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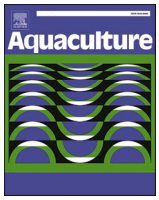

Possibility mitigation of cold stress in Nile tilapia under biofloc system by dietary propylene glycol: Performance feeding status, immune, physiological responses and transcriptional response of delta-9-desaturase gene

Mohamed R. Soaudy [a], Eman Y. Mohammady [b], Mohamed A. Elashry [a], Marwa M. Ali [a], Nasr M. Ahmed [c], Mahmoud H. Hegab [c], Hoda A.S. El-Garhy [d], Ehab R. El-Haroun [e], Mohamed S. Hassaan [a,*]

[a] Department of Animal Production, Fish Research Laboratory, Faculty of Agriculture at Moshtohor, Benha University, Benha 13736, Egypt
[b] Aquaculture Division, National Institute of Oceanography and Fisheries, NIOF, Egypt
[c] Freshwater and Lakes Division, National Institute of Oceanography and Fisheries, NIOF, Egypt
[d] Department of genetic engineering, Faculty of Agriculture at Moshtohor, Benha University, Benha 13736, Egypt
[e] Animal Production Department, Faculty of Agriculture, Cairo University, Cairo, Egypt

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ABSTRACT

This trial was conducted to study the ability of dietary propylene glycol to mitigate winter stress of Nile tilapia under biofloc system. Nile tilapia (average initial weight = 28.50 ± 0.25 g) were fed three isonitrogenous (257.75 g kg^{-1} crude protein) diets for 47 days in winter season. The first diet was free added with propylene glycol (PG) control. The other two diets were supplemented with 5 mL and 7.5 mL PG kg^{-1} diet, respectively. At the end of feeding trial, the highest survival rate ($P < 0.05$) was observed in tilapia fed the diet supplemented with 7.5 ml PG kg^{-1} diet. A linear response in weight gain (WG; $P = 0.034$), specific growth rate (SGR; $P = 0.041$) and protein efficiency ratio (PER; $P = 0.038$) of tilapia were found by the increase in the PG levels in diets. The supplemental diets with PG did not induce any significant differences ($P > 0.05$) on feed conversion ratio (FCR) and feed intake (FI) of fish. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, triglyceride and low density of lipoprotein cholesterol (LDL-C) were linearly decreased in response to the increased the PG level. Also, linear increase in serum total protein, albumin, globulin and lipoprotein cholesterol (HDL—C) were found in response to supplementation of PG. Significant linear increased in the concentration of serum ions; sodium, calcium and chloride were observed with increasing the PG level. Significant linear increased were found in superoxide dismutase (SOD; $P = 0.035$), catalase (CAT; $P = 0.001$), glutathione (GSH; $P = 0.056$) and glutathione peroxidase (GPx; $P = 0.048$) for fish fed 5- or 7.5-ml PG kg^{-1} under cold stress, with the highest values in fish fed 7.5 ml PG kg^{-1}. While, the activity of malondialdehyde (MDA; $P = 0.023$) was linearly reduced with increasing of the PG levels in fish diets, with the lowest value in group fed supplemental diet with 7.5 ml kg^{-1} PG. The response of glucose ($P = 0.026$) and cortisol ($P = 0.193$) of fish in cold stress for 47 days were linearly with increasing PG supplementation. The transcription of Δ 9D gene of fish reared under cold stress was linearly up regulated (linear, $P = 0.001$) with increasing dietary PG level. In conclusion, diet supplemented with 7.5 ml kg^{-1} PG could decrease the mortality, and enhance the physiological status as well as transcription of Δ 9D gene of fish reared under cold stress.

1. Introduction

Cold stress is an essential factor participating in fish disease and mortality, subsequently, decreased the production of aquaculture systems in tropical region (Hassaan et al., 2019). Furthermore, cold stress has negative influences on fish as reducing metabolic rates (Galloway

* Corresponding author.
E-mail address: Mohamed.hassaan@fagr.bu.edu.eg (M.S. Hassaan).

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and Kieffer, 2003), swimming performance (Hocutt, 1973), and harming the immune functions (Hurst, 2007). Concerning, Nile tilapia, *(Oreochromis niloticus*) as a tropical warm water fish are able to tolerate a wide range of temperatures (8–42 °C). It does not grow well at temperature below 16 °C and does not survive at temperature below 10 °C for more than a few days (Bauer and Schlott, 2004; Siddik et al., 2014; Wu et al., 2019). Activity and feeding become reduced when the temperature falls below 20 °C and stops completely at around 16 °C. The temperature in winter season dropped to 16 °C or less, which affected negatively on the survival, the growth and might cause the death of tilapia cultured in aquaculture pond in Egypt (Soltan et al., 2014). Delta-9-desaturase (Δ 9D) gene play a vital role in cold stress mitigation by de saturate membrane lipids in order to sustain membrane fluidity (Zerai et al., 2010; Polley et al., 2003; Murray et al., 2007). Therefore, it is necessary to find new technologies to enhance the tolerance of cultured fish against cold stress and improve its production in winter season (Yokoyama et al., 2005). Several strategies have been conducted to provide a proper temperature in tilapia ponds during winter season by using heated facilities, geothermal water and pond covering with plastic sheet (Dan and Little, 2000; Abdel-Aal, 2008). Also, dietary supplemental feed additives are another strategy to mitigate the cold stress for cultured tilapia through raising the polyunsaturated fatty acids (PUFs) such as linseed or sunflower oils in precool season diets to guarantee its suitable functionality (Hsieh et al., 2007; Correa et al., 2017; Correa et al., 2018). Other studies have indicated that the administration of isolated immunostimulants from plants, animals and microorganisms (Sakai, 1999) in stressful conditions during winter season such as, using natural herbs *Astragalus membranaceus* extract powder (AMEP) in tilapia diet (Wu et al., 2019), β-Glucans in catfish diet (Soltanian et al., 2014) and Propolis (bee glue) in tilapia diet (Hassaan et al., 2019) were reduced their negative effects of stress and boost the immune functions of fish (Ortuno et al., 2003; Sarma et al., 2009).

Propylene glycol (PG) (1,2-propanediol) is a synthetic hydrocarbon produced during the cracking of propane. Few, studies were used PG as a dietary source of energy in broiler diets (Bayley et al., 1967; Persons et al., 1968; Waldroup and Bowen, 1968) and it also improved the physical-chemical properties at level less 10% of the feed pellet as plasticity and texture and preventing the proliferation of bacteria through its effects as antibacterial and antifungal which in turn elevating the feed palatability, weight gain and feed conversion ratio of rainbow trout and Atlantic salmon, *Salmo salar* (Hilton et al., 1986; Hughes, 1988; Nalawade et al., 2015). Similarly, channel catfish, *Ictalurus punctatus* fed diets containing 15 and 20% glycerol as a dietary source of energy had reduced weight gain and feed efficiency while, their levels up to 100 did not induce any changes (Li et al., 2010). On the other hand, using glycerol from 0 to 100 g kg^{-1} as a dietary energy source did not affect significantly on the growth performance and feed utilization of Nile tilapia (Neu et al., 2013). In the same context, glycerol failed to use as a source of energy in rainbow trout, *Oncorhynchus mykiss* diet at level up to 12% (Menton et al., 1986). Also, PG is anti-ketogenic by increasing plasma glucose concentrations through decreased peripheral tissue glucose demand, reducing non-esterified fatty acids (NEFA) and liver triglyceride levels which resulting in a decreasing concentration of ß-hydroxybutyric acid (BHBA) in plasma (Hussein et al., 2015). The optimum safe levels of PG in diets ranged from 2.5 to 8% depending on the animal species (Bayley et al., 1967; Persons et al., 1968; Waldroup and Bowen, 1968). Fortunately,ittel PG could convert to lactic acid, subsequently convert into glucose (Rudney, 1950; Miller et al., 1953) this indicated that PG can be used as a source of energy in practical tilapia diets. To the best author's knowledge, there is no information about dietary PG as a source of energy to mitigate the cold temperature stress in fish; however, previous researches as mentioned above were carried out on broiler to indicate a high level of PG availability (Bayley et al., 1967). Therefore, the purpose of this study was to determine the supplemental effects of PG in cold temperature stress on the survival, growth performance, feed utilization, serum biochemical indices and the expression of Δ9D gene in Nile tilapia, *Oreochromis niloticus* rearing under biofloc system.

