



## Dietary lipid sources affect cold tolerance of Nile tilapia (*Oreochromis niloticus*)

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### ABSTRACT

This study was carried out to evaluate the effects of dietary lipid sources on growth performance, fatty acids composition and cold tolerance of Nile tilapia (*Oreochromis niloticus*) fingerlings ( $7.00 \pm 0.50$  g/fish). The fish were fed four isonitrogenous (28% crude protein), isocaloric (500 kcal/100 g) diets containing four lipid sources; fish oil (FO), corn oil (CO), coconut oil (COCO) or fish oil/ corn oil mixture (1:1 ratio) (oil mix). The diets were offered to the fish at a daily rate of 3% of their body weights (BW), twice a day for two months. After the feeding trial, the fish were exposed to decreasing water temperature from 25 °C until the appearance of death symptoms. The results revealed that FO-based diets (FO and oil mix) produced the best growth rates and feed efficiency, followed by corn oil diet, while COCO resulted in the lowest performance. Fish fed on CO and oil mix showed higher body unsaturated fatty acids (UFA) and lower lethal temperature than those fed on FO- or COCO-based diets. These results indicate that cold shock can modify the lipid metabolism in Nile tilapia by lowering total body saturated fatty acids and raising n-6 and n-3 UFA. This finding suggests that the inclusion of high levels of plant oils in Nile tilapia feeds can enhance their cold tolerance.

### 1. Introduction

Tilapias are among the most important farmed aquatic species in the world. They are currently the second largest farmed finfish group in the world, only after carps. The global production of farmed tilapia increased from 383,654 metric tons (mt) in 1990 representing 4.5% of total farmed fish production to 5,898,793 mt in 2016, representing 11% of total farmed fish production, with an average annual growth rate of 13.5% (FAO, 2018). Nile tilapia (*Oreochromis niloticus*) is the most important farmed tilapia species in the world. The production of Nile tilapia reached 4,199,567 mt representing 71% of total production of farmed tilapia in 2016 (FAO, 2018).

Tilapias are tropical fishes, well adapted to warm water environments (Sun et al., 1992), but they are sensitive to cold stress. Mass mortalities of farmed tilapia have been recorded as a result of severe drop in water temperature during winter months in some subtropical regions, leading to significant economic loss (Sun et al., 1992; Hassan et al., 2013). Mortality has been attributed mainly to the sensitivity of fish cell membranes to thermal stress. Cell membranes are thermally sensitive, and hence can be a potential target of chill and freeze damage (Cao-Hoang et al., 2010). In addition, the reduction or loss of active

transport across cell membranes can severely affect ion and water homeostasis (Overgaard and MacMillan, 2017). Therefore, improving tilapia tolerance to low temperature is highly demanded to prolong their growth period, reduce mortality during winter and avoid economic loss (Charo-Karisa et al., 2005).

Fish can avert freezing by decreasing the degree of saturation of cell membrane phospholipids to compensate the cold-induced solidification of lipid bilayers during cold shock (Craig et al., 1995; Pernet et al., 2007). This process is termed “homeoviscous adaptation” (Sinensky, 1974), which leads to unsaturation of the phospholipids and reduction in the proportion of saturated fatty acids (SFA). It has been reported that fish caught in winter in temperate zones contain higher polyunsaturated fatty acids (PUFA) levels than those caught in warmer months (Farkas and Csengeri, 1976). High PUFA levels have also been recorded in fish from colder geographic regions (Olsen, 1999; Storelli et al., 1998). In addition, when fish are subjected to high temperatures and then acclimated to colder temperatures, they display elevated levels of PUFA in their cell membrane lipids (Wallaert and Babin, 1994). Snyder and Hennessey (2004) found that fish that died during cold shock contained lower levels of PUFA and higher levels of mono-unsaturated fatty acids in their membrane lipids than the controls and

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survivors. Thus, dietary fatty acids can be a valuable tool to enhance the resistance of fish to cold stress, and in turn, to increase fish productivity at low temperatures, since fatty acids are responsible for cell membrane structure, fluidity, and functionality.

The regulation of unsaturated fatty acids composition, in order to maintain cell membrane fluidity, can be a key factor in the physiological response to cold shock (Hazel and Williams, 1990; Craig et al., 1995; Hsieh et al., 2007). Body fatty acid composition in fish is influenced by the food source (Kelly and Kohler, 1999). This simply means that dietary lipid sources have a significant effect on cold tolerance of fish, as has been reported in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) (Hsieh et al., 2007), red drum (*Sciaenops ocellatus*) (Craig et al., 1995) and striped bass, white bass, palmetto bass and sunshine bass (Kelly and Kohler, 1999).

Despite the sensitivity of Nile tilapia to cold, the effect of dietary lipid sources on their resistance to cold stress is not well understood. Therefore, the present study was carried out to investigate the effects of dietary lipid sources on the growth performance, fatty acids composition and cold tolerance of Nile tilapia fingerlings.

