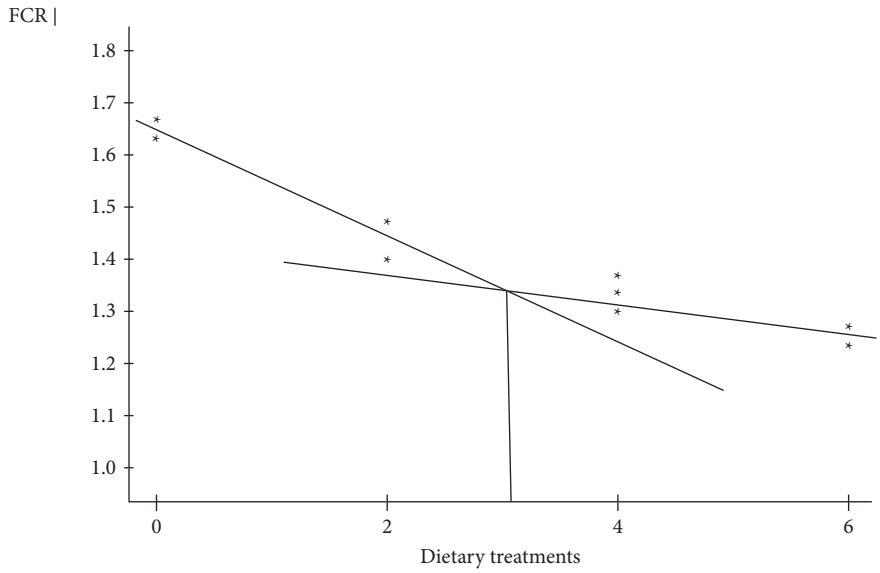


FIGURE 6: Broken-line regression analysis of the feed conversion ratio of Nile tilapia, *O. niloticus*, fed four experimental diets supplemented with α-monolaurin g kg^{-1} diet; $R^2 = 0.902$. The broken-line analysis is 3.2 g of a sericite kg^{-1} diet. $y = 1.65 - 0.087\ x + 0.0165 \times^2$.

alpha-monolaurin had improved feed utilization because of the beneficial effect of the nutrients on the intestinal morphology. Similarly, Hong et al. [61] and Rimoldi et al. [13] noted that MCFA supplementation could improve the efficiency of the capacity of nutrient absorption in the fish intestine. In addition, Magouz et al. [20] reported that carp fed diet supplemented with MCFA significantly improved the villus length and the number of goblet cells. The current results showed that dietary alpha-monolaurin plays a greater role in the intestine of Nile tilapia.

The positive effects of alpha-monolaurin on amylase and lipase enzyme activities in the current research indicate that alpha-monolaurin encourages digestive processes. Furthermore, the actions of intestinal enzymes were attributed to and supported by the growth-promoting effects of alpha-monolaurin. Recently, Wang et al. [22] displayed that a diet containing 750 mg kg-1 of glycerol monolaurate significantly enhanced intestinal lipase activity. MCFA supplementation has positive effects on the architecture of the anterior intestine [43]. On the other hand, lipase activity of black sea bream was lower in a diet supplemented with lauric acid than with those fed a control diet [3]. MCFA may participate in stimulating the secretion of cholecystokinin and a peptide hormone from the intestine, which may arouse the secretion of lipase [62]. Glycerol monolaurate can protect the ester linkage in the intestinal tract; these characteristics are important effects to achieve good digestibility and efficient utilization of nutrients of fish [63]. The high abundance of favorable enzyme-producing bacteria in the gut microbiota is reported to be triggered by dietary glycerol monolaurate [13, 44, 45].

Ichthyo-hematological parameters are considered valuable indices to evaluate fish health and feed quality [56, 57, 64]. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities in the fish diets were improved in the current study, possibly demonstrating the hepatoprotective action of alpha-monolaurin [56, 65–68]. Furthermore, these results can be associated with the normal morphology of the liver tissue (Figure 6). In addition, the increase in total protein and albumin levels can be related to enhanced feed digestibility. The serum total protein of gilthead sea bream fed a diet supplemented with sodium salt MCFA was altered compared to those fed a basal diet [43]. Moreover, Nile tilapia fed a diet supplemented with MCFA displayed improvement in the serum biochemical AST, ALT, albumin, and total protein levels [20]. In a study by Simó-Mirabet et al. [43], however, the hematological parameters of gilthead sea bream were not significantly affected by MCFA supplementation.

