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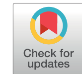
Assessing the effectiveness of CoQ10 dietary supplementation on growth performance, digestive enzymes, blood health, immune response, and oxidative-related genes expression of Nile tilapia (*Oreochromis niloticus*)



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ABSTRACT

The present study was conducted to investigate the effects of CoQ10 dietary supplementation on growth performance, feed utilization, blood profile, immune response, and oxidative status of Nile tilapia (12.4 ± 0.11 g, initial body weight). Five experimental diets were formulated containing CoQ10 at levels of 0, 10, 20, 30, 40 mg kg^{-1} diet (D1, D2, D3, D4, and D5, respectively). The results of a 56-days feeding trial showed that, significantly higher weight gain % (WG %), specific growth rate (SGR), feed intake (FI), and feed efficiency ratio (FER) were recorded in fish groups fed diets supplemented with different levels of CoQ10 than fish fed the control diet, while survival rate (SR%), condition factor (CF), hepatosomatic index (HSI) and viscerasomatic index (VSI) showed no obvious differences ($P > 0.05$) among all experimental groups. The highest activities of digestive enzymes (protease, amylase, and lipase) were recorded in D3, D4, and D5 groups. Moreover, blood status of all experimental fish was within normal rates and significant alterations were only in the case of glucose, cortisol, total cholesterol (T-Chol), triglycerides, and total protein (TP), where fish fed on D3, D4 and D5 diets exhibited lower values of glucose, cortisol, T-Chol, and triglycerides and higher values of TP. Furthermore, the lowest values of immune response [lysozyme, bactericidal, respiratory burst (NBT), and alternative complement pathway activities (ACP)], antioxidant capacity and oxidative related genes expressions [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP_X)] resulted from feeding on the basal diet (D1) compared to CoQ10 diets, especially with its high levels {≥20 mg kg^{-1} diet (D3, D4, and D5)} in most cases. In conclusion, our results suggest that the use of ≥20 mg CoQ10 kg^{-1} diet improves the growth and health being of Nile tilapia.

1. Introduction

Rapid and influential expansions in the aquaculture sector, particularly intensive farming as an ideal option for the production of a considerable amount of safer high-quality animal protein, have increased the focus of the studies on overcoming the unfavorable impacts and stressful conditions of intensification [1–3]. Fish is more susceptible to disease outbreak in intensive aquaculture [4] and the basis for the success of aquaculture is good management [5]. Chemotherapeutics or/and antibiotics were the most common approach to cope with diseases [6,7], but with increased anxiety about the consequences of frequent use (e.g. the development of resistant pathogens strains, accumulation of toxic residues, suppression of immune system, and environmental hazards) [8,9], their use in several countries has prevented [3]. As promising and effective alternatives to antibiotics, eco-friendly natural strategies and/or functional feed additives such as

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medicinal herbs, probiotics, prebiotics, and synbiotics, have attracted researchers' attention [10–13].

One candidate example found naturally in the biological system and most cells is coenzyme Q10 (CoQ10) or ubiquinone, a hydrophobic molecule [14], which exists in the inner membrane of mitochondria where plays a pivotal role in the electron transport chain (ETC) and generates energy (ATP) by transfer electrons from dehydrogenases (NADH) to complex II (succinate) to complex III (ubiquinone cytochrome *c* oxidase) of ETC [15–17]. Coenzyme Q10, especially its reduced form (ubiquinol), is an effective antioxidant and free radical scavenger that provides a protection to DNA, cells membrane, lipids and proteins from the risks of oxidative damage, helps regenerating of vitamin E and support healthy energy levels [18–21].

The bioactive compound CoQ10 is considered as a super-vitamin (vitamin Q) that exhibits potent antioxidant amplitude, which is formed in the body in insufficient quantities especially under stresses and therefore need to be obtained from an external source [22]. The addition of CoQ10 helps to improve human and animal health in the case of many diseases such as diabetes, obesity, muscular dystrophy, heart disease, periodontal disease, cancer, Alzheimer, aging, etc. [23–27]. Also, in vitro studies suggest its anti-inflammatory potency [28,29]. Because of the multiple benefits of CoQ10, the potential therapeutic advantages and the ease of synthesis either in biological or chemical methods made it one of the distinctive material of high demand in the market along with some other compounds such as vitamin C, vitamin E, alpha-lipoic acid, and L-carnitine [15,30]. In addition, CoQ10 solubility in lipids and organic solvents and water insolubility make it an ideal supplement for aquatic animal feed.

To our best knowledge, there is a lack of studies on the use of CoQ10 in the aquatic animals. Therefore, this study is designed to evaluate the use of CoQ10 on Nile tilapia (*Oreochromis niloticus*) performance and health as one of the most prevalent cultivated species in the world (2nd place with > 65% of the total aquaculture production) due to its convenience for farming and high marketability while maintaining a good marketing price [31]. Intensification of tilapia culture makes fish more susceptible to disease and stresses [32,33]. Our hypothesis suggests that the use of CoQ10 as a feed supplement may boost the growth, utilization of nutrients, blood profile, immune response, and oxidative status. This study could be the basis for future research on the use of CoQ10, not only with Nile tilapia but also for other aquatic species.

2. Materials and methods

2.1. Ethical approval

The study was fulfilled in accordance with the standards of animal care and use for scientific purposes approved by the Ethics Committee of Tanta University, Egypt.