## 2. Material and methods

The present study was carried out in two consecutive phases. In phase I, Nile tilapia were fed test diets containing different lipid sources, at an optimum water temperature. Phase II evaluated the response of the fish to cold stress, following the feeding trial.

### 2.1. Test diets

Four isonitrogenous (28% crude protein), isocaloric (500 kcal/100 g) diets containing four different oil sources (at 4% inclusion level) were prepared (Table 1). The lipid sources used were: coconut oil (COCO) (rich in 12- and 14-carbon saturated fatty acids), corn oil (CO) (rich in 18:2 n-6 fatty acids), fish oil (FO) (rich in n-3 polyunsaturated fatty acids) and a mixture of fish oil and corn oil (1:1) (oil mix). The composition, proximate analysis and fatty acid profiles of the diets are shown in Tables 1 and 2.

**Table 1**  
Formulation and proximate analysis of the test diets.

Ingredients (%)	Diets			
	Fish oil	Corn oil	Oil mix	Coconut oil
Fish meal (70% CP)	10	10	10	10
Soybean meal (44% CP)	40	40	40	40
Wheat bran	30	30	30	30
Starch	13	13	13	13
Fish oil	4.0	0.0	2.0	0.0
Corn oil	0.0	4.0	2.0	0.0
Coconut oil	0.0	0.0	0.0	4.0
Vitamins and minerals mix <sup>a</sup>	2.0	2.0	2.0	2.0
Dicalcium phosphate	1.0	1.0	1.0	1.0
<b>Proximate analyses (% dry weight)</b>				
Crude protein	28.17	28.15	27.92	28.09
lipid	7.48	6.99	7.10	7.27
Total ash	6.06	6.10	6.20	6.47
Crude fiber	4.69	4.58	4.63	4.55
NFE <sup>b</sup>	53.60	53.31	54.15	53.62
Gross energy (Kcal/100 g)	449.79	446.32	447.05	447.43

<sup>a</sup> Contains (mg/kg or IU/kg): Vit. A, 2,200,000 IU. Vit. D3, 1100,000 IU. Vit. E, 1,500 I.U. Vit. K 800 mg. Vit. B1 1100 mg. Vit. B2 200 mg. Vit. B6 2,000 mg. Vit. H 15 mg. Vit. B1 24 mg. Vit. C 3,000 mg. Iron, 160 mg. Magnesium, 334 mg. Copper, 21.6 mg. Zinc, 21.6 mg. Selenium, 25 mg. Cobalt, 2.38 mg.

<sup>b</sup> Nitrogen-free extract (calculated by difference).

**Table 2**  
Fatty acid composition (% of total lipids) of the test diets.

Fatty acids	Fish oil	Corn oil	Oil mix	Coconut oil
C6:0	0.20	ND	ND	0.10
C8:0	ND	ND	ND	2.21
C10:0	ND	ND	ND	2.41
C12:0	ND	ND	ND	21.80
C14:0	6.71	1.63	3.72	11.16
C15:0	0.49	ND	ND	ND
C16:0	20.37	15.79	17.95	16.16
C17:0	0.94	ND	0.47	ND
C18:0	5.01	2.86	3.97	3.68
C20:0	0.71	0.62	0.59	0.42
<b>Saturates</b>	<b>34.43</b>	<b>20.9</b>	<b>26.7</b>	<b>57.94</b>
C16:1	8.62	1.69	5.30	1.93
C17:1	1.24	0.26	0.66	0.28
C18:1c	19.13	24.09	22.66	14.51
<b>Monoenes</b>	<b>28.99</b>	<b>26.04</b>	<b>28.62</b>	<b>16.72</b>
C18:2c (ω6)	7.41	50.08	31.67	22.86
<b>n-6 PUFA</b>	<b>7.41</b>	<b>50.08</b>	<b>31.67</b>	<b>22.86</b>
C18:3α(ω3)	2.31	0.50	1.00	0.37
C20:3ω3	3.19	1.43	3.95	1.25
C20:5EPA	10.16	ND	4.60	ND
C22:6DHA	13.50	1.05	3.45	0.87
<b>n-3 PUFA</b>	<b>29.16</b>	<b>2.98</b>	<b>13.00</b>	<b>2.49</b>

### 2.2. Fish, culture facility and feeding regimes

Nile tilapia (*O. niloticus*) fingerlings were obtained from a commercial fish farm near Alexandria, Egypt. The fish were rested in a 500-L fiberglass tank filled with dechlorinated tap water for 24 h. Triplicate groups of 10 fish (7.00 ± 0.50 g/fish) were stocked in 70-L glass aquaria. Each aquarium was provided with an air stone for aeration. Lighting in the culture unit was set at 12:12 light: dark cycle throughout the study. Water temperature was maintained at 25 ± 1 °C. The aquaria were cleaned each morning, and about 50% of the culture water was replaced daily with fresh, dechlorinated water of similar temperature. Water quality parameters including O<sub>2</sub>, pH, and total ammonia (NH<sub>4</sub>) were monitored regularly. The average values of these parameters throughout the study were: 6.1 mg/l, 7.8 and 0.29 ppm, respectively.