2. Materials and methods

2.1. Experimental design and diets

Three diets were formulated to fulfill the dietary requirements of Nile tilapia (NRC, 2011). The experimental diets were assigned as control (Propylene glycol (PG; 99% purity)-free added diet), and another two examined diets containing two levels of PG 5 ml and 7.5 ml g kg^{-1} diet, respectively. Levels of PG used in this study was recommended by Hilton et al. (1986) and Hughes (1988). The experimental diets were 257.75 g kg^{-1} crude protein and 73.45 g kg^{-1} crude lipid (Table 1). For preparation of diets, the ingredients were mixed thoroughly after adding different levels of PG and pellets (with diameter of 2 mm) were made by using pelleting hand-noodle maker, then dried at room temperature for 12 h and kept in cellophane bags at −4 °C until use. The proximate analysis of chemical composition for the tested diets was estimated according to AOAC (1995).

2.2. Fish and experimental management

Nile tilapia, *Oreochromis niloticus* monosex were collected from the fish farm of Faculty of Agriculture, Benha University, Egypt on 15 December 2019. After collection, fish were reared in two cement ponds (2 × 4 × 1 m) for 15-day to acclimate to the experimental conditions and feed a commercial feed (25 g kg^{-1} protein), until to start the feeding trial. The study started on 8 January 2020 and ended until 24 February 2018 (47 days). After the acclimation, 117 healthy mono-sex fish of similar sizes (average initial weight of 28.50 ± 0.25 g) were randomly

Table 1
Formulation and proximate composition of the basal diet (g $kg\ diet^{-1}$, dry matter).

Ingredients	g
Fish meal	50
Soybean meal	390
Yellow corn	350
Wheat bran	130
Fish oil	50
Premix[a]	30
Proximate analysis	
Crude protein	257.6
Crude lipid	73.5
Ash	47.5
Fiber	56.2
NFE[b]	565.2
Gross energy (MJ kg^{-1})[c]	18.7

[a] Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 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. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65 mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).

[b] NFE (Nitrogen free extract) =100-(crude protein + Crude lipid + ash +fiber content).

[c] Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kjg^{-1} for protein, fat and carbohydrate, respectively according to Brett (1971).

distributed into nine fiberglass tanks (0.5 m^3) at a stocking density of 13 fish per tank. The experiment was performed under biofloc system (zero water exchange). Each tank received constant aeration by two air stones. Fish hand-fed to an apparent visual satiety two times daily (12.00 pm and 15.00 pm) after sunrise, six days per week according to (Dan and Little, 2000; Hassaan et al., 2019). A C: N ratio of 10:1 was used in this experiment according to De Schryver et al. (2008). The starch carbon source was weighed and soluble in water and applied daily to all tanks in a split-order manner at intermediate feeding times according to De Schryver et al. (2008).

2.3. Physicochemical parameters of water

Temperature (°C) was monitored by using a mercury thermometer suspended at 15-cm depth and pH was recorded by using a pH meter (Orion pH meter, Abilene, Texas, USA) twice a day (at 08:00 am and 04:00 pm). Dissolved oxygen (DO, mg/L) was measured by Jenway 970 Dissolved Oxygen Meter (Keison Company, UK). Ammonia (NH_4, mg/L), nitrate (NO_3, mg/L) and nitrite (NO_2, mg/L) were monitored once a week according to standard methods APHA (1989). All analyses were triplicated and estimated weekly. Average of ambient temperature and measured water quality parameters are showed in Table 2.

2.4. Growth parameters

Initial body weight (g) (IBW) and final body weight (g) (FBW) of individual fish were recorded for all fish/each tank at the initiation and the termination of the experiment. Growth performance and efficiency of diets were calculated according to the equations in the footnote of the Table 4. Also, cumulative rates of mortality (%) were noted.

2.5. Serum biochemical analysis

At the termination of the experimental trial, three fish of each replicate were anesthetized by 3-aminobenzoic acid ethyl ester (MS 222, 100 mg/L, Sigma, Egypt), and blood samples were collected using clean syringes from the caudal vein of fish. The collected blood samples were centrifuged at 1500*g* for 15 min at 4 °C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957). Total serum protein and albumin were determined according to Doumas et al. (1981). However, the total serum globulin was calculated by subtracting the total serum albumin from the total serum protein according to Coles (1974). Serum cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL—C) and glucose were measured using standard Kits (Modern Laboratory Kits). Cortisol levels (ng ml^{-1}) were determined by chemiluminescence with an immulite kit. The sodium concentration in the blood serum was determined by using a colorimetric detection kit (Coral Clinical Systems) at 530 nm and 630 nm respectively after 5-min incubation at room temperature. Magnesium concentration was measured by the magon sulfonate method (505 nm), calcium by the cresolphthalein method (570 nm) and chloride by the thiocyanate method (470 nm).

Table 2
Average of ambient temperature and water quality parameters during the experimental Period from January to February.

Treatment periods	Average of water quality parameters					
	Temp	DO	NH^{4+}	NO^2	NO^3	pH
8 Jan- 22 Jan	16	7.28	0.27	0.01	0.11	7.98
23 Jan – 7 Feb	17	7.69	0.26	0.01	0.13	8.04
8 Feb – 22 Feb	19.7	7.69	0.26	0.01	0.16	8.11

2.6. Oxidative enzyme activity

Livers of three fish from each replicate were weighed, rinsed and grinded in glass homogenizer tubes with ice-cold saline (0.1 g of liver was added 0.9 mL saline, pH 7.0), and centrifuged at 3000*g* for 10 min. The collected supernatant was used for the activity of superoxide dismutase (SOD) measurement according to the method of Peskin and Winterbourn (2000). The modified method of Beers and Sizer (1952) was used for catalase (CAT) activity assay. Melanodialdehyde (MDA) activity was measured according to Dogru et al. (2008). Glutathione peroxidase (GPx) activity was determined according to of Moin (1986) and expressed as units per milligram protein. Glutathione (GSH) was measured according to the method of Beutler et al. (1963).

2.7. Expression of delta-9-desaturase gene

After fish anesthetized by using 3-aminobenzoic acid ethyl ester (MS 222, 100 mg/L, Sigma, St. Louis, MO), liver samples were removed from all treatments as well as control and homogenized by Tissue Lyser LT apparatus (QIAGEN; Cat No. /ID: 85600). Total ribonucleic acid (RNA) was extracted from these tissues using RNeasy® Mini kit (Qiagen, Cat No. 74104), based on the manufacturer's protocol provided in the kit. The reverse transcriptase reaction of RNA was conducted for complementary DNA (cDNA) synthesizing according to the protocol of High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA), cDNA was stored at −80 °C for further molecular analyses. Primers for Delta-9-desaturase (Δ9D, AY150696.1) gene were used for quantifying the mRNA of the target gene using real time PCR (qRT-PCR) methodology. In this study, both genes, β-actin (AY116536.1) and 18S rRNA (AF497908.1) were used as internal reference genes for qRT-PCR data normalization. The expression levels of Δ9D gene was normalized to that of the housekeeping *18S rRNA* gene, where it was more stable than β-actin gene, primers were ordered from Invitrogen™, Germany (Table 3).