2.2. Experimental design and diets preparation

Five experimental diets (≅30.5% crude protein and ≅18.05 kJ g^{-1}) were prepared by supplementing the basal diet (Table 1) with graded levels of CoQ10 (C9538 Sigma-Aldrich, USA) at 0 (control), 10, 20, 30, and 40 mg kg^{-1} diet at the expense of starch. CoQ10 was thoroughly mixed with fish oil and sunflower oil before adding other ingredients then water was added at 10% with continued mixing for extra 15 min until the texture was pasted and then pelleted using a California mill machine with 2 mm diameters [33]. The pellets were air-dried at room temperature in a dark place to avoid degrading of CoQ10 [34]. All diets were kept at −20 °C in a freezer until use. The chemical composition of the test diets was verified according to the standard analysis methods [35] (Table 1).

Table 1
Basal diet ingredients and proximate chemical analysis (%).

Ingredients		Proximate composition[c]	
Fish meal (72%)	10.5	Dry matter (DM)	90.48
Soybean meal (44%)	42	Crude protein (CP)	30.50
Wheat bran	10	Crude lipids (CL)	7.77
Yellow corn	18.5	Ash	6.97
Rice bran	10	Gross energy (KJ g^{-1})[d]	18.05
Fish oil	2.5		
Sunflower oil	1.5		
Vitamins mixture [a]	1		
Minerals mixture [b]	1		
Starch	3		
Total	100		

[a] Vitamins mixture (kg): vitamin A (3300 IU), vitamin B1(133 mg), vitamin B2 (580 mg), vitamin B6 (410 mg), vitamin B12 (50 mg), biotin (9330 mg), vitamin C (2660 mg), Colin chloride (4000 mg), vitamin D1 (410 IU), inositol (330 mg), niacin (26.60 mg), Para-amino benzoic acid (9330 mg), pantothenic acid (2000 mg).

[b] Minerals mixture (kg): cobalt (5 mg), copper (25 mg), iodine (5 mg), iron (200 mg), manganese (325 mg).

[c] Proximate analyses were performed in triplicates.

[d] Gross energy (KJ g^{-1}): calculated mathematically by multiplying the combustion coefficient of protein (23.6), lipid (39.5), and carbohydrate (17.2) in their ratio in diets.

2.3. Fish maintenance

The present study was conducted under the supervision of Fish production branch, Animal Production department, Faculty of Agriculture, Tanta University in cooperation with Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, Egypt.

Juveniles of Nile tilapia (*O. niloticus*) were purchased from a commercial fish hatchery located in Kafrelsheikh, Egypt and after two weeks of adaptation period to the experimental conditions in a 1000-L tank with feeding on a 30% crude protein diet, 300 fish (initial body weight = 12.4 ± 0.11 g) were distributed into 15 glass aquaria (5 treatments in triplicates; 20 fish/aquarium; 100 l). All tanks were supplied with air generators and 50% of the water was changed daily with clean chlorine-free water at the same temperature under 12: 12hrs light: darkness photoperiod regime for 56 days.

The mean values of daily measurements of water properties during the experiment period were: T°C = 25.2 ± 0.39 (Thermometer), pH = 6.9 ± 0.75 (Portable digital pH meter Martini Instruments Model 201/digital), dissolved oxygen (DO, mg l^{-1}) = 7.3 ± 04 (Waterproof Portable Dissolved Oxygen and BOD Meter model Hanna waterproof IP67), and total ammonia-nitrogen (TAN, mg l^{-1}) = 0.06 ± 02 (Colorimetrically: Spectronic 601, Milton Roy Company, San Diego, CA, USA). All water measured values were within the optimal ranges for rearing Nile tilapia [2,33].

2.4. The feeding trial, sampling schedule, and analytical procedures

2.4.1. Feeding protocol

The feeding trial lasted for 56 days in which fish were hand-fed twice at a specific time of day (08:00 and 16:00) at a percentage of wet body weight (4% for 2 weeks and 3% for 6 weeks) which is re-adjusted every two weeks according to the actual fish biomass in the tanks after the batch-weights of the fish. After 3 h of feeding time, uneaten feed is withdrawn from the tanks by siphoning and dried to calculate the actual feed intake (FI). At the end of the feeding trial, feeding was stopped for 24 h prior to final sampling to reduce fish handling stress.

2.4.2. Growth parameters, feed utilization and biometric indices

The weight and length of each fish were measured separately. Growth parameters and feed utilization were calculated according to

the following formulae [36,37]:

$$\text{Weight Gain (WG \%)} = \frac{W_T - W_0}{W_0} \times 100$$

$$\text{Specific Growth Rate (SGR \% day}^{-1}) = \frac{\text{Ln } W_T - \text{Ln } W_0}{T} \times 100$$

$$\text{Feed Intake (FI, g fish}^{-1}\text{ 56 days}^{-1}) = \frac{\text{Dry diet given} - \text{Dry remaining diet recovered}}{\text{No.of fish}}$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{FI (g)}}{\text{WG (g)}}$$

$$\text{Survival rate (SR \%)} = \frac{N_T}{N_0} \times 100$$

$$\text{Condition factor (CF)} = \frac{W}{L^3} \times 100$$

Where, W_T = final body weight; W_0 = initial body weight; T = the trial period in days (d); N_0 = initial number of fish; N_T = final number of fish; W = total weight (g); L = total length (cm).

Five fish were taken randomly from each aquarium (15 per treatment), anesthetized (3 ml pure clove oil: 10 ml of 40% ethyl alcohol) and the liver and viscera were eviscerated on the ice surface. Liver weight relative to body weight was used to calculate the hepatosomatic index (HSI) and viscera weight relative to body weight was used to calculate the viscerasomatic index (VSI). The gastrointestinal tract was separated from the rest of the viscera and washed with PBS (pH 7.5; 1 g per 10 ml), then homogenized and centrifuged for 5 min at 8000 rpm and the supernatant was then kept at 4 °C for the assessing of digestive enzyme activities. Besides, parts of the collected organs (liver and intestine) were immediately frozen in liquid nitrogen for the further analysis.