The present study showed improvement in immunoparameters which confirmed that the immune-stimulating effects of alpha-monolaurin in the diet of the Nile tilapia. Immune indices such as IgM and IgG are used as a marker of health status, stress, humoral resistance systems, and fish welfare [47, 69]. The present results indicated that the value of serum IgM and IgG was higher in fish fed 4 and 6 g alpha-monolaurin kg^{-1} diets, which may be due to the mode of action of alpha-monolaurin in improving the nonspecific immune response of the Nile tilapia. Simó-Mirabet et al. [43] showed that sodium salt MCFA induced no significant alterations in lysozyme and complement activities and IgM levels of gilthead sea bream. On the other hand, short-chain fatty acids could modulate the immune responses of various species of aquatic animals, such as Nile tilapia [56, 70], common carp [71], zebrafish [72], Pacific white shrimp, [73–76], and tiger shrimp [77].

Liver antioxidant enzymes are considered cellular immune responses and an excellent indicator of stress response [78, 79]. The present study showed greater activities of SOD, GSH, and GPX and lower MDA contents in fish fed dietary alpha-monolaurin. This might imply that the inclusion of alpha-monolaurin boosts the antioxidant defense system against reactive oxygen species. These results are in parallel with the studies of Witcher et al. [80] and Seneviratne and Sudarshana [81] who noted that addition of MCFA enhanced the activity of antioxidant enzymes which deter diseases caused by oxidative stress. Simó-Mirabet et al. [43] observed the decrease in oxidative radicals and respiratory burst which directly reveals the antioxidant effect of alpha-monolaurin for gilthead sea bream (*S. aurata*). In another study, Ullah et al. [3] noted that a diet supplemented with lauric acid increased the biomarker of antioxidant enzymes such as CAT and SOD and total antioxidant capacity and reduced the MDA value of black sea bream.

In the current trial, three genes were selected from the liver tissue, a vital organ in fish used to assess the effect of dietary alpha-monolaurin on transcriptomes of genes. Concisely, proinflammatory INF-γ and IL-1β genes are important markers of immunity and inflammatory response [82–85], while HSP70 is a marker of stress. In this trial, alpha-monolaurin supplementation significantly improved the transcriptions of INF-γ and IL-1β with upregulated INF-γ and IL-1β transcription values at levels of 4 g or 6 g alpha-monolaurin kg^{-1}. The present results are consistent with those obtained by Simó-Mirabet et al. [43] who noted that supplementation of MCFA in gilthead sea bream (*S. aurata*) can encourage anti-inflammatory genes Interleukin-1 beta, interleukin-6, interleukin-8, and interleukin-10 reduce the inflammatory response of the host as well. Following the same trends, the expression of anti-inflammatory cytokine IL-6 and IL-10 genes increased in rats fed MCFA [86]. Capric acid enhanced IL-8 production in humans [87], while caprylic acid inhibited the secretion of IL-8 [88]. In addition, Silva et al. [89] found that genes such as IL-6, IL-18, and TNF presented a higher expression, and the expression of IL-1$\alpha\alpha$ decreased when treated with monolaurin treatments (25 or 50 μM). However, the results are inconsistent with those obtained by the authors of [90] who found no significant changes in the transcription of macrophage inflammatory protein (MIP1-b), IL-1b, IFN-g, TNF-a, and monocyte chemoattractant protein (MCP-1) of piglets fed a diet supplemented with MCFAs. The present results could be attributed to the following scenario: (1) The immune and antibacterial effects of alpha-monolaurin may be related to the favorable proliferation of intestinal microbiota, which may have produced antimicrobial peptides and modulated inflammatory reaction [91–93]. (2) Monolaurin presents antibacterial and antiviral activities in vitro that boost the expression of immunity and inflammatory genes [94, 95], which may be of great interest in the treatment and/or prevention of various infections. (3) Monolaurin can express a variety of cytokines and chemokines such as IL-1α, IL-6, IL-8, and TNF [96, 97]. The results of the current study confirmed that alpha-monolaurin is a useful feed additive for the Nile tilapia. Monolaurin has positive modulation in the inflammatory gene response of these cells such as INF-γ and IL-1β and can reduce HSP70 expression. Up until this point, no published work has clarified how alpha-monolaurin affects the expression of HSP70 and inflammatory gene expressions in fish.

5. Conclusions

Alpha-monolaurin exhibits a strong immunostimulant function as seen in its antiviral and antibacterial effects. This paves the way for its use as a new generation of powerful feed additives. The results highlight the importance of the inclusion of a 4 g alpha-monolaurin kg^{-1} diet to modulate the metabolism and immune system of the Nile tilapia. This may enhance the immune system response and disease resistance and control disease outbreaks which have consequent positive impacts on growth performance, nutrient utilization efficiency, intestinal enzymes, immune response, and related gene expression of the Nile tilapia. These findings demonstrate that monolaurin could be considered a potential feed ingredient to boost the growth performance and cytokines of the Nile tilapia.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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