Quantitative PCR reaction contained 2.5 μl of 1 μg/μl cDNA, 12.5 μl SimplyGreen SYBR Green qPCR Master Mix, Low Rox (Cat SQ102–0100, GeneDireX, Inc), 0.3 μM of each of forward and reverse primers, 1 μl RNase inhibitor and RNase-Free water to a final volume of 25 μl. Reaction was run on Prime Q real time qPCR machine (Techne, UK) using a two steps protocol: hot-start at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min, and ending with a melt curve from 65 to 95 °C. PCR reactions were performed in triplicate in addition to non-template control (NTC) and cDNA template negative, for confirming the results. The expression level of selected target gene was normalized to those of *18S rRNA* gene. Changes in expression levels of the target gene (*Δ9D*) were presented as

Table 3
Oligonucleotide name and sequence of quantitative real time polymerase chain reaction (qRT-PCR) primers.

Target genes	Sequence	Primer length	Gen Bank No.
Δ 9D	F 5′-ATCACCACACGTTCCCATATGAC-3′	23	AY150696.1
	R 5′-CCAGACCCAAGAAACACATGAAG-3′	23	
β-actin (reference gene)	F 5′-CAGTGCCCATCTACGAGGGTTAT-3′	23	AY116536.1
	R 5′-CGGCTGTGGTGGTGAAGGAGT-3′	21	
18 s rRNA (reference gene)	F 5′-GTTGCAAAGCTGAAACTTAAAGG-3′	20	AF497908.1
	R 5′-TTCCCGTGTTGAGTCAAATTAAGC-3′	20	

F: Forward primer.
R: Reverse primer.

n-fold changes relative to the corresponding controls. Relative gene expression ratios (RQ) was estimated using the formula: RQ = $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001).

2.8. Data analysis

Polynomial regression was used to detect linear and quadratic influence of various dietary propylene glycol levels on the observed response variables. The level of significance adopted was 5%. A statistical package SAS (Statistical Analysis System, 1993) was used for all statistical analysis response variables.

3. Results

3.1. Survival

Fig. 1 showed fish survival in winter season, which significantly ($P < 0.05$) affected by propylene glycol (PG) supplementation. The response of fish survival was linearly improved by increasing the PG level. The highest survival ($P < 0.05$) was observed in tilapia fed the diet supplemented with 7.5 ml PG kg diet^{-1}, whereas, fish fed the control diet recorded the lowest ($P < 0.05$) one.

3.2. Growth performance and feed utilization efficiency

Table 4 shows the growth performance and feed utilization of fish as affected by different levels of PG in cold stress under biofloc system. A linear response in final body weight (FBW; $P = 0.032$), weight gain (WG; $P = 0.034$), specific growth rate (SGR; $P = 0.041$), feed conversion ratio (FCR; $P = 0.036$) and protein efficiency ratio (PER; $P = 0.038$) of tilapia were found by increasing the PG levels in the experimental diets, respectively. However, fish fed diet supplemented with 7.5 ml kg diet^{-1} PG recorded the best records of FBW, WG, SGR, FCR and PER. No significant difference was found in feed intake among experimental diets.

3.3. Serum biochemical parameters

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, triglyceride and low density of lipoprotein cholesterol (LDL-C) were linearly decreased in response to the increased the PG level (Table 5). Also, linear increase in serum total protein, albumin, globulin and lipoprotein cholesterol (HDL—C) were found in response to supplementation of PG (Table 5). Addition of 7.5 ml PG improved the values of ALT, AST, ALP, total protein, albumin, globulin, cholesterol, triglyceride, HDL-C and LDL-C. The response of glucose ($P = 0.026$) and cortisol ($P = 0.193$) of fish in cold stress for 47 days were linearly with increasing PG supplementation (Table 5).

Table 4
Growth performance and feed utilization of Nile tilapia fed different levels of propylene glycol (PG) under cold stress for 47 days.

	Experimental treatments			*P*-value	
	Control	5 mL kg^{-1} PG	7.5 mL kg^{-1} PG	Linear	Quadratic
Initial body weight (g fish^{-1})	28.20	28.50	28.25	0.899	0.459
Final body weight (g fish^{-1})	33.79	36.42	38.64	0.032	0.807
Weight gain (g fish^{-1})	5.59	7.93	10.39	0.034	0.946
Specific growth rate (% day^{-1})	0.40	0.55	0.70	0.041	0.967
Feed intake (g fish^{-1})	33.61	35.30	40.41	0.210	0.650
Feed conversion ratio	6.15	4.45	3.89	0.037	0.396
Protein efficiency ratio	0.66	0.90	1.03	0.038	0.469

Means followed by different letters in the same row are significantly different ($P < 0.05$).
WG = final weight (g) – initial weight (g); Specific growth rate (SGR) = $LnW_2 - LnW_1/t$ (days), Where, Ln = the natural log; W_1 = initial fish weight, W_2 = the final fish weight in grams and t = Period in days; Feed conversion ratio (FCR) was calculated according to by the equation: FCR = Feed intake (g)/weight gain (g); Protein efficiency ratio (PER) = Weight gain (g)/protein ingested (g).

3.4. Blood serum ions

PG supplementation either 5 ml or 7.5 ml kg^{-1} diet had no significant effect ($P > 0.05$) on the concentration of serum magnesium (Table 6). Significant linear increased in the concentration of serum ions; sodium, calcium and chloride were observed with increasing the PG level (Table 6).

3.5. Hepatic oxidative stress

The activities of hepatic oxidative stress biomarkers are presented in

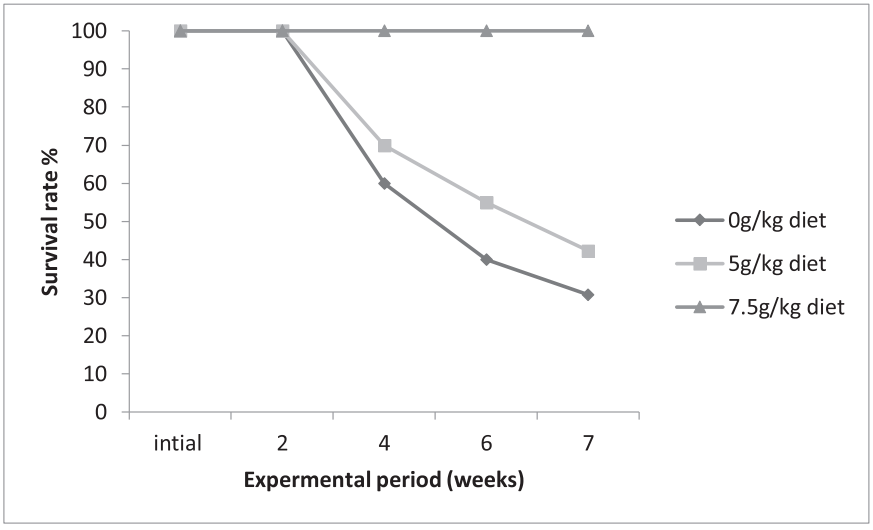

Fig. 1. Survival rate of Nile tilapia fingerlings fed diet supplemented with 5 mL and 7.5 mL propylene glycol kg^{-1} diet during 47 days in winter season under biofloc system.

Table 5
Blood biochemical indices of Nile tilapia fed different levels of propylene glycol (PG) under cold stress for 47 days.

