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The modulatory impact of Arabic gum and lecithin on the efficiency of cold-stressed Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Tilapia are commonly used in aquaculture but are sensitive to cold, limiting their culture in colder regions. Thus, the purpose of this investigation was to examine how lecithin and/or Arabic gum could help Nile tilapia cope with cold stress during the winter season. In a 3×3 factorial feeding study, tilapia fingerlings with an average initial weight of 7.56 ± 0.10 g were given nine different diets (crude protein: 305 g kg^{-1}; gross energy: 19.47 MJ kg^{-1}) for 60 days during the winter. The diet formulations included three levels of Arabic gum (0 g, 2 g, and 4 g kg^{-1}), with each level receiving three different dosages of lecithin (0 g, 5 g, and 10 g kg^{-1}). After the trial, tilapia fed the food enriched with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin showed the highest specific growth rate, fish survival, weight gain, and the lowest feed conversion ratio. Low-density lipoprotein cholesterol and cholesterol levels were highest in fish given the control diet. The groups fed diets with 10 g kg^{-1} lecithin and 4 g kg^{-1} Arabic gum showed higher levels of triglycerides and HDL-C. Significant decreases in alanine aminotransferase, glucose, aspartate aminotransferase, and cortisol activities were also observed ($P < 0.05$) with the same diet. Fish fed a diet enriched with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin showed the highest levels of serum calcium, potassium, sodium, and chlorine, as well as the highest transcription of Δ9D. The same diet was shown to have the lowest levels of malondialdehyde and the highest activity levels for other antioxidant enzymes such as glutathione peroxides, superoxide dismutase, glutathione, catalase, and total antioxidant capacity. Tilapia raised in cold-stressed winter conditions had improved survival and performance when fed a diet containing 4 g kg^{-1} of Arabic gum and 10 g kg^{-1} of lecithin.

1. Introduction

Physiological stress caused by biological, physical, or chemical stimuli, is the main factor contributing to fish diseases and mortality in aquaculture (Acar et al., 2015; Baba et al., 2016; Hassaan et al., 2019a; Soaudy et al., 2021; Yang et al., 2022; Kesbiçç et al., 2022; Mohammady et al., 2023; Elashry et al., 2024; Moawad et al., 2024). Temperature variations are a kind of natural stress that fish may encounter during their life cycle; they are either temporary fluctuations or seasonal changes in fish that are linked to causing disease outbreak and mortality (Ju et al., 2002). Temperature shock can harm fish by disrupting their homeostasis and lowering their metabolic rates (Galloway and Kieffer, 2003; Suski et al., 2006; Vanlandeghem et al., 2010), influencing their swimming ability (Hyvärinen et al., 2004; Suski et al., 2006), weakening immunological syndromes, and increasing opportunistic infections (Chang et al., 2006; Hurst, 2007; Panase et al., 2018). Tropical fish like Nile tilapia optimal temperature range warm water (26–30°C), while cold suboptimal temperatures can decrease feed consumption, growth, and mortality (Bhujel et al., 2007; Azaza et al., 2008; Zerai et al., 2010; Ma et al., 2015;Correa et al., 2017, 2018; Siddik et al., 2014; Nobrega et al., 2017; Wu et al., 2019). Numerous studies have indicated that cold, below-average temperatures have a detrimental effect on Nile tilapia productivity (Ma et al., 2015; Shi et al., 2015; Nobrega et al., 2019; Hassaan et al., 2019a). Therefore, it is critical to increase fish resistance

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to a variety of stressors such as extreme cold (Yokoyama et al., 2005; Shi et al., 2015; Nobrega et al., 2017). Fish, however. can adapt to changes in their immediate environment through altering physiological processes, such as those linked to stress response (Barton, 2002; Siddik et al., 2014).

Various approaches to combat low temperatures include employing greenhouses (Radwan, 2008), using covers made with polyethylene sheets for fish ponds, or utilizing varying pond depths to regulate the water volume (Dan and Little, 2000; Rothbard and Peretz, 2002; Abdel-Aal, 2008). Moreover, techniques for cultivation such as biofloc systems are useful in reducing the negative effects of cold and lowering tilapia mortality over the winter period (Soltan et al., 2015; Soaudy et al., 2021). One promising strategy for preventing environmental stress in aquaculture is the use of functional feed additives (Hassaan et al., 2019b; Bowyer et al., 2019; Refaey et al., 2022; Saleh et al., 2022; Taalab et al., 2022; Abdo et al., 2024), which can help fish that are experiencing growth retardation, inflammatory response, immunosuppression, and oxidative damage. In this context Wu et al., (2019) added *Astragalus membranaceus* extract powder as natural herbs in Nile tilapia diet for recovery cold stress conditions. In cold temperatures, fish increase the unsaturation of fatty acids in their cell membrane phospholipids to maintain fluidity and permeability, aiding in physiological homeostasis (Weber and Bosworth, 2005; Turchini et al., 2009). Numerous fish species have been observed to undergo this process, which is referred to as homeoviscous adaptation (Craig et al., 1995; Fracalossi and Lovell, 1995; Atwood et al., 2003).

Arabic gum is secreted by certain trees, including *Sengalia senegal* and *Acacia senegal*, that contain organic acids, amino acids, a few minerals, and a high-molecular-weight complex of glycoproteins and polysaccharides, such as arabinose, galactose, and rhamnose (Bakhoum et al., 2018; Fouda et al., 2019). Beside the above-mentioned bioactive component in Arabic gum, Imbabi et al. (2023) found another component like, Phosphatidylcholine and phosphatidylserine. The natural prebiotic Arabic gum improves gut health by inhibiting harmful bacteria, promoting nutrient absorption, and activating beneficial bacteria (Al-Baadani et al., 2021). In the study of Naiel et al. (2022), they found that Arabic gum plays a crucial role in promoting antioxidant status, growth, and immunological response of tilapia.

For aquatic species, lecithin is considered a significant source of bioavailable phospholipids (PL). It is an essential component of biological membranes in all eukaryotic cells and plays a crucial function in energy generation through food metabolism (Aničić et al., 2013). In general, diets supplemented with PL resulted in growth-promoting effects and increased survival and stress resistance (Tocher et al., 2008). Adding a lecithin-containing bio emulsifier to the diet of tilapia has been shown to improve the innate immunological response, growth, and feed digestion of the tilapia (El-Sayed et al., 2021). Furthermore, diets supplemented with marine lecithin (mainly PL) and soy lecithin improved the growth and recovery from stress challenge of juvenile cobia and milkfish larvae (Trushenski et al., 2013; Sivaramakrishnan et al., 2021). Regarding cold stress recovery Batista et al., (2023) found that the addition of soy lecithin in tilapia diet at a range of 42–50 g kg^{-1} mitigated the cold stress condition. As far as we are aware, there are no scientific reports on the use of Arabic gum or lecithin in tilapia diets as a mitigation feeding strategy during the winter season. Therefore, a feeding trial was done to assess the effects of dietary supplements containing Arabic gum and lecithin on the antioxidant activity, blood index, innate immune responses, and delta-9-desaturase gene (Δ 9D) expression in farmed Nile tilapia during the winter season.