2.4.3. Digestive enzyme activities

Intestine protease activity was measured using casein as a substrate following the assay of Sigma's non-specific protease activity [38]. Amylase and lipase activities were quantified spectrophotometrically at A_{540} and A_{714} according to the modified methods of Wang et al. [39].

2.4.4. Blood and skin mucus sampling

After 24 h of the final weigh scaling, 15 anesthetized fish per treatment were assigned for sampling skin mucus and blood. Skin mucus was collected from a constant area (200 mm^2) of dorsal side skin using a plastic frame just before blood was collected according to the method of Ross et al. [40]. Blood was sampled using a sterile syringe ± heparin (anticoagulant) from the fish caudal vessels. Haematocrit was assessed in the partial heparinized whole blood by the centrifugation (13,000 rpm for 5 min) of samples placed into micro-haematocrit tubes according to the methods of Goldenfarb et al. [41]. Non heparinized syringes were used to collect blood for serum separation. Serum and plasma were collected undercooling (4 °C) at a speed of 3000 rpm for 10 min. Plasma and serum samples were kept at −20 °C until the analysis.

2.4.5. Blood hematological and biochemical indices

Plasma hematology [haemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs)] and serum biochemical profile [glucose, cortisol, total protein, cholesterol, triglycerides, alanine transaminase (ALT) and aspartate transaminase (AST)] were determined using Semi-automatic analyzer for clinical chemistry and hematology tests- Model 2000 Evolution, EMEG using Bayer Diagnostics Reagents strips following the manufactory guidelines.

2.4.6. Non-specific immune responses

Serum and mucus lysozyme activities were diagnosed with 96-well microplate turbidimetric assay utilizing *Micrococcus lysodeikticus* (lyophilized cell, Sigma-Aldrich, India) as described by Lygren et al. [42]. Serum or mucus samples (10 μl) were placed into 96 microplate tubes, and then the substrate mixture [190 μl of 0.2 mg of *Micrococcus lysodeikticus* ml^{-1} PSB, pH = 7.4] were added with soft shaking at room temperature. Changes in turbidity values were measured after 1 and 5 min, at 450 nm. A unit of enzyme activity was defined as the amount of enzyme that causes a decrease in absorbance of 0.001/min.

Bactericidal activities of serum and mucus were assessed spectrophotometrically at 570 nm according to the modified methods of Gallage et al. [43] as described by Wang et al. [44]. Briefly, serum or mucus samples were mixed with the bacterial suspension (*Streptococcus agalactiae* 1.4 × 10^8) in a 1: 1 ratio (50 μl sample: 50 μl bacterial suspension) at 25 °C for 2.5 h by using micro-tube rotator (Wavex – Tube Rotator E11270). After incubation, mixtures were placed into 96-microplate tubes with 15 μl 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide MTT (Sigma-Aldrich, Egypt) (5 mg ml^{-1}) for 15 min at 25 °C with soft shaking and the resulted formazan was dissolved with 50 μl of dimethyl sulfoxide (DMSO). Bacterial suspension in PBS without serum or mucus samples served as a positive control. The optical density (OD_{570}) for each sample was assessed in triplicate and the antimicrobial activities presented as a percentage of *S. agalactiae* inhibition relative to the positive control as follow:

$$\textit{S. agalactiae}\ \text{inhibition \%} = \frac{OD_{Control} - OD_{Sample}}{OD_{Control}} \times 100$$

Whole blood respiratory burst activities were quantified spectrophotometrically at 630 nm by performing nitro blue tetrazolium (NBT) assay according to the modified method of Secombes [45]. Alternative complement pathway activities of serum samples (ACP) were defined following the method described by Yanno [46] and Van Doan et al. [47].

2.4.7. Antioxidant potential assessment and oxidative related genes

Tissues samples (liver and intestine of 15 fish/treatment) were homogenized (Homogenizer VEVOR, FSH-2A) in cold iced NaCl (0.86%), then centrifuged (10 min, 12,000 rpm, 4 °C) and the supernatant was collected for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) colorimetric analyses with a specific detection kits (JianCheng, Nanjing, China) using a microplate spectrophotometer at 550, 280, 412 nm following the manufacturer's instructions. One unit of SOD enzyme activity equals the amount of superoxide dismutase needed to inhibit 50% of the nitrobluete trazolium reduction [48]. One unit of CAT enzyme activity was defined as the needed amount of CAT to transform 1 μmol of H_2O_2 min^{-1} [49]. GPx enzyme activity unit is the required amount of GPx to oxidize 1 μl of NADPH min^{-1} [50].

The gene expression trial focused on the genes associated with oxidation (SOD, GP_X and CAT) [51–53] and was performed in triplicates using quantitative real-time PCR (qRT-PCR) in the collected tissues (liver and intestinal samples kept at −80 °C; 15 fish per treatment). About 50–100 mg of collected samples were utilized to extract RNA using RNeasy Mini (Qiagen, Hilden, Germany) following manufacturer's guidance. The extracted RNAs were subjected to 1.5% agarose gel electrophoresis in TAE buffer under a stable voltage condition (100 V) using E-Gel Agarose System to verify RNA integrity, while the concentration was defined using a spectrophotometer. First-strand cDNA was obtained by the reverse transcriptase of 1–2 μg pure RNA using SuperScript IV Reverse Transcriptase cDNA synthesis kit as described by Amin et al. [33].