The test diets were fed to the fish at a daily rate of 3% of their live body weight (BW), twice a day (09:00 and 16:00 h) for 8 weeks. Fish were weighed at 10-day intervals, their average weights were recorded and the daily amount of feed for each aquarium was readjusted accordingly.

### Calculation of fish performance

Fish performance was calculated as follows:  
Survival (%) = 100 (Final fish number/Initial fish number)

### Growth rates

$$\text{Percent weight gain (\%WG)} = \frac{W_f(g) - W_i(g)}{W_i(g)} \times 100$$

$$\text{Average daily gain (ADG)} = \frac{W_f(g) - W_i(g)}{t}$$

$$\text{Specific growth rate (\%SGR)} = \frac{\ln W_f - \ln W_i}{t} \times 100$$

where: W<sub>i</sub> and W<sub>f</sub> are initial and final weights (g), and *t* is time of experiment (days).

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry feed intake (g)}}{\text{Fish live weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Fish weight gain (g)}}{\text{Protein intake (g)}}$$

### 2.3. Cold tolerance challenge

After the termination of the feeding trial, three fish were collected from each treatment and frozen at  $-20^{\circ}\text{C}$  for body composition and fatty acid analyses. The rest of the fish were then subjected to a gradual decrease in water temperature using a thermostat. Initially, the temperature was decreased one degree every 12 h until it reached  $16^{\circ}\text{C}$ . At  $16^{\circ}\text{C}$  the fish stopped eating, and therefore, feeding was terminated. At  $15^{\circ}\text{C}$ , 3 fish were collected from each treatment and frozen for fatty acid analysis. After  $15^{\circ}\text{C}$  water temperature was decreased one degree every 2 h.

Fish mortality was recorded during the cold challenge. Death was defined as the point at which the fish loose balance and direction, swim on their backs, loose response to external stimuli and stay on the bottom on their sides. Dead fish were removed from the tanks and water temperature at death was recorded. Subsequently, total mortality was calculated at each temperature. Mean temperature at death (TAD) and lethal temperature for 50% of fish ( $\text{LT}_{50}$ ) were calculated.

### 2.4. Body composition and fatty acid analyses

Proximate analyses of the test diets and fish carcasses were determined according to standard methodology (AOAC, 1990). Lipid extraction was made according to Pearson's chemical analysis of foods (1981). The lipid samples were transmethylated to their corresponding fatty acid methyl esters by acidified methylation in 1%  $\text{H}_2\text{SO}_4$  in absolute methanol solution. Analysis of fatty acid methyl esters was performed using a Hewlett-Packard 6890 gas chromatograph equipped with flame ionization detector and a flexible fused silica capillary column (30 m, 32 mm, 0.25  $\mu\text{m}$ ) using nitrogen as a carrier gas. Peak identification was performed by comparing retention times with those of authentic standards using equivalent chain length values.

### 2.5. Statistical analysis

All data, except fish mortality data obtained during the cold shock, were subjected to a one-way analysis of variance (ANOVA) at a 95% confidence limit, using SPSS software (v 20.0, SPSS Inc., Chicago, IL). Duncan's multiple range test was used to compare means when  $F$ -values from the ANOVA were significant ( $P < 0.05$ ). Least significant difference was used to compare means at  $P < 0.05$ . Arcsine-transformed percent mortality data for Nile tilapia during cold shock were analyzed using the General Linear Model (GLM) (SAS software).

## 3. Results

### 3.1. Growth performance

The present results indicated that dietary lipid sources significantly affected ( $P < 0.05$ ) the growth rates and feed utilization efficiency of