2. Materials and methods

2.1. Determination of bioactive compounds in Arabic gum

Following slight adjustments to the procedure previously described by Folch et al. (1957), the major phospholipids were isolated. Prior to HPLC analysis, the isolated phospholipid was diluted in a mobile phase solvent that contained 20 % chloroform. With a Porasil silica gel column (10-lm particle size), an Agilent 1200 Series HPLC system with a computerized solvent supply system and UV detector (Santa Clara, CA, USA) was used to perform the isocratic high-performance liquid chromatographic separation of various phospholipids. The degassed mobile phase, which was supplied at a flow rate of 0.80 mL/min and consisted of acetonitrile, methanol, and 85 % phosphoric acid (96:3:1, v/v/v), was used to elute samples (20 μL) for HPLC analysis. Using comparable phospholipid standards, the concentration of each sample was determined while the effluent was being observed at a wavelength of 203 nm. The source of the standard (GSH: 200–725–4) was the Sigma Chemical Company located in St. Louis, MO, USA (Fig. 1).

2.2. Design of experiment and diets

Benha University, Faculty of Agriculture's Fish Nutrition Laboratory served as the site of the current experiment. The effects of Arabic gum (Acaci gum; 0 g, 2 g, and 4 g kg^{-1} diet), lecithin (0 g, 5 g, and 10 g kg^{-1} diet), and their combinations on the growth, survival rate, serum biochemical parameters, oxidative response, and Δ 9D gene of Nile tilapia fingerlings were investigated in a factorial experiment (3×3). Soybean lecithin was provided by Aceitera Feneral Deheza S. A., Argentina, while Arabic gum was obtained from local market. Nine isonitrogenous (305 g kg^{-1} crude protein) and isocaloric (19.45 MJ kg^{-1} gross energy) diets were created by mixing each level of Arabic gum (Table 1). After adding varied amounts of Arabic gum and lecithin, the diet components were thoroughly blended. A pelleting hand-noodle maker was then used to make the 2-mm diameter pellets, which were then left for 12 h at ambient temperature to dry, and finally stored in cellophane bags at −4°C until required, according to Ali et al. (2023). The AOAC (1995) was used to estimate the proximate composition of the diets.

2.3. Fish and experimental management

On 15 November 2021, monosex Nile tilapia, *Oreochromis niloticus* were obtained from the fish farm of the Faculty of Agriculture at Benha University in Egypt. The fish were kept in two cement ponds (2 × 4 × 1 m) for 15 days after being collected to acclimate to the experimental conditions and fed a commercial feed (25 g kg^{-1} protein) before the feeding trial started. Following acclimation, 18 fiberglass tanks (0.5 m^3) were randomly filled with 270 uniformly sized fish, with 15 fish per tank as stocking density. The investigation began on 1 December 2021, and it was finished on 1 February 2022 (60 days). The initial body weight of the fish was 7.56 ± 0.10 g. Two air stones continuously aerated each tank. Fish were manually fed twice daily to maintain the warm water temperature, six days a week (Hassaan et al., 2019a). Table 2 shows the ambient temperature during the experiment period.

Throughout the trial period, water quality data were recorded using various instruments. This included mercury thermometers, an Orion pH meter, and a Jenway 970 dissolved oxygen meter. Standard techniques were used to quantify nitrate (NO_3, 0.55 ± 0.02 mg L^{-1}), ammonia (NH_4; 0.25 ±0.04, mg L^{-1}), and nitrite (NO_2, 0.021 ± 0.01 mg L^{-1}) once per week according to APHA (1989).

2.4. Growth indices

The growth performance and feed utilization were evaluated by measuring specific growth rate, final body weight, protein efficiency ratio, weight gain, feed conversion ratio, and feed intake as detailed in the footnote of Table 3.

2.5. Serum biochemical analysis

Blood samples were taken from the caudal vein of three fish from

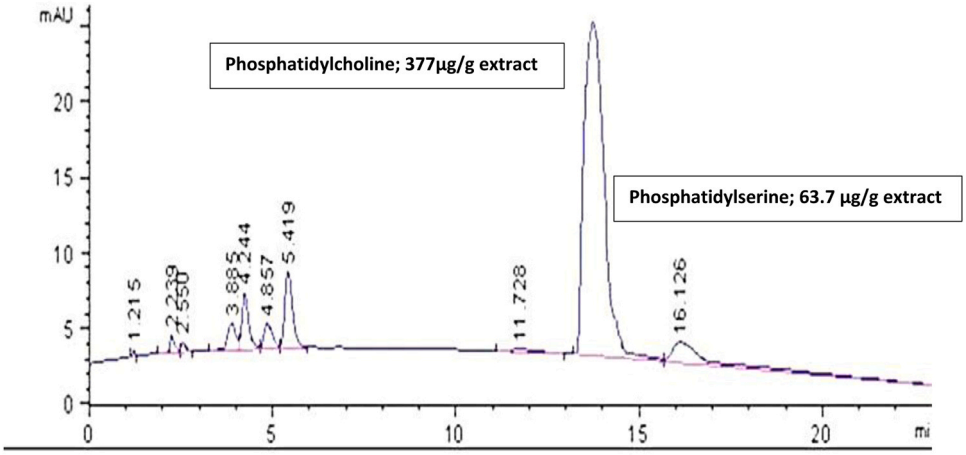

Fig. 1. HPLC chromatogram of Arabic gum. For HPLC analysis, samples (20 mL) were injected and eluted using the degassed mobile phase, which was supplied at a flow rate of 0.80 mL/min and consisted of acetonitrile, methanol, and 85 % phosphoric acid (96:3:1, v/v/v). Using comparable phospholipid standards, the concentration of each sample was determined while the effluent was being monitored at a wavelength of 203 nm. The source of the standard (GSH: 200–725–4) was the Sigma Chemical Company located in St. Louis, MO, USA.

Table 1
Composition and proximate analysis of experimental diets (g kg^{-1} dry matter).