Selected genes and a stable internal housekeeping standard (β-actin) were amplified using a specific primer set (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (Table 2) [53] and amplification products were tested by melting curve analysis at the end of each PCR reaction. SOD, GP_X and CAT mRNA expressions in liver and intestine were assessed by real-time quantitative PCR (qRT-PCR) and the amplification

Table 2
Oligonucleotide primers set used for qRT-PCR analysis.

Gene	Sequence (5′-3′) F= Forward – R = Reverse		Annealing T	Amplicons size (Pb)	Accession number	Reference
SOD	F	GGTGCCCTGGAGCCCTA	60	377	JF801727.1	[53]
	R	ATGCGAAGTCTTCCACTGTC				
CAT	F	TCCTGAATGAGGAGGAGCGA	60	232	JF801726.1	[53]
	R	ATCTTAGATGAGGCGGTGATG				
GPx	F	CCAAGAGAACTGCAAGAGA	60	180	FF280316.1	[53]
	R	CAGGACACGTCATTCCTACAC				
β-actin	F	CAATGAGAGGTTCCGTTGC	60	280	EF206801	[53]
	R	AGGATTCCATACCAAGGAAGG				

mixture and thermal profile were designed following the manufacturer's protocol of the SYBR® Premix Ex Taq™ II Kit (Takara Bio, Otsu, Japan). The relative values of mRNA expression were calculated using $2^{-\Delta\Delta CT}$ algorithm with β-actin as the house keeping endogenous reference [54].

2.5. Statistical analysis

The data were analyzed statistically using IBM® SPSS® Statistics program version 22 (SPSS Inc., IL, USA) and expressed as means ± standard errors (S.E.) of triplicate values. The homogeneity and normality of variance were inspected by Shapiro-Wilk and Levene tests and then one-way ANOVA and Duncan's post hoc tests were performed at a 5% probability level.

3. Results

3.1. Fish growth, feed efficiency and biometric indices

Table 3 summarizes the growth, feed utilization, and biometric indices of Nile tilapia fed the experimental diets for 56 days. Significantly higher growth performance and feed utilization were recorded in fish groups fed diets supplemented with CoQ10 compared to fish fed the control diet, while SR, CF, HSI, and VSI shown no obvious differences ($P > 0.05$). Moreover, there were no appreciable differences ($P > 0.05$) in weight (WG and SGR) and feed efficiency (FI and FER) among fish groups fed on diets with different levels of CoQ10.

3.2. Digestive enzyme activities

Intestine enzymes activities (protease, amylase, and lipase) of Nile tilapia after 56 days trial period were shown in Table 4. CoQ10 supplementations at a rate of ≥20 mg kg^{-1} diet (D3, D4, and D5) maximize the activity of protease, amylase, and lipase. Conversely, fish fed the basal diet (D1) had the lowest significant lipase and protease performance compared to their analogs. Meanwhile, no statistically difference ($P > 0.05$) in amylase activity between fish groups fed D2 diet (10 mg CoQ10 kg^{-1} diet) and D1.

3.3. Blood hematological and biochemical indices

Table 5 represents the blood hematological and biochemical parameters of Nile tilapia fish after 56 days experimental period. No significant differences were found in Ht (%), Hb (g dl^{-1}), RBCs (10^6 μl^{-1}), WBCs (10^3 μl^{-1}), ALT (IU l^{-1}), and AST (IU l^{-1}) between the experimental groups. Interestingly, fish fed diet contain CoQ10 a rate of ≥20 mg kg^{-1} diet (D3, D4, and D5) exhibited lower values of glucose (mg dl^{-1}), cortisol (ng dl^{-1}), T-Chol (mg dl^{-1}), and triglycerides (mg dl^{-1}) and higher values of TP (g dl^{-1}).

3.4. Non-specific immune responses

Serum and mucus lysozyme activity of Nile tilapia is presented in Fig. 1-A. The lowest values of lysozyme activity in both serum and mucus were recorded in fish fed on the basal diet (D1) and the highest value of mucosal lysozyme was observed in fish groups fed on CoQ10 diets, while the highest activity of serum lysozyme was found in fish groups fed on diets with high levels of CoQ10 ≥ 20 mg kg^{-1} diet (D3, D4, and D5).

Data on serum and mucus bactericidal activity of Nile tilapia are presented in Fig. 1-B. Serum and mucus bactericidal activity positively exhibited higher values in the groups reared on CoQ10 diets and the lower values were recorded in fish fed with D1.

Respiratory burst (Nitro-blue Tetrazolium, NBT) showed higher values in CoQ10 groups compared with the control (Fig. 2-A). Values of serum alternative complement pathway activities (ACP) were higher ($P < 0.05$) in fish groups fed on D3, D4, and D5 when compared with other groups (D1, D2) (Fig. 2-B).

3.5. Antioxidant potential assessment and oxidative related genes

Results of SOD, CAT and GP_X are shown in Fig. 3. Significantly higher SOD, CAT, and GPx values were observed in fish groups fed on D3, D4, and D5 diets, while the lower values were in fish fed D1. Gene expression analysis detected a significant ($P < 0.05$) upregulation of liver and intestine SOD, CAT, and GPx expressions in fish fed CoQ10 diets at different levels compared to control group, with high values in

Table 3
Growth, nutrient efficiency and biometric indices of Nile tilapia fed test diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56 days.