	Experimental treatments			P-value	
	Control	5 mL kg^{-1} PG	7.5 mL kg^{-1} PG	Linear	Quadratic
Alanine aminotransferase (UL^{-1})	130	121.50	120.50	0.029	1.000
Aspartate aminotransferase (UL^{-1})	17.35	12.20	11.75	0.037	0.500
Alkaline phosphatase (UL^{-1})	170.50	152	147.50	0.015	0.083
Total protein (g L^{-1})	1.70	2.50	2.95	0.011	0.188
Albumin (g L^{-1})	0.55	1.30	1.45	0.038	0.521
Globulin (g L^{-1})	1.15	1.20	1.60	0.013	0.071
Cholesterol (mmol L^{-1})	193.50	139.50	173.50	0.033	0.075
Triglyceride (mmol L^{-1})	626	583.50	531	0.004	0.065
HDL-C[a](mmol L^{-1})	94.50	92.50	106.50	0.027	0.369
LDL-C[b](mmol L^{-1})	148	133	101	0.018	0.064
Cortisol (ng mL^{-1})	5.75	5.20	4.25	0.193	0.794
Glucose (mg dl^{-1})	171.50	100.50	95	0.026	0.594

Means followed by different letters in the same row are significantly different ($P < 0.05$).
[a] HDL-C, High-density lipoprotein Cholesterol.
[b] LDL-C, Low-density lipoprotein Cholesterol.

Table 6
The blood serum ions of Nile tilapia fed different levels of propylene glycol (PG) under cold stress for 47 days.

	Experimental treatments			P-value	
	Control	5 mL kg^{-1}PG	7.5 mL kg^{-1}PG	Linear	Quadratic
Magnesium (mg dl^{-1})	1.85	2.20	2.35	0.108	0.165
Sodium ($mEq1^{-1}$)	146	151	154	0.039	0.552
Calcium (mg dl^{-1})	10.65	14.15	17.30	0.036	0.113
Chloride ($mEq1^{-1}$)	9	12.40	13.35	0.039	0.083

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

Table 7
Hepatic oxidative response (U/g protein) of Nile tilapia, *O. niloticus,* fed different levels of propylene glycol (PG) under cold stress for 47 days.

	Experimental treatments			P-value	
	Control	5 mL kg^{-1} PG	7.5 mL kg^{-1} PG	Linear	Quadratic
[b]SOD	102.16	382.42	388.45	0.035	0.090
[e]CAT	194.32	248.01	449.43	0.001	0.081
[c]GSH	57.50	70	73.03	0.056	0.0312
[d]GPx	56.73	86.10	88.17	0.048	0.089
[a]MDA	723.84	260.58	94.46	0.023	0.091

Means followed by different letters in the same row are significantly different ($P < 0.05$).
[a] MDA, *Malondialdehyde.*
[b] SOD, Superoxide dismutase.
[c] GSH, *Glutathione.*
[d] GPx, Glutathione peroxidase.
[e] CAT, Catalase.

Table 7. Significant linear increased were found in superoxide dismutase (SOD; $P = 0.035$), catalase (CAT; $P = 0.001$), glutathione (GSH; $P = 0.056$) and glutathione peroxidase (GPx; $P = 0.048$) for fish fed 5- or 7.5-ml PG kg^{-1} under cold stress, with the highest values in fish fed 7.5 ml PG kg^{-1}. While, the activity of malondialdehyde (MDA; $P = 0.023$) was linearly reduced with increasing of the PG levels in fish diets, with the lowest value in group fed supplemental diet with 7.5 ml kg^{-1} PG.

3.6. Gene expression

Fig. 2 illustrated the influence of different PG levels on gene expression of delta-9-desaturase (Δ 9D) in livers of Nile tilapia. The transcription of Δ 9D gene of fish reared under cold stress was linearly up regulated (linear, P = 0.001) with increasing dietary PG level.

4. Discussion

One of the major risks in aquaculture production sector is cold stress, which causes physiological dysfunction and generates negative impacts on performance and fish survival (Cheng et al., 2018; Hassaan et al., 2019). While scarce studies have been concerning on the potential effect of using propylene glycol (PG) on fish performance (Hilton et al., 1986; Hughes, 1988), but no published data on the PG role as a source of energy in mitigating the cold stress of fish under biofloc system. The present study showed that the diet supplemental with 7.5 ml kg^{-1} PG could mitigate cold stress of Nile tilapia and improve survival fish. This finding perhaps due to the transformed ability of PG to lactic acid, which was utilized as a dietary source of energy (Rudney, 1950; Miller et al., 1953; Persons et al., 1968; Hilton et al., 1986). Up to now, no existing literature about the effect of PG on survival rate and growth indices of fish exposed to cold temperature stress in order to approve the results detected herein. However, the present study observed indicated that performance of tilapia fed diet supported with PG was improved, which may be regarded to the PG role in enhancing the nutrient absorption and digestion through the intestinal microflora and also their effects as antibacterial, antifungal and antiviral were participated also in the improvement of fish intestine (Robinson and Sprayberry, 2009; Thorgeirsdottir et al., 2003; Nalawade et al., 2015). As it is cleared from the present results the growth performance improvement was corresponded with a change in the palatability of the diet as a response to the PG supplementation in tilapia diet. Correspondingly to the present results, PG supplementation less than 10% in diets enhanced the physical-chemical properties of the feed pellet as plasticity and texture and preventing the proliferation of bacteria in pellet which in turn elevating the feed palatability, weight gain and feed conversion ratio of rainbow trout and Atlantic salmon, *Salmo salar* (Hilton et al., 1986; Hughes, 1988). Moreover, Bayley et al. (1967) used PG up to 8% as a replacer to carbohydrate source; corn starch in White Leghorn chick's diets, while, PG at 16% lowered the growth of the chicks to subnormal levels. On the other hand, channel catfish, *Ictalurus punctatus* fed diets containing 15 and 20% glycerol as a dietary source of energy had reduced weight gain, feed efficiency (Li et al., 2010). Using glycerol in tilapia diets from 0 to 100 g kg^{-1} as a dietary energy source did not affect significantly on the growth performance and feed utilization (Neu et al., 2013). In the same context, Menton et al. (1986) found that rainbow trout, *Oncorhynchus mykiss* did not utilize glycerol as an energy source at level up to 12% (by replacing part of wheat middling) in low-energy and diets did not affect the growth and feed efficiency.

Serum biochemical parameters is a necessary indicator for assessing the nutritional status, health status, immune response and metabolic adjustment during acclimation to seasonal variations of fish (Faggio et al., 2014; Hassaan et al., 2018). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are important enzymes in cellular nitrogen metabolism, oxidation of amino acids, liver gluconeogenesis, and they could be used as a tool for liver dysfunction during cold stress (Hassaan et al., 2019). Their promotion may indicate leakage of enzymes across damaged plasma membranes and/or increased the synthesis of liver enzymes (Yang and Chen, 2003). In short-term cold stress, Shi et al. (2015) reported that the

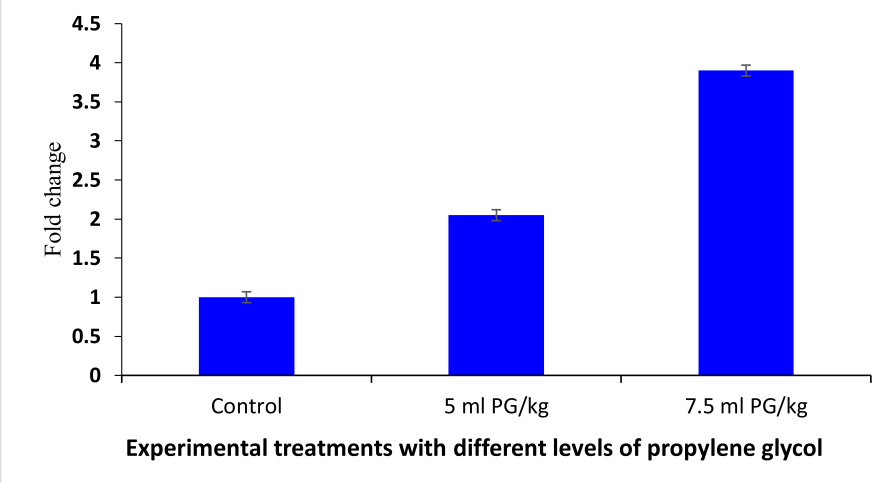

Fig. 2. Gene expression of delta-9-desaturase (Δ 9D; linear response, $P = 0.001$) in liver of Nile tilapia fingerlings fed diet supplemented with 5 mL and 7.5 mL propylene glycol kg^{-1} diet during 47 days in winter season under biofloc system.

activities of ALT, AST and LDH become prominently elevated in tilapia. In the current study, the supplemental diets with PG improved the activity of AST, ALT and ALP enzymes with increasing the PG levels in fish diet perhaps indicating the hepatoprotective role of PG. In accordance to the current results, Nile tilapia exposed to cold stress elevated the activities of hepatic enzymes and could be induced liver injury (Panase et al., 2018; Wu et al., 2019; Hassaan et al., 2019).