**Table 3**  
Growth performances and feed utilization (mean  $\pm$  SE) of Nile tilapia fed different lipid sources.

Diets	Average body weight (g/fish)		Gain (g/fish)	ADG (g/fish/day)	SGR (%/day)	Feed intake g/fish	FCR	PER	Survival (%)
	IBW	FBW							
Fish oil	7.81 $\pm$ 0.22 <sup>a</sup>	20.23 $\pm$ 0.32 <sup>a</sup>	12.42 $\pm$ 0.30 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	1.60 $\pm$ 0.05 <sup>a</sup>	20.46 $\pm$ 0.16 <sup>a</sup>	1.65 $\pm$ 0.01 <sup>c</sup>	2.11 $\pm$ 0.04 <sup>a</sup>	100.0
Corn oil	7.52 $\pm$ 0.17 <sup>a</sup>	17.50 $\pm$ 0.29 <sup>b</sup>	9.98 $\pm$ 0.17 <sup>b</sup>	0.16 $\pm$ 0.01 <sup>b</sup>	1.26 $\pm$ 0.02 <sup>b</sup>	18.27 $\pm$ 0.23 <sup>a</sup>	1.83 $\pm$ 0.11 <sup>b</sup>	1.95 $\pm$ 0.07 <sup>b</sup>	100.0
Oil mix	7.37 $\pm$ 0.12 <sup>a</sup>	19.37 $\pm$ 0.32 <sup>a</sup>	11.99 $\pm$ 0.38 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	1.58 $\pm$ 0.04 <sup>a</sup>	18.16 $\pm$ 0.27 <sup>a</sup>	1.52 $\pm$ 0.01 <sup>c</sup>	2.36 $\pm$ 0.04 <sup>a</sup>	100.0
Coconut oil	7.31 $\pm$ 0.16 <sup>a</sup>	16.40 $\pm$ 0.20 <sup>c</sup>	9.09 $\pm$ 0.17 <sup>c</sup>	0.15 $\pm$ 0.01 <sup>b</sup>	1.32 $\pm$ 0.03 <sup>c</sup>	19.21 $\pm$ 1.08 <sup>a</sup>	2.12 $\pm$ 0.02 <sup>a</sup>	1.70 $\pm$ 0.13 <sup>c</sup>	100.0

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

**Table 4**  
Whole body composition (mean  $\pm$  SE) (dry weight basis) of Nile tilapia fed different lipid sources.

Diets	Dry matter	Crude protein	Crude fat	Ash
Initial	21.05 $\pm$ 0.37	54.22 $\pm$ 1.97	19.46 $\pm$ 0.60	16.76 $\pm$ 0.61
Fish oil	25.68 $\pm$ 0.74 <sup>a</sup>	55.10 $\pm$ 0.42 <sup>a</sup>	22.69 $\pm$ 0.24 <sup>a</sup>	16.70 $\pm$ 0.20 <sup>a</sup>
Corn oil	25.82 $\pm$ 0.93 <sup>a</sup>	55.18 $\pm$ 0.43 <sup>a</sup>	23.18 $\pm$ 0.68 <sup>a</sup>	17.17 $\pm$ 1.17 <sup>a</sup>
Oil mix	27.11 $\pm$ 0.60 <sup>a</sup>	54.80 $\pm$ 0.32 <sup>a</sup>	23.77 $\pm$ 1.23 <sup>a</sup>	15.67 $\pm$ 0.33 <sup>a</sup>
Coconut oil	26.11 $\pm$ 0.40 <sup>a</sup>	53.93 $\pm$ 1.84 <sup>a</sup>	24.61 $\pm$ 0.68 <sup>a</sup>	17.42 $\pm$ 0.24 <sup>a</sup>

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Nile tilapia (Table 3). Fish oil (FO) and oil mix resulted in the best growth rates ( $P < 0.05$ ), followed by corn oil (CO), while coconut oil (COCO) produced the lowest growth rates. Feed utilization efficiency was also significantly affected by dietary treatments ( $P < 0.05$ ). The best feed conversion ratio (FCR) and protein efficiency ratio (PER) were achieved in fish fed FO and oil mix, followed by CO diet. On the other hand, COCO-based diet resulted in the lowest feed utilization efficiency. No mortality occurred in any treatment throughout the feeding trial.

### 3.2. Body composition and fatty acid profiles

Body composition of Nile tilapia was not significantly affected ( $P > 0.05$ ) by dietary lipid source (Table 4). Fatty acid profiles of fish fed the test diets are shown in Table 5. The highest saturated fatty acids (SFA) levels were found in fish fed COCO-based diet, followed by fish fed FO and oil mix diets, while CO produced the lowest SFA concentration. Monoenes were slightly higher in fish fed CO-based diet than in those fed oil mix and COCO diets.

Body n-6 and n-3 highly unsaturated fatty acids (HUFA) in Nile tilapia were significantly affected by dietary treatment ( $P < 0.05$ ) (Table 5). The highest n-6 HUFA level was recorded in fish fed the corn oil diet, followed by fish fed the blend oil- and coconut oil-based diets. On the other hand, dietary FO resulted in the lowest body n-6 HUFA value. FO diet also produced the highest level of n-3 HUFA, followed by oil mix, coconut oil and corn oil, respectively.

The cold shock at  $15^{\circ}\text{C}$  significantly reduced the body SFA content at all dietary lipid sources (Table 5). On the other hand, monoenes in fish fed FO and COCO diets were significantly increased, while cold shock did not significantly change monoenes levels in the blend oil- and CO-fed groups. However, cold stress at  $15^{\circ}\text{C}$  significantly increased n-3 HUFA and n-6 HUFA levels ( $P < 0.05$ ) in fish tissues in all treatments.