Item	Test diets				
	D1	D2	D3	D4	D5
WG (%)	197.64 ± 4.30^b	240.81 ± 5.21^a	246.35 ± 6.62^a	249.35 ± 6.81^a	242.80 ± 6.01^a
SGR (% WG day^{-1})	1.95 ± 0.03^b	2.19 ± 0.03^a	2.22 ± 0.03^a	2.23 ± 0.04^a	2.20 ± 0.03^a
FI (g/fish/56 days)	37.92 ± 0.10^b	40.08 ± 0.17^a	40.13 ± 0.22^a	39.95 ± 0.31^a	39.69 ± 0.17^a
FER	0.64 ± 0.01^b	0.74 ± 0.01^a	0.76 ± 0.03^a	0.77 ± 0.02^a	0.76 ± 0.02^a
SR (%)	95.00 ± 2.89	96.67 ± 1.67	100.00 ± 0.00	98.33 ± 1.67	98.33 ± 1.67
CF (%)	1.79 ± 0.12	1.81 ± 0.08	1.82 ± 0.03	1.82 ± 0.01	1.81 ± 0.01
HSI (%)	2.01 ± 0.15	2.10 ± 0.20	2.04 ± 0.18	2.05 ± 0.11	2.10 ± 0.06
VSI (%)	6.32 ± 0.32	6.55 ± 0.52	6.71 ± 0.83	7.02 ± 0.44	6.47 ± 0.54

Table 4
Digestive enzymes activities (amylase, lipase, protease) of Nile tilapia fed test diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56 days.

Enzyme activit (U mg^{-1})	Test diets				
	D1	D2	D3	D4	D5
Amylase	23.02 ± 0.59^{b}	25.30 ± 0.57^{b}	31.73 ± 0.92^{a}	31.70 ± 0.94^{a}	30.95 ± 1.04^{a}
Lipase	24.80 ± 0.75^{c}	27.11 ± 0.56^{b}	36.44 ± 0.59^{a}	35.61 ± 0.74^{a}	35.87 ± 0.67^{a}
Protease	17.62 ± 0.72^{c}	19.53 ± 0.28^{b}	24.87 ± 0.09^{a}	24.70 ± 0.55^{a}	24.09 ± 0.58^{a}

Values represent Means ± S.E. ($n = 5$). Means in the same row bearing different superscript are significantly different at ($P < 0.05$).

Table 5
Blood hematological and biochemical parameters of Nile tilapia fed test diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56 days.

Item	Test diets				
	D1	D2	D3	D4	D5
Haematocrit (Ht, %)	29.23 ± 1.21	29.95 ± 0.54	30.36 ± 0.26	29.74 ± 1.29	29.61 ± 1.38
Hemoglobin (Hb, g dl^{-1})	7.24 ± 0.07	7.80 ± 0.15	7.71 ± 0.34	7.77 ± 0.18	7.48 ± 0.30
Red blood cells (RBCs, 10^6 µl^{-1})	1.98 ± 0.02	2.47 ± 0.09	2.46 ± 0.26	2.57 ± 0.29	2.04 ± 0.04
White blood cells (WBCs, 10^3 µl^{-1})	68.77 ± 3.95	76.96 ± 8.89	79.46 ± 2.91	80.33 ± 3.24	79.69 ± 0.62
Glucose (mg dl^{-1})	82.92 ± 1.11^{a}	76.72 ± 0.44^{b}	69.49 ± 0.52^{c}	70.13 ± 0.74^{c}	71.39 ± 0.62^{c}
Cortisol (ng ml^{-1})	42.59 ± 0.36^{a}	35.39 ± 0.67^{b}	34.69 ± 0.51^{b}	35.05 ± 0.12^{b}	35.74 ± 0.32^{b}
Total protein (TP, g dl^{-1})	2.19 ± 0.02^{c}	2.66 ± 0.06^{ab}	2.89 ± 0.07^{a}	2.82 ± 0.06^{a}	2.45 ± 0.14^{b}
Total cholesterol (T-Chol, mg dl^{-1})	87.40 ± 0.63^{a}	84.84 ± 1.19^{ab}	80.26 ± 1.22^{c}	83.11 ± 0.57^{bc}	82.71 ± 1.42^{bc}
Triglyceride (mg dl^{-1})	102.59 ± 1.17^{a}	98.93 ± 1.80^{ab}	94.58 ± 1.48^{b}	97.57 ± 0.94^{b}	96.77 ± 1.66^{b}
Alanine transaminase (ALT, IU l^{-1})	81.55 ± 0.91	77.69 ± 1.80	77.02 ± 1.74	76.98 ± 1.99	76.77 ± 0.71
Aspartate transaminase (AST, IU l^{-1})	32.77 ± 0.44	31.60 ± 0.73	31.32 ± 0.71	31.31 ± 0.81	31.09 ± 0.28

Results are expressed as Means ± S.E. ($n = 5$). Means in the same row bearing different superscript are significantly different at ($P < 0.05$).

fish fed on D3, D4, and D5 diets (Fig. 4).