The lipid profile; cholesterol and triglyceride are varying depending upon the nutritional status (Regost et al., 2001). Triglycerides (TG) are synthesized in the hepatic cells and assessed to evaluate the lipid metabolism, and its high concentrations may cause nephritic syndrome or glycogen storage disease and liver dysfunction (Coz-Rakovac et al., 2005; Osman et al., 2010). The present findings revealed higher TG levels in fish fed diets incorporated with PG than the control diet which could indicate the ability of PG in mitigating the cold stress response of fish. Whereas, the reduction in TG levels in the control diet might due to TG degradation to produce adenosine triphosphate (ATP) (Sun et al., 2019), disturb lipid mobilization, change the membrane fluidity and circulation between liver and tissue to cope with the energy demands under cold stress (Chang et al., 2006). Contrary to these results, TG content was increased in GIFT strain of Nile tilapia, *Oreochromis niloticus* under cold stress (Liu et al., 2011) and the CHOL content in common carp, *Cyprinus carpio* (Shikata et al., 1995). In our results, the levels of cholesterol (CHOL), low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) were reduced in fish fed dietary PG as compared with the control diet, indicating the role of PG in strengthen tilapia to alleviate the cold stress and prevent the liver tissue injuries. Hence, our results indicated that adding PG at level 7.5 ml kg^{-1} or the control diet promoted the level of HDL-C for transporting CHOL from peripheral cells to the liver to reduce the lipid peroxidation damage because of cold shock (Fredenrich and Bayer, 2003).

Glucose is an energetic substrate stored and organized as glycogen in the hepatic and muscular cells to provide energetic support to fish and it is more sensitive to temperature changes than serum protein and cholesterol (Lucas, 1996; Lermen et al., 2004). Furthermore, cortisol triggers the glycogenolysis and gluconeogenesis process and motivates the release of catecholamines from chromaffin cells which increasing the glycogenolysis and modulates cardiovascular and respiratory function (Vijayan et al., 1997; Reid et al., 1998; Mommsen et al., 1999; Panase et al., 2018). Cortisol as well as glucose levels in blood serum are commonly used to indicate the stress response of fish (Wendelaar Bonga, 1997; Pacheco and Santos, 2001). In the present study, PG supplementation improved the contents of serum glucose and cortisol of tilapia under cold stress while, the highest values of glucose and cortisol were recorded in the control diet. This increase in the glucose and cortisol concentrations in the control diet could explain the high need of energy to struggle the cold stress and recompense the lower enzymatic reaction rate (Atwood et al., 2003). In line with our finding, Tandon and Joshi (1974); Best et al. (2001) and Cho et al. (2015) indicated that low temperatures induced high blood glucose levels in *Clarias batrachus; Salmo trutta* and Red spotted grouper, *Epinephelus akaara* that caused retardation in fish metabolism. Otherwise, the lowest values of glucose and cortisol were recorded in fish fed diet supplemented with 7.5 ml kg^{-1} PG, indicating the anti-stress effect of PG. Corroborating with this study, glucose was not altered by crude glycerol supplementation in the Amazon catfish (female *Pseudoplatystoma punctifer* x male *Leiarius marmoratus* diet (da Silva Rôxo et al., 2018). In contrast to our results, the dietary supplemental diet with 5% glycerol was significantly increased blood glucose level in Channel Catfish, *Ictalurus punctatus* (Li et al., 2010). However, glucose is highly variable among species and different stages of life or under certain feeding diets (Hemre et al., 2002).

The levels of total protein (albumin and globulin) are considered as one of the most stable blood components; albumin is important for maintaining oncotic pressure in the plasma of vertebrate (Andreeva, 2010; Peres et al., 2015), and globulins involved in the processes of metabolism, transport, coagulation, inflammatory and immune (Hoseini et al., 2014). In this experiment, the observed low concentration of globulin in the control diet could be related to the high cortisol level causing impairment in the immune system (Akhtar et al., 2013; Ghelichpour et al., 2017). Serum total protein and albumin in this work did not affect ($P > 0.05$) by dietary PG supplementation. In this context, Menton et al. (1986) revealed glycerol can be used as a source of energy in trout diets with positive effect on plasma total protein. Accordingly, De Costa et al. (2015), diets containing glycerol protected protein catabolism for energy purposes in Nile tilapia.

Also, temperature alters the plasma electrolytes in several conditions such as exposure to cold temperature stress (Kristoffersson et al., 1972; Cerqueira and Fernandes, 2000). Plasma electrolytes such as sodium (Na), magnesium (Mg), calcium (Ca) and chloride (Cl) and potassium (K) level can be used as blood osmolality stress indicators in tropical fish (Eliassen et al., 1960). In the current study, the concentration of blood ions such as Na and Ca were elevated in fish fed dietary supplemented with 7.5 ml kg^{-1} PG while, Mg and Cl increased with adding PG at level 5 ml kg^{-1} in comparison with control, this may be due to the beneficial effect of PG to modulate the blood osmatic under cold temperature stress condition. Elevation of blood electrolytes contents could be corresponded to the decrease of the osmotic pressure as a response to cold temperature stress condition (Doudoroff, 1945; Tyagi et al., 2013). In

line with our data, the concentration of Na^+ and K^+ in plasma played an important role in the survival of snow trout, *Shizothorax richardsonii* at low temperature. There was a little information explained the mechanism of plasma electrolytes in fish under cold stress.

The increase in the rate of energy metabolism, reflecting to cold temperature stress exposure which altered the antioxidant capacity (Kaushik and Kaur, 2003; Ye et al., 2015). Liver antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPX) and malondialdehyde (MDA) are considered cellular immune response, which can scavenge the superoxide anions, hydroxyl-free radicals, and other free radicals, reducing oxidative damage and protecting the body from injury (Bartoskova et al., 2013; Lortz et al., 2000). MDA is a product of lipid peroxidation and its higher level in liver can cause the inactivation of enzymes and reduce cell membrane permeability leading to oxidative damage to DNA, protein and cytoplasm (Tüzgen et al., 1998; Yao et al., 2010). In this study, the higher activities of SOD, GSH, and GPX with accompanied with lower MDA content were detected in diets supplemented with PG than control diet. These results indicated that dietary PG supplementation may be improved the antioxidant defense and reduced the oxidative impairment under low temperature stress. No previous reports were found to clarify the effect of PG supplementation on the antioxidant enzymes in tilapia exposed to cold temperature stress, in order to support this study. Hence, further investigations are warranted to show the effect of dietary PG on the physiological response of different fish species in monitoring the cold stress.