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Fish meal	50	50	50	50	50	50	50	50	50
Soybean meal	450	450	450	450	450	450	450	450	450
Corn gluten	50	50	50	50	50	50	50	50	50
Yellow corn	290	288	286	295	298	291	300	293	296
Wheat bran	100	100	10	100	100	100	100	100	100
Vit&Min[a]	20	20	20	20	20	20	20	20	20
Soyabean oil	40	40	40	30	20	30	20	30	20
Arabic gum	0	2	4	0	2	4	0	2	4
Lecithin	0	0	0	5	10	5	10	5	10
Proximate analysis (%)									
Crude protein	30.02	29.98	29.91	29.88	29.95	30.12	30.10	29.90	30.20
Crude lipid	6.02	6.01	6.02	6.03	6.04	6.01	6.02	6.02	6.04
Ash	4.50	4.40	4.50	4.50	4.50	4.60	4.50	4.50	4.60
Total carbohydrates	59.46	59.61	59.57	59.59	59.51	59.27	59.38	59.58	59.16
Gross energy (MJ kg^{-1})[b]	19.45	19.51	19.47	19.49	19.47	19.39	19.40	19.48	19.36

[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 B12, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 g Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; D-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3077 mg; ferrous sulfate (FeSO4.7H2O, 20 % Fe), 65 mg; manganese sulfate (MnSO4, 36 % Mn), 89 mg; zinc sulfate ($ZnSO_4.7\ H_2O$, 40 % Zn), 150 mg; copper sulfate ($CuSO_4.5\ H_2O$, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I).

[b] 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).

Table 2
Ambient water temperature average during experimental period from 1 December to 1 February.

Treatment periods	Temperature range	Ambient temperature average (°C)
1 Dec – 14 Dec (1–2 weeks)	16–19.5	18
15 Dec – 29 Dec (2–4 weeks)	14–17	16
30 Dec – 14 Jan (4–6 weeks)	15–18.5	17
15 Jan – 30 Jan (6–8 weeks)	16–21	18.5

each replicate. Enzymes like AST and ALT were measured according to the method of Reitman and Frankel (1957) and serum triglycerides, glucose, LDL-C, and HDL-C were tested by using standardized kits (Modern Laboratory Kits). Cortisol levels were measured using an immulite kit and chemiluminescence, while sodium concentration was determined using a colorimetric detection kit. Potassium, calcium, and chloride contents were measured using the magon sulfonate method, the cresolphthalein method, and the thiocyanate method, respectively.

2.6. Oxidative enzymes activity

Three fish livers from each replicate were weighed, cleaned, and ground with ice-cold saline (0.1 g of liver in 0.9 mL saline, pH 7.0), then centrifuged at 3000 g for 10 min. The collected supernatant was used to assess the activity of superoxide dismutase (SOD) following Peskin and Winterbourn (2000) approach. For catalase (CAT) activity, Beers and Sizer's (1952) modified procedure was used. Malondialdehyde (MDA) activity was assessed according to Dogru et al. (2008). Glutathione peroxidase (GPx) activity was measured and expressed as units per milligrams of protein following Moin (1986) method. Glutathione (GSH) was measured using the Beutler et al. (1963) technique. The total antioxidant capacity was evaluated using the Prieto et al. (1999) method.

2.7. Expression of delta-9-desaturase gene

Liver samples from fish were taken and homogenized. Total RNA was

Table 3
Growth performance and feed utilization of fish fed supplemented with Arabic gum and lecithin and their interaction.

T	AG¶ g kg^{-1} diet	Lecithin g kg^{-1}	IBW[1] (g fish^{-1})	FBW[2] (g fish^{-1})	WG[3] (g fish^{-1})	SGR[4] (% day^{-1})	FCR[5]	FI[6] (g fish^{-1})	SR[7]%
Individual treatments mean									
T1	0	0	7.74	13.11^{d}	5.38^{e}	0.91^{d}	3.46^{a}	18.50^{b}	60.50 ^{f}
T2	2	0	7.70	18.23^{c}	10.53^{d}	1.49^{c}	2.29^{b}	24.00^{a}	82.50^{e}
T3	4	0	7.67	19.71^{b}	12.04^{b}	1.63^{b}	1.79 ^{f}	22.50^{a}	85.50^{d}
T4	0	5	7.53	18.97^{c}	11.43^{c}	1.60^{b}	2.06^{c}	23.50^{a}	91.00^{c}
T5	2	5	7.44	18.76^{c}	11.33^{c}	1.60^{b}	1.99^{d}	22.50^{a}	92.50^{c}
T6	4	5	7.44	19.88^{b}	12.45^{b}	1.70^{b}	1.81^{e}	22.50^{a}	98.00^{ab}
T7	0	10	7.40	21.78^{a}	14.38^{a}	1.86^{a}	1.60^{e}	23.00^{a}	96.50^{b}
T8	2	10	7.57	20.07^{ab}	12.51^{b}	1.68^{b}	1.88^{e}	23.50^{a}	99.00^{a}
T9	4	10	7.54	21.60^{a}	14.07^{a}	1.82^{a}	1.67 ^{g}	23.50^{a}	99.50^{a}
Pooled SE			0.10	0.58	0.59	0.05	0.23	0.68	1.23
Means of the main effects									
	0		7.61	17.38^{r}	9.77^{q}	1.40^{q}	83.50^{r}	0.29	21.83^{q}
	2		7.51	19.85^{q}	12.33^{p}	1.67^{p}	90.50^{q}	0.27	23.17^{p}
	4		7.55	20.17^{p}	12.63^{p}	1.69^{p}	94.33^{p}	0.35	22.83^{p}
		0	7.70	17.05^{z}	9.35^{z}	1.34 ^{y}	76.17 ^{y}	0.31	21.67 ^{y}
		5	7.47	19.20 ^{y}	11.73 ^{y}	1.63^{x}	93.83^{x}	0.35	22.83^{x}
		10	7.50	21.15^{x}	13.65^{x}	1.78^{x}	99.33^{x}	0.26	22.33^{x}
ANOVA (P-value)									
Arabic gum			0.374	0.034	0.027	0.024	0.030	0.2764	0.406
Lecithin			0.353	0.015	0.011	0.008	0.001	0.2166	0.026
Arabic gum × lecithin			0.264	0.001	0.001	0.001	0.001	<.0001	0.011

†Treatments means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different.
‡Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test; p, q and r for Arabic gum and x, y and z for lecithin levels.
: Arabic gum
IBW[1] = initial body weight; FBW[2] = final body weight; Weight gain (WG)[3] = final weight (g) – initial weight (g) Specific growth rate (SGR)[4]: SGR = $\frac{LnW2 - LnW1}{t} x100$ Where: Ln = the natural log W_1 = first fish weight W_2 = the following fish weight in grams t = period in days. Feed conversion ratio (FCR)[5]: FCR = Feed ingested (g)/Weight gain (g). FI[6] = feed intake; SR[6] = survival fish rate.

isolated from liver samples (three fish from each replicate) by the standard Promega RNA Isolation Kit (Cat No. Z3100, USA) and reagent extraction method according to the manufacturer's instructions. The quantity of the RNA was assessed using a Nano-Drop spectrophotometer (NANODROP 1000, Thermo Scientific, USA). The integrity (quality) was checked by denaturing gel electrophoresis (1 % agarose gel) and the purity by measuring the OD260/OD280 absorption ratio (>1.95).

cDNA was generated from 1 μg of total RNA using High Capacity cDNA (Thermo Fisher Scientific, Cat. No.436, 8814) reverse transcriptase kit for reverse transcriptase polymerase chain reaction (RT-PCR) following the manufacturer's protocol. The product of the first strand cDNA synthesis was stored at −80°C until the quantitative RT-PCR (qRT-PCR) runs.