### 3.3. Cold tolerance challenge

The results of cold tolerance challenge are shown in Table 6 and Figs. 1 and 2. The results reveal that mortality started to occur in Nile tilapia fed COCO-based diet at  $16^{\circ}\text{C}$ ; earlier than in other treatments. In this group, the mortality continued until all fish died at  $9^{\circ}\text{C}$ . This group also showed the highest lethal temperature ( $\text{LT}_{50}$ ) and mean temperature at death (TAD). In FO group, mortality started to appear at  $12^{\circ}\text{C}$ , while all fish died at  $7^{\circ}\text{C}$ . Fish fed the oil mix and CO started to die at

**Table 5**Fatty acid composition (mean  $\pm$  SE) (% of total lipids) of the whole body of Nile tilapia fed different lipid sources at 25 and 15 °C.

	25 °C				15 °C			
	Fish oil	Corn oil	Oil mix	Coconut oil	Fish oil	Corn oil	Oil mix	Coconut oil
C6:0	0.063 $\pm$ 0.01 <sup>a,w</sup>	ND	ND	ND	ND	ND	ND	ND
C8:0	ND	ND	ND	ND	ND	ND	ND	ND
C10:0	ND	ND	ND	0.29 $\pm$ 0.01 <sup>a,w</sup>	ND	ND	ND	0.22 $\pm$ 0.02 <sup>a,x</sup>
C12:0	0.28 $\pm$ 0.02 <sup>b,w</sup>	0.22 $\pm$ 0.03 <sup>b,w</sup>	ND	8.22 $\pm$ 0.20 <sup>a,w</sup>	ND	ND	ND	7.97 $\pm$ 0.09 <sup>a,w</sup>
C14:0	4.54 $\pm$ 0.20 <sup>b,x</sup>	1.33 $\pm$ 0.15 <sup>d,w</sup>	2.50 $\pm$ 0.09 <sup>c,w</sup>	8.64 $\pm$ 0.23 <sup>a,w</sup>	4.17 $\pm$ 0.12 <sup>b,w</sup>	1.87 $\pm$ 0.02 <sup>c,w</sup>	1.90 $\pm$ 0.04 <sup>c,x</sup>	8.30 $\pm$ 0.40 <sup>a,w</sup>
C15:0	0.55 $\pm$ 0.06 <sup>a,w</sup>	0.33 $\pm$ 0.05 <sup>b,w</sup>	0.40 $\pm$ 0.03 <sup>ab,w</sup>	0.34 $\pm$ 0.04 <sup>b,w</sup>	0.52 $\pm$ 0.03 <sup>a,w</sup>	0.27 $\pm$ 0.03 <sup>b,w</sup>	0.32 $\pm$ 0.03 <sup>b,w</sup>	0.25 $\pm$ 0.02 <sup>b,w</sup>
C16:0	21.98 $\pm$ 0.93 <sup>a,w</sup>	16.85 $\pm$ 0.25 <sup>c,w</sup>	19.45 $\pm$ 0.4 <sup>b,w</sup>	20.50 $\pm$ 0.54 <sup>ab,w</sup>	19.66 $\pm$ 0.60 <sup>a,w</sup>	14.34 $\pm$ 0.40 <sup>c,w</sup>	17.89 $\pm$ 0.50 <sup>b,w</sup>	19.07 $\pm$ 0.10 <sup>ab,x</sup>
C17:0	0.72 $\pm$ 0.06 <sup>b,w</sup>	1.10 $\pm$ 0.07 <sup>a,w</sup>	0.890 $\pm$ 0.03 <sup>c,w</sup>	0.370 $\pm$ 0.03 <sup>c,w</sup>	0.51 $\pm$ 0.02 <sup>a,x</sup>	0.45 $\pm$ 0.04 <sup>ab,x</sup>	0.41 $\pm$ 0.02 <sup>ab,x</sup>	0.34 $\pm$ 0.03 <sup>b,w</sup>
C18:0	6.83 $\pm$ 0.60 <sup>a,w</sup>	4.45 $\pm$ 0.40 <sup>b,w</sup>	5.54 $\pm$ 0.20 <sup>b,w</sup>	7.94 $\pm$ 0.30 <sup>a,w</sup>	5.28 $\pm$ 0.15 <sup>a,w</sup>	2.15 $\pm$ 0.10 <sup>c,x</sup>	4.65 $\pm$ 0.20 <sup>b,x</sup>	6.58 $\pm$ 0.40 <sup>ab,x</sup>