Lipids play a vital role to protect poikilotherms from low temperature, therefore, changes in the expression of Δ 9D gene are required for adaptation and tolerance to cold stress, due to its working on desaturating membrane lipids to sustain membrane fluidity during cold temperature (Zerai et al., 2010; Hassaan et al., 2019; Wu et al., 2020). Our results revealed that fish fed diets supplemented with different levels of propylene glycol enhanced transcriptome of mRNA of Δ 9D gene in the liver tissue comparing with those fed control diet under cold stress (Fig. 2). The highest expression of Δ 9D gene was recorded in the liver tissue samples of fish fed 7.5 ml PG kg^{-1} supplemented diet, while fish fed the control diet recorded the lowest. In this context, increased the expression of Δ 9D could improve the ability of tilapia against cold stress (Hassaan et al., 2019). Previous studies reported that the expression of Δ 9D gene was affected by feed quality especially, fatty acids types (Hsieh et al., 2007), as well as thermal conditions (Tocher et al., 1996). Reduction of fatty acid synthase, acetyl-CoA carboxylase as well as carnitine palmitoyl transferase expression genes reflect the deleterious effect of cold stress such as inhibition of the digestion, synthesis, and oxidation of lipids were showed under cold stress, while, increased the expression of sphingolipid delta- 4 desaturase gene (DEGS) resulted in an increment within the request for highly unsaturated fatty acids under cold stress (Wu et al., 2020).

5. Conclusion

Propylene glycol is a dietary source of energy, which can be supplemented to Nile tilapia, *Orechromis niloticus* diets to alleviate the cold stress in winter season, decrease the mortality, and enhance the physiological status. This finding provided valuable information about the fish health under cold stress. However, further studies are required to investigate their genetically effects on other fish species in winter season because, this study was the first study done on this issue.

Declaration of Competing Interest

None.

Acknowledgement

None.

References

Abdel-Aal, M.M., 2008. Effects of over-wintering and water depth on growth performance of Nile tilapia (*Oreochromis niloticus*). In: Proceedings of the 8th International Symposium on Tilapia in Aquaculture. Cairo: The Central Laboratory for Aquaculture Research. Ministry of Agriculture and Land Reclamation, pp. 297–306.

Akhtar, M.S., Pal, A.K., Sahu, N.P., et al., 2013. Thermal tolerance, oxygen consumption and haemato-biochemical variables of *Tor putitora* juveniles acclimated to five temperatures. Fish Physiol. Biochem. 39, 1387–1398. https://doi.org/10.1007/s10695-013-9793-7.

Andreeva, A.M., 2010. The role of structural organization of blood plasma proteins in the stabilization of water metabolism in bony fish (Teleostei). J. Ichthyol. 50, 552–558. https://doi.org/10.1134/S0032945210070076.

AOAC, 1995. Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

APHA, 1989. Water pollution control federation. Standard methods for the examination of water and wastewater, 17.

Atwood, H.L., Tomasso, J.R., Webb, K., Gatlin III, D.M., 2003. Low-temperature tolerance of Nile tilapia, *Oreochromis niloticus*: effects of environmental and dietary factors. Aquac. Res. 34 (3), 241–251.

Bartoskova, M., Dobsikova, R., Stancova, V., Zivna, D., Blahova, J., Marsalek, P., Faggio, C., 2013. Evaluation of ibuprofen toxicity for Zebrafish (*Danio rerio*) targeting on selected biomarkers of oxidative stress. Neuro Endocrinol. Lett. 34, 102–108.

Bauer, C., Schlott, G., 2004. Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility—measurement of activity by radio telemetry. Aquaculture 241, 301–317.

Bayley, H.S., Slinger, S.J., Summers, J.D., 1967. The use of propylene glycol as a source of energy for the chick. Poult. Sci. 46, 19–22.

Beers, R.F., Sizer, I.W., 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195, 133–140.

Best, J.H., Eddy, F.B., Codd, G.H., 2001. Effects of purified microcystin-LR and cell extracts of Microcystis strains PCC 7813 and function in brown trout (*Salmo trutta*) alevins. Fish Physiol. Biochem. 24, 171–178.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–890.

Brett, J.R., 1971. Energetic responses of salmon to temperature - study of some thermal relations in physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am. Zool. 11 (1), 99–113.

Cerqueira, C.C.C., Fernandes, M.N., 2000. Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*. Ecotoxicol. Environ. Saf. 52, 83–91.

Chang, Y.M., Cao, D.C., Sun, X.W., Liang, L.Q., 2006. Changes of serum biochemical indices of common carp affected by cold temperatures. Chin. J. Fish. 2, 71–75.

Cheng, C.-H., Guo, Z.-X., Wang, A.-L., 2018. The protective effects of taurine on oxidative stress, cytoplasmic free-Ca2þ and apoptosis of pufferfish (*Takifugu obscurus*) under low temperature stress. Fish Shellfish Immunol. 77, 457–464. https://doi.org/10.1016/j.fsi.2018.04.022.

Cho, H.C., Kim, J.E., Kim, H.B., Baek, H.J., 2015. Effects of water temperature change on the hematological responses and plasma cortisol levels in growing of red spotted grouper, *Epinephelus akaara*. Dev. Reprod. 19, 19–24.

Coles, E.H., 1974. Plasma proteins. In: Veterinary Clinical Pathology, 2nd ed. W.B. Saunders Co, Philadelphia, Pennsylvania, USA, pp. 558–560.

Correa, C.F., Nobrega, R.O., Mattioni, B., Block, J.M., Fracalossi, D.M., 2017. Dietary lipid sources affect the performance of Nile tilapia at optimal and cold, suboptimal temperatures. Aquac. Nutr. 23 (5), 1016–1026.

Correa, C.F., Nobrega, R.O., Block, J.M., Fracalossi, D.M., 2018. Mixes of plant oils as fish oil substitutes for Nile tilapia at optimal and cold suboptimal temperature. Aquaculture 497, 82–90.

Coz-Rakovac, R., Strunjak-Perovic, I., Hacmanjek, M., Lipej, Z., Sostaric, B., 2005. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the North Adriatic Sea. Vet. Res. Commun. 29 (8), 677–687.

da Silva Rôxo, V.B., Moron, S.E., Ferreira, D.A., Jorge, M.P.B., 2018. Crude glycerol in the diets of the juveniles of Amazon catfish (female *Pseudoplatystoma punctifer* x male *Leiarius marmoratus*). Int. J. Environ. Agricu. Biotechnol. (IJEAB) 3 (5), 1640–1655.

Dan, N.C., Little, D.C., 2000. The culture performance of monosex and mixed-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in northern Vietnam. Aquaculture 184 (3–4), 221–231.

De Costa, D.V., Paulino, R.R., Okamura, D., Oliveira, M.M., Rosa, P.V., 2015. Growth and energy metabolism of Nile tilapia juveniles fed glycerol. Brazilian Agricu. Res. 50 (5), 347–354.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., Verstraete, W., 2008. The basics of bio-flocs technology: the added value for aquaculture. Aquaculture 277, 125–137.

Dogru, M.I., Dogru, A.K., Gul, M., Esrefoglu, M., Yurekli, M., Erdogan, S., Ates, B., 2008. The effect of adrenomedullin on rats exposed to lead. J. Appl. Toxicol. 28 (2), 140–146.

Doudoroff, P., 1945. The resistance and acclimatization of marine fishes to temperature change. Experiment with Fundulus and Antherinops. Biol. Bull. 88, 194–206.

Doumas, B.T., Bayse, D.D., Borner, K., Carter, R.J., Elevitch, F., Garber, C.C., Graby, R.A., Hause, L.L., Mather, A., Peters Jr., T., Rand, R.N., 1981. A candidate reference method for determination of total protein in serum. II. Test for transferability. Clin. Chem. 27 (10), 1651–1654.