Delta-9-desaturase (Δ 9D) and 18S rRNA gene primers were purchased from Invitrogen, Germany. The primer sequences of 18S rRNA was F: GTTGCAAAGCTGAAACTTAAAGG; R: TTCCCGTGTTGAGTCAAATTAAGC (GenBank no. AF497908.1) and F: ATCACCACACGTTCCCATATGAC; R: CCAGACCCAAGAAACACATGAAG (GenBank no. AY150696) for Δ 9D. Triplicate qPCR reactions were performed on an AriaMx Real-Time PCR System (Agilent Technologies). Reactions containing 5 μl of 5 × diluted cDNA, 10 pmol each of forward and reverse primers, 0.4 μl ROX dye solution (1:500 dilution) and 10 μl SYBR Green PCR MasterMix (Maxima SYBR Green qPCR, Thermo Fisher Scientific, Cat. No # k0251) were performed in a four-step experimental run protocol: a denaturation program (10 min at 95 °C); an amplification and quantification program repeated 40 times (30 s at 95 °C, 50 s at 55°C and 40 s at 72 °C); a melting curve program (55–95°C with a heating rate of 0.10 °C/s and a continuous fluorescence measurement) and finally a cooling step. Melt curve analyses of the target genes and reference genes resulted in single products with specific melting temperatures. In addition, "no-template" controls (i.e. with water sample) for each set of genes was also run to ensure no contamination of reagents, no primer–dimer formation. Moreover, 18S rRNA gene was used as an internal standard. The relative mRNA expression levels were calculated by a standard curve method. The expression levels of gene were normalized to the levels of 18S rRNA gene in the same sample. Standard curve was generated by serial dilution of a random mixture of control samples. Relative gene expression ratios (RQ) were calculated using the method of Livak and Schmittgen (2001).

2.8. Data analysis

Before being evaluated, the data were subjected to homogeneity and normality tests. Following that, results were examined using a two-way ANOVA with values for Arabic gum and lecithin. The limit for statistical significance was established at 5 %. Using the SAS ANOVA technique, Duncan's multiple range test was used to determine how the means differed (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1993).

3. Results

3.1. Growth and feed indices

The effect of different levels of Arabic gum or lecithin and their interaction in tilapia diet on growth and feed indices are presented in Table 3. Addition of Arabic gum or lecithin individually significantly ($P < 0.05$) improved final body weight (FBW), survival rate (SR), specific growth rate (SGR), weight gain (WG), and feed conversion ratio (FCR). Positive significant responses were found in WG and SR of tilapia fed diet containing Arabic gum and lecithin ($P < 0.05$). The fish fed diet with 4 g/kg Arabic gum and 10 g/kg lecithin showed the highest FBW, SGR, WG, and SR, as well as the best FCR.

3.2. Serum biochemical indices

Biochemical indices of fish fed diet supplemented with Arabic gum or lecithin and their interaction are shown in Table 4. Aspartate amino transferase (AST), glucose, alanine aminotransferase (ALT), and cortisol in the blood serum of tilapia were lowered considerably by using Arabic gum or lecithin separately as a dietary supplement ($P < 0.05$). Adding 4 g/kg of Arabic gum and 10 g/kg of lecithin to the fish diet resulted in the lowest levels of ALT, AST, glucose, and cortisol. Individually, there are significant differences in lipid profile including cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides, and low-density lipoprotein-cholesterol (LDL-C) with the addition of Arabic gum and lecithin at varying levels ($P < 0.05$) (Table 5). The lowest values of LDL-C and cholesterol were noted in fish fed control diet, while the highest HDL-C and triglycerides were noted in group fed diet supplemented with 10 g kg^{-1} lecithin and diet supplemented with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin, respectively

3.3. Blood serum ions

Fish serum ions given a diet enriched with Arabic gum or lecithin and their interaction are presented in Table 6. Sodium, potassium, calcium, and chlorine contents were significantly affected by Arabic gum and lecithin and their interaction. The highest content of sodium, potassium, calcium, and chlorine were obtained by feeding fish a diet enriched with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin.

3.4. Hepatic oxidative biomarkers

Diet supplemented with Arabic gum or lecithin individually significantly ($P < 0.05$) increased the activities of SOD, GPX, CAT, GSH, TAC, and decreased MDA in tilapia (Table 7). The highest CAT, GPX, GSH, SOD, and TAC were recorded in fish fed dietary supplemented with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin, whereas fish fed the same supplements had the lowest levels of MDA.

Table 4
Biochemical blood indices of fish fed dietary supplemented with Arabic gum and lecithin and their interaction.

T	AG g kg^{-1} diet	Lecithin g kg^{-1}	Biochemical blood indices			
			ALT (UL^{-1})	AST (UL^{-1})	Glucose (mg dl^{-1})	Cortisol (ng mL^{-1})
Individual treatments mean						
T1	0	0	45.25^{a}	26.90^{a}	119^{a}	14.5^{a}
T2	2	0	40.25^{b}	24.65^{b}	111^{b}	12.5^{cb}
T3	4	0	38.75^{c}	23.10^{c}	101.5^{c}	12.65^{cb}
T4	0	5	38.10^{d}	21.25^{d}	91.5^{d}	12.5^{cb}
T5	0	10	34.10^{g}	13.90^{g}	73.5^{g}	13^{b}
T6	2	5	37.40^{e}	19.65^{e}	87^{e}	11.5^{d}
T7	2	10	33.10^{h}	13.15^{h}	69^{h}	12.25^{c}
T8	4	5	35.25^{f}	14.65^{f}	81.5^{f}	12.25^{c}
T9	4	10	31.10^{i}	12.25^{i}	62.5^{i}	10.75^{e}
Pooled SE			0.17	0.17	0.36	0.14
Means of the main effects						
	0		391.5^{p}	206.83^{p}	94.67^{p}	13.33^{p}
	2		369.17^{q}	191.50^{q}	89.00^{q}	$12.\ 08^{q}$
	4		350.33^{r}	166.67^{r}	81.83^{r}	11.88^{q}
		0	414.17^{x}	248.83^{x}	110.50^{x}	13.22^{x}
		5	369.17^{y}	185.17^{y}	86.67^{y}	12.08^{y}
		10	327.67^{z}	131^{z}	68.33^{z}	12^{y}
ANOVA (P-value)						
Arabic gum			0.0001	0.0002	0.1133	0.0262
Lecithin			0.0001	0.0001	0.0725	0.0031
Arabic gum× Lecithin			0.0001	0.0001	0.0001	0.0001

†Treatments means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different.
‡Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test; p, q and r for Arabic gum and x, y and z for lecithin levels.
: Arabic gum

Table 5
Serum lipid profile of fish fed dietary supplemented with Arabic gum and lecithin and their interaction.