C20:0	1.05 $\pm$ 0.07 <sup>b,w</sup>	0.30 $\pm$ 0.03 <sup>c,w</sup>	1.25 $\pm$ 0.08 <sup>a,w</sup>	1.47 $\pm$ 0.07 <sup>a,w</sup>	0.90 $\pm$ 0.08 <sup>a,w</sup>	0.26 $\pm$ 0.03 <sup>c,w</sup>	1.22 $\pm$ 0.03 <sup>b,w</sup>	1.41 $\pm$ 0.07 <sup>ab,w</sup>
C22:0	0.84 $\pm$ 0.05 <sup>b,w</sup>	0.88 $\pm$ 0.02 <sup>a,w</sup>	0.89 $\pm$ 0.04 <sup>a,w</sup>	0.76 $\pm$ 0.03 <sup>b,w</sup>	0.72 $\pm$ 0.03 <sup>a,w</sup>	0.69 $\pm$ 0.04 <sup>bc,x</sup>	0.73 $\pm$ 0.04 <sup>b,w</sup>	0.58 $\pm$ 0.02 <sup>c,x</sup>
<b>Saturates</b>	36.85 $\pm$ 0.26 <sup>b,w</sup>	25.46 $\pm$ 0.10 <sup>d,w</sup>	30.92 $\pm$ 0.59 <sup>c,w</sup>	48.53 $\pm$ 0.26 <sup>a,w</sup>	31.76 $\pm$ 0.77 <sup>b,x</sup>	20.03 $\pm$ 0.64 <sup>d,x</sup>	27.12 $\pm$ 0.28 <sup>c,x</sup>	44.72 $\pm$ 0.07 <sup>a,x</sup>
C14:1	1.81 $\pm$ 0.20 <sup>a,w</sup>	0.49 $\pm$ 0.03 <sup>b,x</sup>	0.43 $\pm$ 0.03 <sup>b,w</sup>	0.47 $\pm$ 0.02 <sup>b,w</sup>	0.48 $\pm$ 0.04 <sup>b,x</sup>	0.87 $\pm$ 0.03 <sup>a,w</sup>	0.41 $\pm$ 0.02 <sup>b,w</sup>	0.36 $\pm$ 0.04 <sup>b,w</sup>
C15:1	0.21 $\pm$ 0.05 <sup>a,w</sup>	0.11 $\pm$ 0.01 <sup>a,w</sup>	ND	ND	0.26 $\pm$ 0.02 <sup>a,w</sup>	ND	ND	ND
C16:1	5.32 $\pm$ 0.20 <sup>a,x</sup>	3.53 $\pm$ 0.09 <sup>b,w</sup>	4.20 $\pm$ 0.25 <sup>b,w</sup>	4.17 $\pm$ 0.20 <sup>b,w</sup>	7.24 $\pm$ 0.26 <sup>a,w</sup>	3.66 $\pm$ 0.07 <sup>c,w</sup>	4.45 $\pm$ 0.08 <sup>b,w</sup>	4.34 $\pm$ 0.06 <sup>b,w</sup>
C17:1	0.630 $\pm$ 0.07 <sup>a,w</sup>	0.410 $\pm$ 0.02 <sup>b,w</sup>	0.470 $\pm$ 0.03 <sup>ab,w</sup>	0.340 $\pm$ 0.03 <sup>ab,w</sup>	0.70 $\pm$ 0.03 <sup>b,w</sup>	1.20 $\pm$ 0.04 <sup>a,x</sup>	0.52 $\pm$ 0.04 <sup>c,w</sup>	0.32 $\pm$ 0.03 <sup>d,w</sup>
C18:1c	21.48 $\pm$ 0.49 <sup>c,w</sup>	26.99 $\pm$ 1.00 <sup>a,w</sup>	24.27 $\pm$ 0.70 <sup>b,w</sup>	23.63 $\pm$ 0.40 <sup>b,w</sup>	21.63 $\pm$ 0.30 <sup>c,w</sup>	27.04 $\pm$ 0.09 <sup>a,w</sup>	24.96 $\pm$ 0.60 <sup>b,w</sup>	24.18 $\pm$ 0.20 <sup>b,w</sup>
C20:1	2.07 $\pm$ 0.10 <sup>a,w</sup>	1.58 $\pm$ 0.09 <sup>b,x</sup>	1.24 $\pm$ 0.06 <sup>c,w</sup>	1.21 $\pm$ 0.05 <sup>c,w</sup>	1.05 $\pm$ 0.04 <sup>c,x</sup>	2.09 $\pm$ 0.04 <sup>a,w</sup>	1.28 $\pm$ 0.03 <sup>b,w</sup>	1.22 $\pm$ 0.03 <sup>b,w</sup>