4. Discussion

Cultivation of aquatic animals at high densities requires precise strategies to achieve the desired production and quality and also to cope with the consequences of intensification. Recently there are many approaches used in boosting and modulating fish growth and well-being, including fermentation [13], modern aquaculture systems [e.g. aquaponics, recirculatory aquaculture system (RAS), biofloc technology (BFT), compensatory growth technology} [55–57], and nutraceuticals feed additives {probiotics, prebiotics, synbiotics, immunostimulant agents, exogenous enzymes, hormones, nucleotides, medicinal plants, organic acids or acidifiers, and antioxidants] [1]. Dietary immunostimulants are known to be a useful application for enhancing performance and disease resistance in fish [58]. CoQ10 is a natural

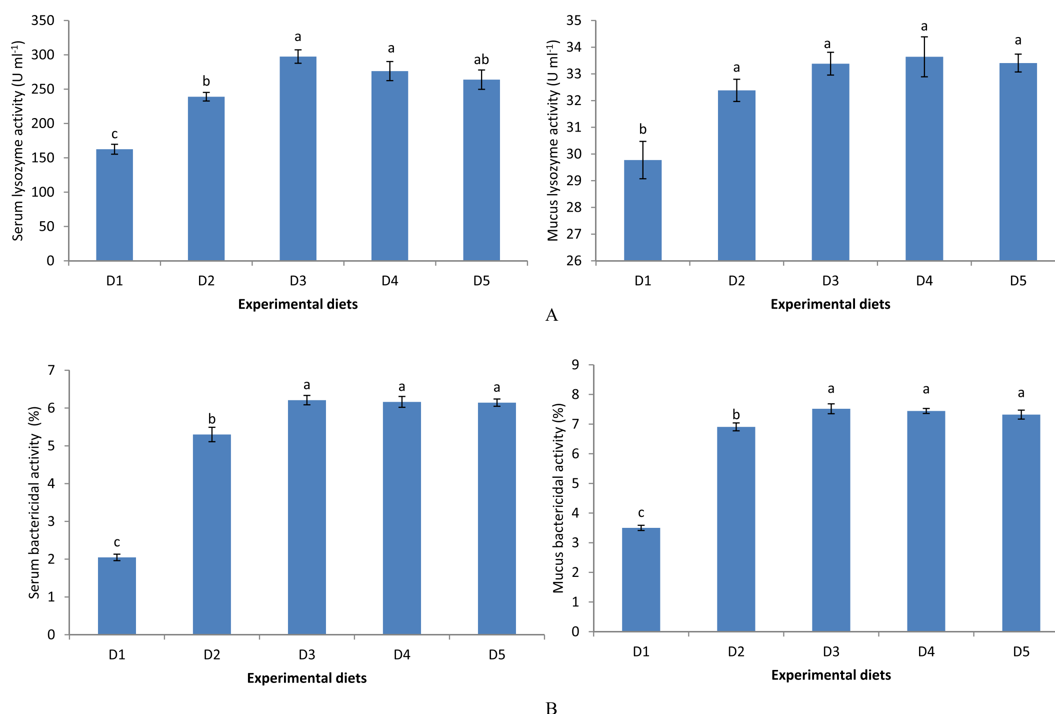

Fig. 1. Lysozyme and bactericidal activities in serum and mucus of Nile tilapia fed the experimental diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56-days.

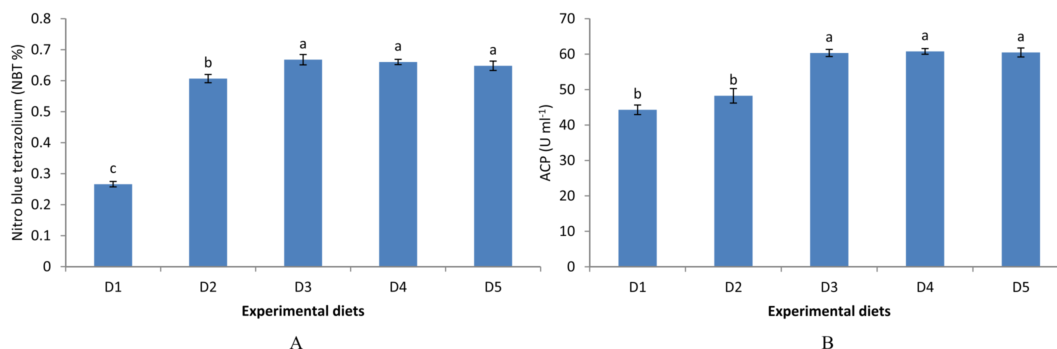

Fig. 2. Respiratory burst (Nitro-blue Tetrazolium, NBT) and serum alternative complement pathway (ACP) values for Nile tilapia fed the experimental diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56-days. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

substance with a strong antioxidant impact that has been studied significantly in humans [25] and some animals [59,60], while there is a lack of studies on its effect on farmed fish. To this end, in the present study Nile tilapia was used as a widespread model worldwide to test the efficacy of CoQ10 in modifying and improving growth and health status. CoQ10 as an inexpensive obtainable dietary supplement has been proven to yield a broad range of advantageous impacts [61]. The natural sources of CoQ10 vary from prokaryotic organisms to eukaryotic organisms including a large number of bacteria, some of which use as probiotics [15].

The results of growth and utilization of nutrients showed an improvement with the addition of CoQ10 in the diet. The enhanced feed utilization in terms of feed intake (FI) and feed efficiency ratio (FER) might be one of the reasons for the boosted growth in fish fed on CoQ10 diets. Increased nutritional efficiency may also be associated with the improved intestine condition due to modulating intestine microflora or CoQ10 anti-inflammatory properties [28]. Also, the results of digestive enzymes activity (protease, amylase, and lipase) confirm this improvement as enzymes activity could extend further insight into the prospective impacts of diets on growth performance and feed utilization