T	AG g kg^{-1} diet	Lecithin g kg^{-1}	Lipid profile			
			Cholesterol (mmol L^{-1})	Triglyceride (mmol L^{-1})	HDL (mmol L^{-1})	LDL (mmol L^{-1})
Individual treatments mean						
T1	0	0	119.00^{h}	202.50^{f}	44.50^{d}	49.00^{e}
T2	2	0	186.00^{f}	216.50^{e}	29.50^{g}	69.01^{a}
T3	4	0	175.50^{g}	180.50^{g}	34.00^{f}	59.01^{c}
T4	0	5	211.50^{e}	178.50^{g}	44.50^{d}	63.50^{b}
T5	0	10	401.00^{a}	331.50^{a}	52.50^{b}	52.00^{d}
T6	2	5	316.50^{c}	281.00^{c}	36.00^{f}	42.50^{f}
T7	2	10	399.00^{a}	299.00^{b}	49.02^{c}	68.51^{a}
T8	4	5	311.00^{d}	201.00^{f}	41.00^{e}	52.02^{d}
T9	4	10	387.00^{b}	273.00^{d}	59.00^{a}	64.00^{b}
Pooled SE			1.31	0.86	0.87	0.42
Means of the main effects						
	0		243.83^{q}	237.50^{pq}	47.00^{p}	54.83
	2		300.50^{p}	265.50^{p}	38.17^{q}	60.00
	4		291.17^{p}	218.17^{q}	44.67^{p}	58.33
		0	160.17^{z}	199.83^{y}	35.83^{z}	59.00
		5	279.67^{y}	220.17^{y}	40.50^{y}	52.67
		10	395.67^{x}	301.17^{x}	53.50^{x}	61.50
ANOVA (P-value)						
Arabic gum			0.0063	0.0400	0.0032	0.6327
Lecithin			0.0001	0.0001	0.0001	0.2800
Arabic gum× Lecithin			0.0001	0.0001	0.0001	0.0001

†Treatments means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different.
‡Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test; p, q and r for Arabic gum and x, y and z for lecithin levels.
: Arabic gum

3.5. Gene expression

Fig. 2 shows the effect of the different levels of Arabic gum or lecithin on the expression of the delta-9-desaturase (Δ 9D) gene in Nile tilapia livers. The transcription of Δ 9D was upregulated in groups fed with diets supplemented with the test ingredients than those fed diets without supplementation. Also, fish fed Arabic gum levels showed significantly higher Δ 9D expression compared with those fed lecithin levels. The highest transcription of Δ 9D was seen in fish given diet that included 4 g kg^{-1}Arabic gum and 10 g kg^{-1} lecithin.

4. Discussion

4.1. Growth performance and survival

Cold stress is one of the main hazards in the aquaculture production industry. It results in physiological dysfunction and has a detrimental impact on fish performance and survival (Bhujel et al., 2007; Nobrega et al., 2017; Cheng et al., 2018; Hassaan et al., 2019a). The current study shown that adding 4 gkg^{-1}Arabic gum and 10 gkg^{-1} lecithin to the feed could reduce the effects of cold stress and increase the survival of Nile tilapia. Very little research has looked at the potential effect of dietary Arabic gum usage on fish performance (Faggio et al., 2015; El-faki, 2016; Naiel et al., 2022). Moreover, there is no information available on the use of Arabic gum to reduce cold stress in Nile tilapia. The improvement in survival of Nile tilapia in the present study may be due to bioactive compounds in Arabic gum (Fig. 1). Naiel et al. (2022)

Table 6
Serum blood ions of fish fed dietary supplemented with Arabic gum and lecithin and their interaction.

T	AG g kg^{-1} diet	Lecithin g kg^{-1}	Blood ions			
			Na (mEq1^{-1})	K (mEq1^{-1})	Ca (mg dl^{-1})	Cl (mEq1^{-1})
Individual treatments mean						
T1	0	0	139.00^{e}	2.30^{d}	9.13^{d}	91.50 ^{f}
T2	2	0	142.00^{d}	2.90^{c}	11.10^{c}	111.00^{e}
T3	4	0	146.15^{c}	3.20^{b}	12.30^{b}	113.00^{d}
T4	0	5	148.50^{c}	3.10^{b}	11.60^{c}	112.00^{d}
T5	0	10	153.00^{b}	3.50^{b}	12.90^{b}	120.00^{b}
T6	2	5	152.00^{b}	3.90^{b}	12.70^{b}	122.00^{a}
T7	2	10	149.00^{c}	3.20^{b}	11.05^{c}	110.00^{e}
T8	4	5	147.40^{c}	3.80^{b}	12.90^{b}	118.00^{c}
T9	4	10	166.00^{a}	4.20^{a}	13.20^{a}	123.00^{a}
Pooled SE			0.34	0.83	0.19	0.71
Means of the main effects						
	0		145.50^{r}	2.87^{r}	10.59^{r}	104.50^{r}
	2		147.47^{q}	3.40^{q}	12.30^{q}	116.33^{q}
	4		154.72^{p}	3.77^{p}	12.73^{p}	119.33^{p}
		0	142.38^{z}	2.80^{z}	10.84 ^{y}	105.17 ^{y}
		5	151.17 ^{y}	3.50 ^{y}	12.40^{x}	118.00^{x}
		10	154.72^{x}	3.77^{x}	12.73^{x}	119.33^{x}
ANOVA (P-value)						
Arabic gum			0.4277	0.0304	0.0	0.0217
Lecithin			0.0576	0.0388	0.0304	0.0159
Arabic gum× Lecithin			0.0001	0.0195	0.0004	0.0001

[†]Treatments means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different.
[‡]Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test; p, q and r for Arabic gum and x, y and z for lecithin levels.
: Arabic gum

detected natural chemicals such as glucuronic acid, polysaccharides, minerals, and neutral sugars (e.g., rhamnose, arabinose, and galactose) in Arabic gum. These compounds are reported to impart anti-inflammatory, antioxidant, and immunostimulatory effects in fish. Furthermore, earlier research showed that Arabic gum is a natural prebiotic that stimulates growth (Calame et al., 2008). Arabic gum contains polysaccharides, glucuronic acid, and minerals that promote performance by fostering the growth of beneficial bacteria (Al-Baadani et al., 2021).