<b>Monoenes</b>	31.51 $\pm$ 0.37 <sup>ab,w</sup>	33.11 $\pm$ 0.82 <sup>a,w</sup>	30.61 $\pm$ 0.45 <sup>b,w</sup>	29.82 $\pm$ 0.10 <sup>b,x</sup>	31.36 $\pm$ 0.03 <sup>b,w</sup>	34.86 $\pm$ 0.01 <sup>a,w</sup>	31.62 $\pm$ 0.43 <sup>c,w</sup>	30.42 $\pm$ 0.16 <sup>d,w</sup>
C18:2c	3.30 $\pm$ 0.35 <sup>d,x</sup>	36.75 $\pm$ 0.40 <sup>a,x</sup>	22.02 $\pm$ 0.50 <sup>b,x</sup>	16.38 $\pm$ 0.40 <sup>c,x</sup>	5.56 $\pm$ 0.05 <sup>d,w</sup>	39.60 $\pm$ 0.70 <sup>a,w</sup>	23.79 $\pm$ 0.40 <sup>b,w</sup>	18.29 $\pm$ 0.20 <sup>c,w</sup>
C20:2	1.06 $\pm$ 0.08 <sup>a,x</sup>	1.05 $\pm$ 0.04 <sup>a,x</sup>	1.06 $\pm$ 0.06 <sup>a,w</sup>	1.23 $\pm$ 0.10 <sup>w</sup>	2.01 $\pm$ 0.07 <sup>a,w</sup>	1.24 $\pm$ 0.04 <sup>b,w</sup>	1.25 $\pm$ 0.06 <sup>b,w</sup>	1.39 $\pm$ 0.07 <sup>b,w</sup>
<b>n<sub>6</sub>HUFA</b>	4.36 $\pm$ 0.43 <sup>d,x</sup>	37.80 $\pm$ 0.36 <sup>a,x</sup>	23.08 $\pm$ 0.44 <sup>b,x</sup>	17.61 $\pm$ 0.30 <sup>c,x</sup>	7.57 $\pm$ 0.01 <sup>d,w</sup>	40.84 $\pm$ 0.65 <sup>a,w</sup>	25.04 $\pm$ 0.34 <sup>b,w</sup>	19.68 $\pm$ 0.13 <sup>c,w</sup>
C18:3 ω 3	1.51 $\pm$ 0.07 <sup>a,w</sup>	0.40 $\pm$ 0.02 <sup>a,x</sup>	0.59 $\pm$ 0.05 <sup>c,w</sup>	0.77 $\pm$ 0.03 <sup>b,x</sup>	1.72 $\pm$ 0.10 <sup>a,w</sup>	0.83 $\pm$ 0.04 <sup>b,w</sup>	0.66 $\pm$ 0.02 <sup>b,w</sup>	0.53 $\pm$ 0.04 <sup>c,w</sup>
C20:3ω3	3.57 $\pm$ 0.40 <sup>a,x</sup>	0.190 $\pm$ 0.03 <sup>c,x</sup>	2.68 $\pm$ 0.08 <sup>b,x</sup>	ND	5.01 $\pm$ 0.10 <sup>a,w</sup>	0.36 $\pm$ 0.04 <sup>c,w</sup>	2.98 $\pm$ 0.08 <sup>b,w</sup>	ND
C20:4	ND	0.22 $\pm$ 0.02 <sup>a,w</sup>	ND	ND	ND	ND	ND	ND
C20:5EPA	9.88 $\pm$ 0.80 <sup>a,w</sup>	0.83 $\pm$ 0.05 <sup>b,x</sup>	5.72 $\pm$ 0.30 <sup>b,w</sup>	1.01 $\pm$ 0.08 <sup>c,x</sup>	10.58 $\pm$ 0.48 <sup>a,w</sup>	1.01 $\pm$ 0.02 <sup>c,w</sup>	6.03 $\pm$ 0.10 <sup>b,w</sup>	1.51 $\pm$ 0.02 <sup>c,w</sup>
C22:6DAH	12.30 $\pm$ 0.33 <sup>a,w</sup>	2.08 $\pm$ 0.09 <sup>d,w</sup>	6.40 $\pm$ 0.4 <sup>b,w</sup>	2.34 $\pm$ 0.20 <sup>c,x</sup>	12.50 $\pm$ 0.40 <sup>a,w</sup>	2.09 $\pm$ 0.08 <sup>d,w</sup>	6.54 $\pm$ 0.20 <sup>b,w</sup>	3.16 $\pm$ 0.10 <sup>c,w</sup>
<b>n<sub>3</sub>HUFA</b>	27.26 $\pm$ 0.13 <sup>a,x</sup>	3.72 $\pm$ 0.05 <sup>d,x</sup>	15.39 $\pm$ 0.23 <sup>b,x</sup>	4.12 $\pm$ 0.15 <sup>c,x</sup>	29.81 $\pm$ 0.08 <sup>a,w</sup>	4.29 $\pm$ 0.06 <sup>d,w</sup>	16.21 $\pm$ 0.04 <sup>b,w</sup>	5.20 $\pm$ 0.04 <sup>c,w</sup>