Eliassen, E., Leivestad, H., Moller, D., 1960. Effect of low temperature on the freezing point of plasma and on the potassium /sodium ratio in muscles of some boreal and subarctic fishes. Arbok Univ. Bergen, Mat. Nat., Ser. (14), 1–24.

Faggio, C., Piccione, G., Marafioti, S., Arfuso, F., Fortino, G., Fazio, F., 2014. Metabolic response to monthly variations of *Sparus aurata* reared in mediterranean on-shore tanks. Turk. J. Fish. Aquat. Sci. 14, 567–574.
Fredenrich, A., Bayer, P., 2003. Reverse cholesterol transport, high density lipoproteins and HDL cholesterol: recent data. Diabetes Metab. 29 (3), 201–205.
Galloway, B.J., Kieffer, J.D., 2003. The effects of an acute temperature change on the metabolic recovery from exhaustive exercise in juvenile Atlantic salmon (*Salmo salar*). Physiol. Biochem. Zool. 76, 652–662.
Ghelichpour, M., Mirghaed, A.T., Mirzargar, S.S., Joshaghani, H., Mousavi, H.E., 2017. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquac. Res. 48, 5666–5676.
Hassaan, M.S., Mahmoud, S.A., Jarmolowicz, S., El-Haroun, E.R., Mohammady, E.Y., Davies, S.J., 2018. Effects of dietary baker's yeast extract on the growth, blood indices and histology of Nile tilapia (*Oreochromis niloticus* L.) fingerlings. Aquac. Nutr. 24 (6), 1709–1717.
Hassaan, M.S., Nagar, A.G.E., Salim, H.S., Fitzsimmons, K., El-Haroun, E.R., 2019. Nutritional mitigation of winter thermal stress in Nile tilapia by propolis-extract: associated indicators of nutritional status, physiological responses and transcriptional response of delta-9-desaturase gene. Aquaculture 511, 734256.
Hemre, G.I., Mommsen, T.P., KrogdahL, A., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes., 2002. Aquac. Nutr. 8, 175–194.
Hilton, J.W., Atkinson, J.L., Slinger, S.J., 1986. Effect of propylene glycol on feed digestibility and the growth and physiological response of rainbow trout. Can. J. Anim. Sci. 66, 1057–1063.
Hocutt, C.H., 1973. Swimming performance of three warm-water fishes exposed to a rapid temperature change. Chesap. Sci. 14, 11–16.
Hoseini, S.M., Hedayati, A., Ghelichpour, M., 2014. Plasma metabolites, ions and thyroid hormones levels, and hepatic enzymes [U + 05F3] activity in Caspian roach (*Rutilus rutilus caspicus*) exposed to waterborne manganese. Ecotoxicol. Environ. Saf. 107, 84–89. https://doi.org/10.1016/j. ecoenv.2014.05.002.
Hsieh, S.L., Hu, C.Y., Hsu, Y.T., 2007. Influence of dietary lipids on the fatty acid composition and expression of stearoyl-CoA desaturase in hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, under cold shock. Comp. Biochem. Physiol. B-Biochem. & Mol. Biol. 147 (3), 438–444.
Hughes, S.G., 1988. Effect of dietary propylene glycol on growth, survival, histology, and carcass composition of Atlantic Salmon. Progr. Fish-Cultu. 50, 12–15.
Hurst, T.P., 2007. Causes and consequences of winter mortality in fishes. J. Fish Biol. 71 (2), 315–345.
Hussein, H.A., Abdel-Raheem, S.M., Abd-Allah, M., Senosy, W., 2015. Effects of propylene glycol on the metabolic status and milk production of dairy buffaloes. Tierärztliche Praxis Großtiere 43 (G), 1–10.
Kaushik, S., Kaur, J., 2003. Chronic cold exposure affects the antioxidant defense system in various rat tissues. Clin. Chim. Acta 333, 69–77.
Kristoffersson, R., Broberg, S., Oikari, A., 1972. Effect of temperature change on some blood constituents and osmotic balance in the pike (*Esox lucius* L.) in brackish water. Ann. Zool. Fenn. 9 (4), 212–218.
Lermen, C.L., Lappe, R., Crestani, M., Vieira, V.P., Gioda, C.R., Schetinger, M.R.C., Baldisserotto, B., Moraes, G., Morsch, V.M., 2004. Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. Aquaculture 239, 497–507.
Li, M.H., Minchew, C.D., Oberle, D.F., Robinson, E.H., 2010. Evaluation of glycerol from biodiesel production as a feed ingredient for Channel catfish, *Ictalurus punctatus*. J. World Aquacult. Soc. 41 (1), 130–136. Baton Rouge.
Liu, B., Wang, M.Y., Xie, J., Xu, P., Ge, X.P., He, Y.J., Pan, L.K., 2011. Effects of acute cold stress on serum biochemical and immune parameters and liver HSP70 gene expression in GIFT strain of Nile tilapia (*Oreochromis niloticus*). Acta Ecol. Sin. 31, 4866–4873 (in Chinese).
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25 (4), 402–408.
Lortz, S., Tiedge, M., Nachtwey, T., Karlsen, A.E., Nerup, J., Lenzen, S., 2000. Protection of insulin-producing rinm5f cells against cytokine-mediated toxicity through over expression of antioxidant enzymes. Diabetes 49, 1123–1130.
Lucas, A., 1996. Physical concepts of bioenergetics. In: Lucas, A. (Ed.), Bioenergetics of Aquatic Animals, English ed. Taylor & Francis, France.
Menton, D.J., Slinger, S.J., Hilton, J.W., 1986. Utilization of free glycerol as a source of dietary energy in rainbow trout (*Salmo gairdneri*). Aquaculture 56 (2), 215–227. Amsterdam.
Miller, O.N., Huggins, C.G., Arai, K., 1953. Studies on the metabolism of 1.2-dipropanediol- 1 -phosphate. J. Biol. Chem. 202, 263–271.
Moin, V.M., 1986. A simple and specific method for determining glutathione peroxidase activity in erythrocytes. Lab Delo 12, 724–727.
Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9 (3), 211–268.
Murray, P., Hayward, S.A.L., Govan, G.G., Gracey, A.Y., Cossins, A.R., 2007. An explicit test of the phospholipid saturation hypothesis of acquired cold tolerance in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. 104 (13), 5489–5494.
Nalawade, T.M., Bhat, K., Sogi, S.H.P., 2015. Bactericidal activity of propylene glycol, glycerin, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms. J. Int. Soc. Prevent. & Commu. Dentistry 5, 114–119.
Neu, D.H., Furuya, W.M., Boscolo, W.R., Potrich, F.R., Lui, T.A., Feiden, A., 2013. Glycerol inclusion in the diet of Nile tilapia (*Oreochromis niloticus*) juveniles. Aquac. Nutr. 19, 211–217.
NRC, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington DC.
Ortuno, J., Esteban, M.A., Meseguer, J., 2003. The effect of dietary intake of vitamins C and E on the stress response of gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol. 14, 145–156.
Osman, A.G., Koutb, M., Sayed, A.E.D.H., 2010. Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga* Miller) leaf extract in alleviation of the effect of ultraviolet–a radiation on African catfish *Clarias gariepinus* (Burchell, 1822). J. Photochem. Photobiol. B Biol. 99 (1), 1–8.
Pacheco, M., Santos, M.A., 2001. Biotransformation, endocrine, and genetic responses of *Anguilla Anguilla* L. to petroleum distillate products and environmentally contaminated waters. Ecotoxicol. Environ. Saf. 49, 64–75.
Panase, P., Saenphet, S., Saenphet, K., 2018. Biochemical and physiological responses of Nile tilapia, *Oreochromis niloticus* Lin subjected to cold shock of water temperature. Aquac. Rep. 11, 17–23.
Peres, H., Costas, B., Perez-Jimenez, A., et al., 2015. Reference values for selected hematological and serum biochemical parameters of Senegalese sole (*Solea senegalensis* Kaup, 1858) juveniles under intensive aquaculture conditions. J. Appl. Ichthyol. 31, 65–71. https://doi.org/10.1111/jai.12641.
Persons, J.N., Damron, B.L., Waldroup, W., Harms, R.H., 1968. Propylene glycol as an energy source for poultry. Poult. Sci. 74 (2), 351–353.
Peskin, A.V., Winterbourn, C.C., 2000. A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1). Clin. Chim. Acta 293 (1–2), 157–166.
Polley, S.D., Tiku, P.E., Trueman, R.T., Caddick, M.X., Morozov, I.Y., Cossins, A.R., 2003. Differential expression of cold- and diet-specific genes encoding two carp liver Delta 9-acyl-CoA desaturase isoforms. Am. J. Phys. Regul. Integr. Comp. Phys. 284 (1), 41–50.
Regost, C., Arzel, J., Cardinal, M., Laroche, M., Kaushik, S.J., 2001. Fat deposition and flesh quality in seawater reared, triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation. Aquaculture 193 (3–4), 325–345.
Reid, S.G., Bernier, N.J., Perry, S.F., 1998. The adrenergic stress response in fish: control of catecholamine storage and release. Comp. Biochem. Physiol. C 120, 1–27.
Reitman, A., Frankel, S., 1957. Determination of aspartate glutamic aminotransferase and alanine aminotransferase. Am. J. Clin. Pathol. 28, 56.
Robinson, E., Sprayberry, K.A., 2009. Current Therapy in Equine Medicine. Elsevier Health Sciences, USA.
Rudney, H., 1950. The metabolism of 1.2. Propanediol. Arch. Biochem. 29, 231–232.
Sakai, M., 1999. Current research status of fish immunostimulants. Aquaculture 172, 63–92.
Sarma, K., Pal, A.K., Sahu, N.P., Ayyappan, S., Baruah, K., 2009. Dietary high protein and vitamin C mitigates endosulfan toxicity in *Channa punctatus*. Sci. Total Environ. 407, 3668–3673.
Shi, G.C., Dong, X.H., Chen, G., Tan, B.P., Yang, Q.H., Chi, S.Y., Liu, H.Y., 2015. Physiological responses and HSP70 mRNA expression of GIFT strain of Nile tilapia (*Oreochromis niloticus*) under cold stress. Aquac. Res. 46, 658–668.
Shikata, T., Iwanaga, S., Shimeno, S., 1995. Metabolic response to acclimation temperature in carp. Fish. Sci. 61, 512–516.
Siddik, M.A.B., Nahar, A., Ahamed, F., Hossain, Md.Y., 2014. Over-wintering growth performance of mixed-sex and mono-sex Nile tilapia *Oreochromis niloticus* in northeastern Bangladsh. Croatian J. Fish. 72, 70–76.
Soltan, M.A., Abdella, M.M., El-Sayaad, G.A., Abu-El Wafa, M.H., 2014. Effect of over-wintering regimes on survival and growth performance of Nile tilapia, *Oreochromis niloticus*. In: Pro. of the 2nd Inter. Conf. on Biotech. Applic. in Agric, 8-12. Moshtohor and Hurghada, Egypt, pp. 41–47.
Soltanian, S., Adloo, M.N., Hafeziyeh, M., Ghadimi, N., 2014. Effect of β-Glucan on cold-stress resistance of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878). Vet. Med. 59 (9), 440–446.
Statistical Analysis system (SAS), 1993. SAS/STAT user Guide Release 6.03 Edition. SAS Institute Inc. Cary, North Carolina, USA.
Sun, Z., Tan, X., Liu, Q., Ye, H., Zou, C., Xu, M., Ye, C., 2019. Physiological, immune responses and liver lipid metabolism of orange-spotted grouper (*Epinephelus coioides*) under cold stress. Aquaculture 498, 545–555.
Tandon, R.S., Joshi, B.D., 1974. Effect of trypanosome infection on blood glucose levels of some fresh water teleosts. J Inland Fish Soc India 6, 81–82.
Thorgeirsdottir, T.O., Thormar, H., Kristmundsdottir, T., 2003. Effects of polysorbates on antiviral and antibacterial activity of mono glyceride in pharmaceutical formulations. Pharmazie 58, 286–287.
Tocher, D.R., Bell, J.G., Sargent, J.R., 1996. Induction of Delta 9-fatty acyl desaturation in rainbow trout, *Oncorhynchus mykiss*, liver by dietary manipulation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 113 (1), 205–212.
Tüzgen, S., Kaynar, M.Y., Güner, A., Gümüstas, K., Belce, A., Etus, V., Ozyurt, E., 1998. The effect of epidural cooling on lipid peroxidation after experimental spinal cord injury. Spinal Cord 36, 654–657.
Tyagi, A., Ali, D.S., Barat, D.A., 2013. Impact of exposure of cold temperature on hematological parameters and plasma electrolytes of cold water fish *Shizothorax richardsonii*. J. Inform. Knowledge Res. Biomed. Eng. 2 (2), 38–41.
Vijayan, M.M., Pereira, C., Graul, E.G., Lwama, G.K., 1997. Metabolic responses to confinement stress in tilapia: the role of cortisol. Comp. Biochem. Physiol. 116C, 89–95.
Waldroup, P.W., Bowen, T.E., 1968. Evaluation of propylene glycol as an energy source in broiler diets. Poult. Sci. 47, 1911–1917.
Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77 (3), 591–625.
Wu, F., Yang, C., Wen, H., Chen Zhang, C., Jiang, M., Liu, W., Tian, J., Yu, L., Xing Lu, X., 2019. Improving low-temperature stress tolerance of tilapia, *Oreochromis niloticus*: A functional analysis of *Astragalus membranaceus*. J. World Aquacult. Soc. 50, 749–762.