On the other hand, lecithin plays an important role in energy production through nutritional metabolism, notably fat digestion and absorption via emulsification and facilitation of the storage of lipids in the hepatopancreas; it is also a necessary part of the biological membranes of all eukaryotic cells (Hamre et al., 2013). The feed digestion, growth, and innate immune status of tilapia were all significantly strengthened by the dietary addition of bioemulsifiers including lecithin (El-Sayed et al., 2021). Lecithin also plays a cooperative function in the intestinal absorption of cholesterol, which enhances animal development and survival (Coutteau et al., 1997; Haghparast et al., 2019). Furthermore, phospholipids have growth-promoting, antioxidant, and chemo-attractant qualities that enhance resilience to stress, particularly in the early stages of development (Hertrampf and Piedad-Pascual, 2000; Tocher et al., 2008;El-Naggar et al., 2021). Dietary lecithin increases lipoprotein synthesis and secretion as well as enhances feed digestibility. Thus, fish somatic development is improved using dietary lipids (Seiliez et al., 2006). The findings of the current study on improved development and survival may be due to lecithin though its crucial function in energy production through nutritional metabolism, particularly fat digestion and absorption via emulsification (Gisbert et al., 2005; Morais et al., 2007).

4.2. Serum biochemical parameters and lipid metabolism

Biochemical blood indices can indicate the health of fish. Monitoring the biochemical fluctuations in hepatic enzyme activities, such as ALT and AST can provide insight into liver function (Zhai et al., 2014; Hassaan et al., 2015; Hassaan et al., 2019b; Hassaan et al., 2021; Inanan et al., 2021; Alandiyjany et al., 2022; Navruz et al., 2023; Awad et al., 2024). It well known that the activities of aminotransferase enzymes are

Table 7
Hepatic oxidative response (U/g protein) of fish fed dietary supplemented with Arabic gum and lecithin and their interaction.

T	AG g kg^{-1} diet	Lecithin g kg^{-1}	Antioxidant enzymes					
			SOD	CAT	GPX	GSH	MDA	TAC
Individual treatments mean								
T1	0	0	586.21 ^{f}	478.60^{d}	99.00 ^{g}	49.00 ^{g}	50.33^{a}	4.62^{d}
T2	2	0	759.29^{e}	488.60^{d}	121.00 ^{f}	70.03 ^{f}	33.47^{b}	7.05^{c}
T3	4	0	1005.20^{b}	510.00^{c}	131.00^{e}	73.00^{e}	29.00^{b}	8.00^{c}
T4	0	5	903.44^{c}	512.00^{c}	146.00^{d}	75.00^{e}	19.27^{c}	8.20^{c}
T5	0	10	992.23c	540.00^{b}	181.50^{b}	89.50^{d}	12.00^{d}	9.30^{b}
T6	2	5	892.47^{d}	562.00^{a}	161.50^{d}	91.00^{d}	14.00^{d}	10.30^{b}
T7	2	10	946.34^{c}	511.00^{c}	190.50^{a}	110.00^{c}	7.55^{e}	9.30^{b}
T8	4	5	1106.45^{b}	567.00^{a}	171.50^{c}	119.00^{b}	7.00^{e}	11.80^{a}
T9	4	10	1129.40^{a}	570.00^{a}	188.00^{a}	128.00^{a}	6.10 ^{f}	12.90^{a}
Pooled SE			1.09	8.91	0.79	0.84	0.89	0.55
Means of the main effects								
	0		812.00^{r}	500.53^{r}	145.17^{r}	78.00^{r}	25.72^{r}	7.37^{r}
	2		952.66^{q}	531.87^{q}	158.00^{q}	92.84^{q}	17.49^{q}	9.38^{q}
	4		1009.02^{p}	547.33^{p}	160.17^{p}	97.33^{p}	16.37^{p}	10.40^{p}
		0	783.57^{z}	492.40^{z}	117.00^{z}	64.01^{z}	37.60^{x}	6.56^{z}
		5	929.38 ^{y}	538.00 ^{y}	163.00 ^{y}	85.17 ^{y}	15.09 ^{y}	9.27 ^{y}
		10	1060.73^{x}	549.33^{x}	183.33^{x}	119.00^{x}	6.88^{z}	11.33^{x}
ANOVA (P-value)								
Arabic gum			0.0001	0.0424	0.0001	0.0001	0.0109	0.04877
Lecithin			0.0001	0.0285	0.0001	0.0001	0.0001	0.0487
Arabic gum× Lecithin			0.0001	0.0001	0.0001	0.0001	0.0001	0.0144

[†]Treatments means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different.
[‡]Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test; p, q and r for Arabic gum and x, y and z for lecithin levels.
: Arabic gum

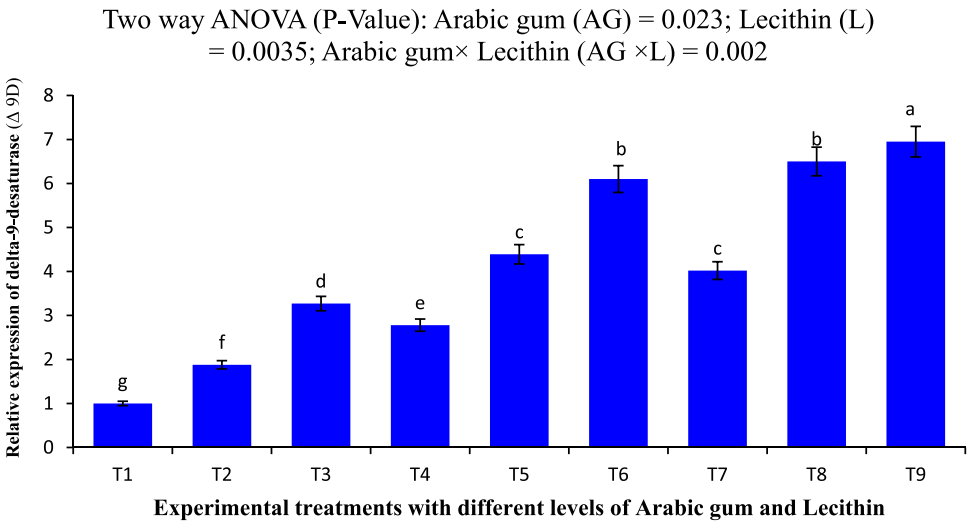

Fig. 2. Gene expression of delta-9-desaturase in liver of Nile tilapia fingerlings fed diet supplemented with different levels of Arabic gum and lecithin during the winter season. T1 = (0 g AG + 0 g kg^{-1} diet); T2 = (2 g AG + 0 g kg^{-1} diet); T3 (4 g AG + 0 g kg^{-1} diet); T4 = (0 g AG + 5 g kg^{-1} diet); T5 (0 g AG + 10 g kg^{-1} diet); T6 = (2 g AG + 5 g kg^{-1} diet); T7 = (2 g AG + 10 g kg^{-1} diet); T8 = (4 g AG + 5 g kg^{-1} diet); T9 = (4 g AG + 10 g kg^{-1} diet).