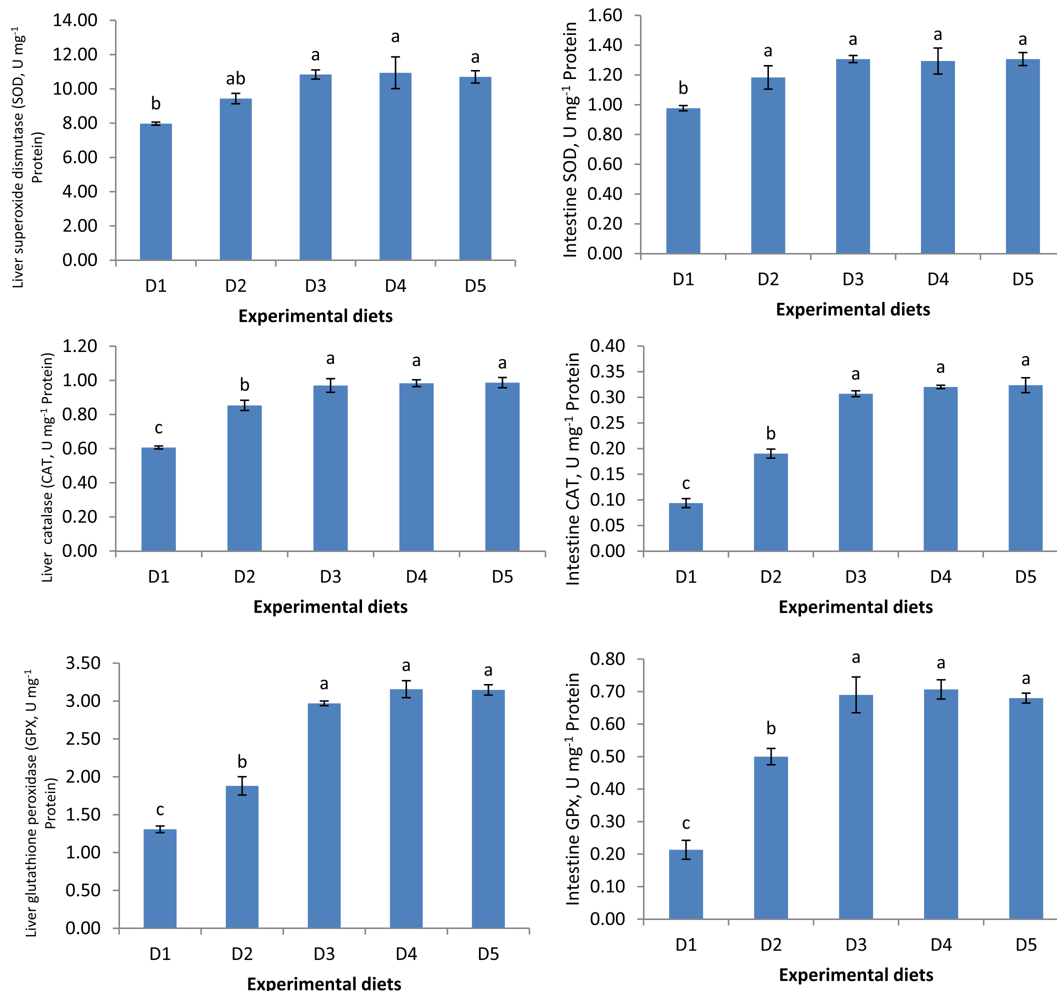

Fig. 3. Antioxidant indicators (SOD, CAT, and GPx) in Nile tilapia fish reared on experimental diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56-days.