Wu, D., Liu, Z., Yu, P., Huang, Y., Cai, M., Zhang, M., Zhao, Y., 2020. Cold stress regulates lipid metabolism via AMPK signalling in Cherax quadricarinatus. J. Therm. Biol. 102693.

Yang, J.L., Chen, H.C., 2003. Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere 53 (8), 877–882.

Yao, J., Wang, J.Y., Liu, L., Li, Y.X., Xun, A.Y., Zeng, W.S., Jia, C.H., Wei, X.X., Feng, J.L., Zhao, L., Wang, L.S., 2010. Anti-oxidant effects of resveratrol on mice with DSS-induced ulcerative colitis. Arch. Med. Res. 41 (4), 288–294.

Ye, C.X., Wan, F., Sun, Z.Z., Cheng, C.H., Ling, R.Z., Fan, L.F., Wang, A.L., 2015. Effect of phosphorus supplementation on cell viability, anti-oxidative capacity and comparative proteomic profiles of puffer fish (*Takifugu obscurus*) under low temperature stress. Aquaculture 452, 200–208.

Yokoyama, S., Koshio, S., Takakura, N., Oshida, K., Ishikawa, M., Gallardo-Cigarroa, F.J., Teshima, S., 2005. Dietary bovine lactoferrin enhances tolerance to high temperature stress in Japanese flounder *Paralichthys olivaceus*. Aquaculture 249, 367–373.

Zerai, D.B., Fitzsimmons, K.M., Collier, R.J., 2010. Transcriptional response of delta-9-desaturase gene to acute and chronic cold stress in Nile tilapia, *Oreochromis niloticus*. J. World Aquacult. Soc. 41 (5), 800–806.