sensitive to cold temperature. Previous studies found that higher activities of ALT and AST when fish are exposed to low temperatures (Shi et al., 2015; Panase et al., 2018; Wu et al., 2019; Hassaan et al., 2019a; Soaudy et al., 2021). As far as we know, there have been no studies on the effect of dietary Arabic gum and lecithin on the activities of aminotransferase enzymes and serum biomarkers of stress in fish under cold stress conditions. While, there are other feed additives improved the levels of ALT and AST such as chia seed powder when added in tilapia diet significantly decreased the levels of ALT and AST (Mahmoud et al., 2023). In the present study dietary Arabic gum and lecithin at level 4 g kg^{-1}Arabic gum and 10 g kg^{-1} lecithin improved AST and ALT activities of Nile tilapia reared in cold stress. This finding may indicate the hepatoprotective role of Arabic gum and lecithin. While, rise of ALT and AST might be a sign of increased hepatic enzyme production or of enzymes leaking via harmed plasma membranes (Yang and Chen, 2003). The reduction of ALT and AST in tilapia fed diet supplemented with Arabic gum and lecithin in the present study reflect the positive effect of the diet additives for cold stress recovery. In line with the present study Naiel et al., (2022) found that addition of Arabic gum at 1 % in tilapia diet resulted in decreased ALT and AST activities. Furthermore, lecithin could improve the metabolism activities and maintain health status of fish (Attia and Kamel, 2012; Shang et al., 2018).

Complex hormonal interactions, including those involving cortisol, regulate the amount of glucose in the blood (Pacheco and Santos, 2001; Panase et al., 2018). Cortisol regulates glucose mobilization through gluconeogenesis and is considered an indicator of stress (Vijayan et al., 1997; Wendelaar Bonga, 1997; Mommsen et al., 1999). Fish use glucose as a fuel source that is stored as glycogen in their muscles and liver. Compared to serum cholesterol and protein, it is more susceptible to temperature variations (Lucas, 1996; Lermen et al., 2004). The current study found that dietary supplemented with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin decreased the levels of cortisol and glucose of tilapia serum compared to the other treatments. While, increased cortisol and glucose levels in fish fed diet devoid of Arabic gum and/or lecithin may be the cause of the increased energy need to combat cold stress and compensate for the decreased enzyme response (Atwood et al., 2003). Correspondingly with the present data, Tandon and Joshi (1974), Best et al. (2001), and Cho et al. (2015) reported that cold stress caused elevated blood glucose levels in *Clarias batrachus*, *Salmo trutta*, red spotted grouper, and *Epinephelus akaara*, resulting in retarded fish metabolism. Our data indicates that lecithin or Arabic gum supplements have an anti-stress effect. Fish given a diet containing 4 g kg^{-1} Arabic gum and 10 g kg^{-1} lecithin had lower levels of cortisol and glucose. According to Šegvić-Bubić et al. (2013), adding 2.5 g kg^{-1} of propolis to diets could help prevent oxidative stress in sea bass when exposed to short-term (24-hour) cold stress. This is based on the impact of propolis on the levels of serum cortisol and glucose in fish experiencing short-term cold stress. Additionally, according to Acar (2018), Nile tilapia reared under standard rearing conditions and given varied doses of propolis extract showed no appreciable variations ($P > 0.05$) in glucose content. Further studies needed to clarify the role of bioactive compounds to decrease the serum biochemical parameters as a biomarker.

LDL-C carries cholesterol from the liver to tissues, while HDL-C carries cholesterol from peripheral tissues into hepatic cells for additional metabolism (Jafari et al., 2018). Elevated triglyceride (TG) levels can result from glycogen storage or nephrotic syndrome. Monitoring these levels helps assess lipid metabolism, nutritional status, and liver health (Coz-Rakovac et al., 2005; Osman et al., 2010). The current study showed high triglyceride and cholesterol levels in fish fed diets supplemented with 10 g kg^{-1} lecithin than diets free of the test ingredients. This could suggest the capacity of lecithin to lessen the cold stress response of tilapia. The breakdown of TG to create adenosine triphosphate may cause a decrease in TG levels in the diet without lecithin (Sun et al., 2019). This can interfere with the liver's ability to mobilize fat, alter the fluidity of membranes, and affect how the liver and tissues circulate blood in response to cold stress (Chang et al., 2006). Also, Jafari et al. (2018) found that higher cholesterol in fish fed dietary supplemented with soya lecithin, due to higher hepatic synthesis of cholesterol. Also, the present study showed that the level of HDL-C content tends to increase in the serum of fish fed dietary supplemented with 4 g kg^{-1} Arabic gum and 10 g kg^{-1} diet lecithin, while LDL-C increased in fish fed 2 g kg^{-1} Arabic gum followed by fish fed 2 g kg^{-1} diet Arabic gum and 10 g kg^{-1} lecithin. The rise in HDL-C indicates that it is transferring cholesterol from peripheral cells to the liver to reduce damage from lipid peroxidation due to cold stress (Fredenrich and Bayer, 2003). Higher levels of blood triglycerides, LDL-C, cholesterol, and HDL-C herein suggest that lecithin and Arabic gum are beneficial in avoiding liver tissue damage under cold stress circumstances. In a study by Šegvić-Bubić et al. (2013), there was a decrease in triglyceride levels in *Dicentrarchus labrax* before and after experiencing acute low-temperature stress for 24 h. Furthermore, propolis extract in varying amounts was added to the Nile tilapia diets and showed no significant variations in their triglyceride levels (Acar, 2018). According

to Hassaan et al. (2019a), tilapia fed diets with propolis extract tend to increase blood HDL-C, triglyceride, LDL-C, and cholesterol contents at a faster pace than fish fed diets without the supplement. Additionally, a study on *Sparus aurata*, revealed that serum triglyceride contents rose following cold stress, indicating enhanced energy requirements to deal with the restoration processes as shown with the use of triglycerides (Kyprianou et al., 2010). The disagreement between our results and others could be caused by variations in the species, exposure period to cold stress, and water temperature.