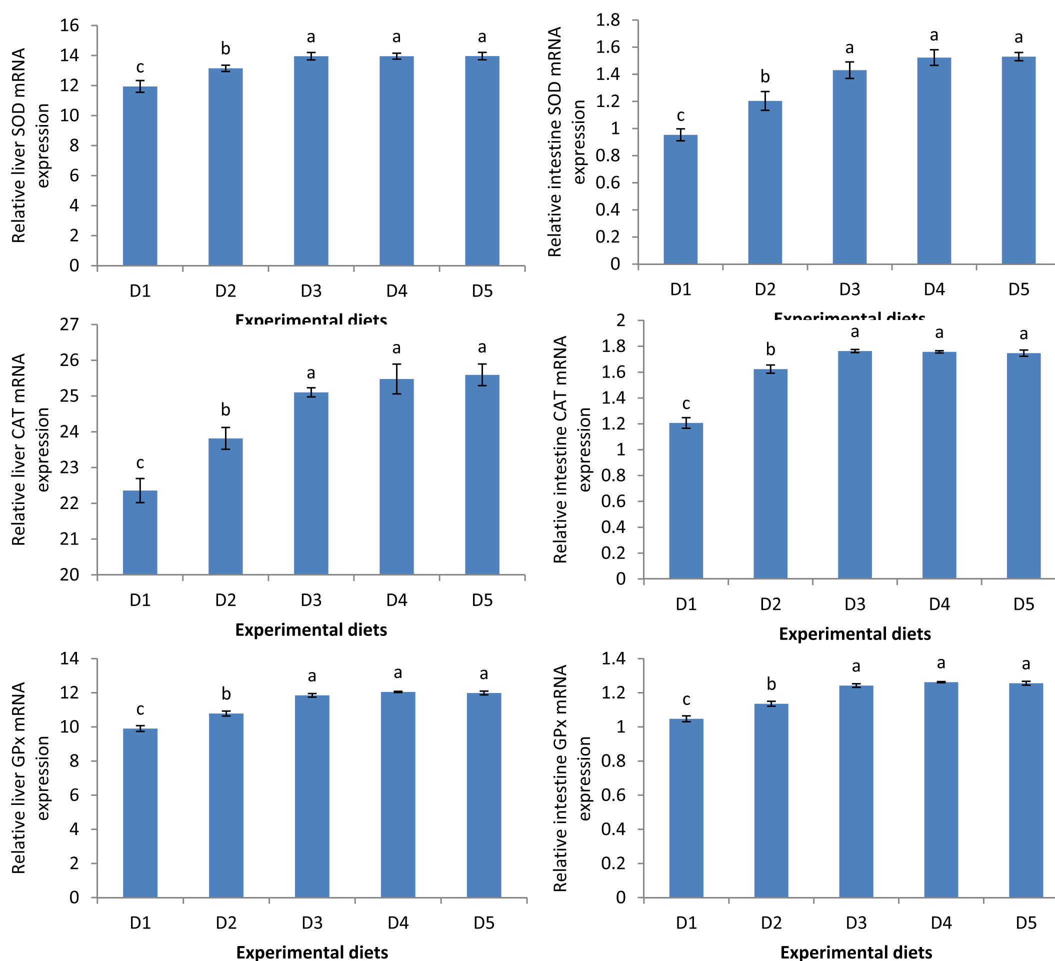

Fig. 4. Analysis of oxidative related genes (SOD, CAT, and GPx) expression in the liver and intestine of Nile tilapia fed the experimental diets (D1 = 0; D2 = 10; D3 = 20; D4 = 30; D5 = 40 mg CoQ10 kg^{-1} diet) for 56-days.

[2]. This amelioration in growth, utilization of feed and digestive enzymes may be due to the indirect effect of CoQ10 on re-synthesis of vitamin E [18–21]. Moreover, the role of CoQ10 in the carbs cycle makes it an important pivot in the metabolism of carbohydrates, protein, and lipids [15–17]. Also, the effects of CoQ10 treatment on insulin, glucagon and cortisone hormones support the positive role in improving the performance of fish [62]. In this context, Gopi et al. [63], reported better feed efficiency and production score with high energy diet supplemented with CoQ10 at 20 mg kg^{-1} diet in broiler. Also, Geng et al. [64], observed remarkable higher weight gain with comparable feed intake and feed efficiency with CoQ10 supplement at the level of 40 mg kg^{-1} diet. moreover, Huang et al. [65], reported an enhancement in the weight gain at the level of 20–40 mg CoQ10 kg^{-1} diet.

Blood indicators are accurate tools that reflect the health status of fish as well as the response to external stimuli and stressors [36,37]. In general, measurement values of blood indices recorded in the present study are within the acceptable limits for Nile tilapia [2]. Also, no considerable changes were noticed in the activities of liver metabolic enzymes (ALT and AST) reflecting the absence of CoQ10 toxicity at any levels used in this study. Glucose and cortisol are realistic indicators of the existence of stressors as they increase with stress and decreases in well-being [66,67]. Interestingly, levels of glucose and cortisol have been decreased in fish fed CoQ10 supplemented diets when compared with fish fed un-supplemented diet; this could be linked either to hypoglycemic hormone stimulation (insulin) or reduction in glucose absorption [36,68]. Also, cholesterol and triglyceride levels were decreased and this may linked to the hypocholesterolemic effect of CoQ10 [68,69]. Conversely, higher total protein (TP) contents were observed in fish fed with high levels of CoQ10 ($\geq$20 mg kg^{-1} diet), which may indicate an improvement in fish health or could be a post-injury or infection adaptation response [70].

Nutrients supplementation may alter the immune responses of fish by impressing directly or indirectly on immune cells through metabolic, neurological, or endocrine pathways [13]. Phagocytosis is a vital defense mechanism line in fish which utilizes in lysozyme activity, bactericidal activity (BA), respiratory burst activity (Nitroblue Tetrazolium, NBT), and alternative complement pathway activities (ACP) [70,71]. Lysozyme is considered one of the most significant non-specific defense lines of natural immune system and used to assess the ability of the immune system in many fish species as a result of its anti-microbial activities [5]. Leucocytes cell produce lysozyme, which lysis the wall of microbial cells and thus stimulate the lysozyme production, activates the immunity complement system [72]. It is worth to be mentioned NBT activity issued as an important indicator of the innate immune defense mechanism in fish [73]. Bactericidal activity (BA) serves as a pivotal factor of the host to withstand pathogens [74]. The results of improved immunological parameters with CoQ10 supplements can be explained by maintaining a healthy energy level, reducing stresses, enhancing mitochondrial respiration, protecting cells membranes, and maximizing the level of vitamin E [61].

The antioxidant defense system is highly linked with fish health status and the immune system [36]. Oxidative stress arises from the lack of balance between production and disposal of reactive oxygen species (ROS) [75]. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) as vital components of the antioxidant

defense system play important roles in removing excessive ROS, sustaining the homeostasis of the cell [2,70]. SOD, CAT and GPx values and their gene expression were significantly higher in fish groups fed at ≥20 mg CoQ10 kg^{-1} diets indicating the high capacity of CoQ10 as an antioxidant. Also, the enzyme activities (SOD, CAT, and GPx) and their gene expressions were higher in the liver compared to the intestine, demonstrating the important role of the liver in the antioxidant defense system as previously mentioned [27,51]. It is well known that, CoQ10 is considered as a super-vitamin (vitamin Q) that exhibits potent antioxidant amplitude, free radical scavenger that provides a protection to DNA, cells membrane, lipids and proteins, helps regenerating of vitamin E and support healthy energy levels, improve health in case of many disease [18–27].

5. Conclusion

Dietary inclusion of CoQ10 at level of ≥20 mg CoQ10 kg^{-1} diet improves the growth, health being and compensate for the harmful impacts of oxidative stress in Nile tilapia.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2020.01.052.

Values are represented as Means ± S.E ($n = 3$, except for CF, HSI, and VSI where $n = 5$). Means in the same row with different superscript letters are significantly different ($P < 0.05$).

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