4.3. Blood serum ions

Cold stress conditions adversely alter the plasma electrolytes stress (Cerqueira and Fernandes, 2000). Trophic fish blood osmolality stress indicators include plasma levels of Ca. Weyh et al. (2022) reported that minerals play a crucial role in regulating inflammation and supporting immune function. The present study showed that the blood ion concentrations of Na, Ca, Cl, and K were higher in fish that received nutritional supplements containing 4 g kg^{-1} of Arabic gum and 10 g kg^{-1} of lecithin in diet. This may be because Arabic gum and lecithin have a beneficial effect on regulating blood osmotic pressure during cold temperature stress (Tyagi et al., 2013, Soaudy et al., 2021). The concentration of K and Na ions in the plasma was shown to be crucial for the survival of *Shizothorax richardsonii* at low temperatures, as supported by our observations. The levels of Na and K in fish fed control diet decreased during cold stress, according to Hassaan et al. (2019a), but their levels in fish fed diets supplemented with propolis extract increased significantly. Stress and disturbances can alter electrolyte levels (such as K and Na) and osmoregulation by activating the pituitary-internal axis in the neuroendocrine system, leading to the release of stress hormones like corticosteroids and catecholamines.

4.4. Hepatic oxidative stress

Exposure to cold temperature stress alters antioxidant capacity, which leads to an increase in energy metabolism (Ye et al., 2015; Kaushik and Kaur, 2003). The liver contains antioxidant enzymes that are capable of scavenging free radicals such as hydroxyl radicals and superoxide anions to avoid being damaged by oxidation and shield the body from harm (Lortz et al., 2000; Zikić et al., 2001; Rudneva et al., 2010; Sandamalika et al., 2021; Mohammady et al., 2024). Lipid peroxidation produces MDA, which can be elevated to the point that it inactivates enzymes and decreases the permeability of cell membranes, causing oxidative damage to proteins, DNA, and cytoplasm, particularly when fish are exposed to unfavorable climatic circumstances (Tüzgen et al., 1998; Yao et al., 2010; Garcia et al., 2020). The current investigation revealed increased CAT, GSH, SOD, and TAC activities in tilapia fed a diet enriched with 4 g kg^{-1} of Arabic gum with 10 g kg^{-1} lecithin. The addition of Arabic gum and lecithin to the fish diets may result in improved antioxidant defense when exposed to cold stress. In this context, Naiel et al. (2020) found that diets supplemented with Arabic gum improved the antioxidant enzymes activities of GSH, SOD, and CAT, and decreased MDA of tilapia. Moreover, Arabic gum can enhance the antioxidant activity of liver cells by regulating the expression of genes related to oxidative stress (Musa et al., 2013). Also, Mirghani et al. (2018) found that the potent antioxidant properties of Arabic gum may be attributed to its high concentration of phenolic compounds. Furthermore, Arabic gum has high antioxidant activity and a protective effect against oxidative damage and a slight immunostimulatory effect when added as food supplement in fish diet. In the study by Kumar et al. (2014), lecithin was found to have a positive effect on the liver's GSH, CAT, and SOD in milkfish at different temperatures. It also protected cells from stress-induced damage, as supported by the current data.

4.5. Gene expression

Certain genes, such as delta 9 desaturase (Δ 9D), are highly expressed in a variety of poikilothermic species throughout the cold acclimation process and vary in expression to adapt to the cold (Zerai et al., 2010; Hassaan et al., 2019; Wu et al., 2020). According to earlier research, temperature, feed quality, and the various fatty acid types have an impact on the expression of the Δ 9D gene (Tocher et al., 1996; Hsieh et al., 2007). Desaturation membrane lipids is one of Δ 9D primary roles in maintaining membrane fluidity in the cold. Our results showed that fish fed diets with Arabic gum and lecithin had enhanced mRNA transcriptome of the Δ 9D gene in liver tissue compared to fish fed control diet under cold stress (Fig. 2). The fish liver tissue samples supplemented with 4 g kg^{-1} of Arabic gum and 10 g kg^{-1} of lecithin showed the highest Δ 9D expression. Low temperatures enhance the fluidity of the cell membrane by increasing the unsaturation of phospholipids more than high temperatures (Hagar and Hazel, 1985b; Ruyter et al., 2003; Soaudy et al., 2021). Decreased expression of genes for acetyl-CoA carboxylase, fatty acid synthase, and carnitine palmitoyl transferase indicates the harmful effects of cold stress, such as inhibition of lipid synthesis, digestion, and oxidation. Conversely, there was a rise in the need of *Cherax quadricarinatus* for highly unsaturated fatty acids due to elevated expression of the sphingolipid delta-4 desaturase gene under cold stress (Wu et al., 2020). The decline in fish survival herein when fed diets without supplementation might have prevented the phosphatidylcholine from being replaced by phosphatidylinositol in cell membranes, which might have also made it more difficult for the fish to withstand the cold (Greene and Selivonchick, 1987; Henderson and Tocher, 1987). This may not be solely due to cold shock, but rather a decrease in metabolic rate caused by reduced feed consumption affecting liver metabolism. Furthermore, under optimal temperature conditions, Δ 9D expression was considerably higher in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) fed diet containing coconut oil than in those provided diets containing palmitoleic or fish oil (Hsieh et al., 2007). The amount of unsaturated fat in rainbow trout liver can be changed by modifying diets, according to Tocher et al. (1996). The current study found that Arabic gum levels had significantly higher Δ 9D expression compared to lecithin levels due to higher levels of phosphatidylcholine and phosphatidylserine. This finding is intriguing and requires further investigation.

5. Conclusion

The feeding of Nile tilapia supplemented with 4 gkg^{-1} Arabic gum and 10 gkg^{-1} lecithin has the potential to improve fish physiological status, reduce mortality, and boost resilience against harsh wintertime temperatures. These results could be applied in the aquatic feed industry as additives for winter diets. New feed technologies are necessary to increase the tilapia output in subtropical regions. To increase production in these areas, research on winter feed designs, sustainable husbandry practices, and cold-resistant Nile tilapia breeds are recommended.

Ethical approval

All experiments were approved by the authority of NIOF Committee for Institutional Care of Aquatic Organisms and Experimental Animals (NIOF-AQ4-F-23-R-051).

CRediT authorship contribution statement

Mohamed Elashry: Data curation. **Jiance Ragaza:** Writing – review & editing. **Eman mohammady:** Writing – review & editing, Writing – original draft, Software, Methodology. **Mohamed R. Soaudy:** Conceptualization. **Mohamed Hassaan:** Writing – review & editing, Writing – original draft, Software. **Janice Alano Ragaza:** Funding acquisition. **Hoda Elgarhy:** Formal analysis. **Marwa M. Ali:** Data curation.

Declaration of Competing Interest

None

Data Availability

Data will be made available on request.

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