Values in the same column with different superscripts are significantly different ( $P < 0.05$ ). The letters (a, b, c and d) represent the differences among lipid sources while the letters (w and x) represent the differences between water temperature.

much lower temperature (10 °C) and complete mortality occurred at 7 and 6 °C, respectively. In the meantime, the lowest LT<sub>50</sub> was recorded in CO and mix oil groups. The lowest TAD was also recorded in fish fed oil mix (8.25 °C), followed by CO (9 °C) and FO (10 °C).

#### 4. Discussion

The results of the present study revealed that fish fed plant oil-based diets (corn oil and oil mix) exhibited higher cold tolerance than fish fed fish oil-and COCO-based diets. This might have been due to the effect of dietary fatty acid contents which are well reflected in the whole body fatty acid profiles. UFA were highest in fish fed plant oil-based diets (corn oil and oil mix), particularly linoleic acid (50%). This finding may suggest that UFA induce high cell membrane fluidity, which is a key mechanism in the physiological compensation to cold shock in living organisms (Hazel and Williams, 1990). Similarly, Skuladottir et al. (1990) found that UFA: SFA ratio was higher in Atlantic salmon fed diets consisting of plant oils, which induced high membrane fluidity.

**Table 6**Mean percent mortality (mean  $\pm$  SE) of Nile tilapia (*O. niloticus*) fed different lipid sources and exposed to gradual decrease in water temperature.

Temperature (°C)	Lipid source			
	Fish oil	Corn oil	Oil mix	Coconut oil
25	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
16	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	33.13 $\pm$ 4.43
15	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	31.60 $\pm$ 2.27
12	22.79 $\pm$ 0.00	9.22 $\pm$ 3.03	0.00 $\pm$ 0.00	26.56 $\pm$ 0.00
10	24.67 $\pm$ 2.66	24.67 $\pm$ 2.66	22.79 $\pm$ 0.00	0.00 $\pm$ 0.00
9	22.50 $\pm$ 5.74	26.39 $\pm$ 5.09	31.60 $\pm$ 2.27	28.28 $\pm$ 2.43
8	24.67 $\pm$ 2.66	26.56 $\pm$ 0.00	43.55 $\pm$ 6.11	–
7	35.90 $\pm$ 4.70	24.02 $\pm$ 2.05	29.88 $\pm$ 4.70	–
6	–	24.67 $\pm$ 2.66	–	–

Least significance difference (LSD) for interaction = 7.05,  $P = 0.05$ . Percentage data were transformed by arcsine (angular) transformation.

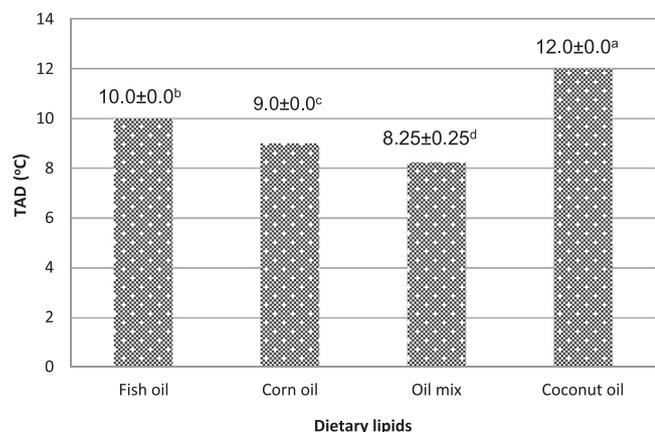


Fig. 1. Mean temperature (°C) at death (TAD) of Nile tilapia (*O. niloticus*) fed different lipids sources and subjected to gradual decrease in water temperature. Data are presented as mean ± SE. Data with different superscripts are significantly different ( $P < 0.05$ ).

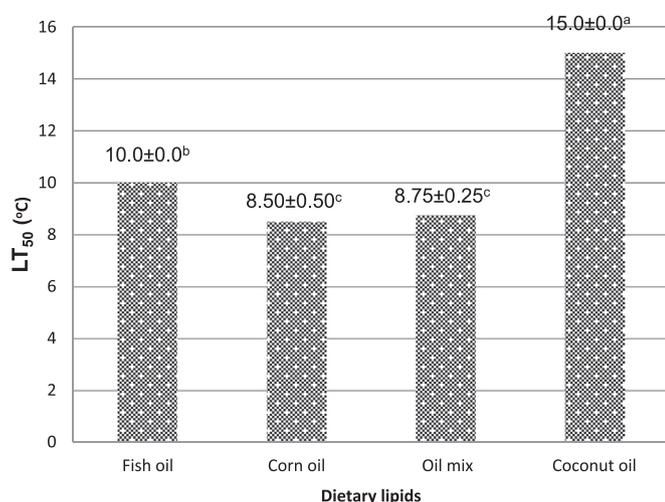


Fig. 2. Lethal temperature (°C) for 50% of fish (LT<sub>50</sub>) of Nile tilapia (*O. niloticus*) fed different lipids sources and subjected to gradual decrease in water temperature. Data are presented as mean ± SE. Data with different superscripts are significantly different ( $P < 0.05$ ).

lowest lethal temperature range (10–6 °C). As far as we know, the TAD reported in the present study is the lowest recorded value for the Egyptian Nile tilapia strain. Previous reports regarding lowest TAD of Nile tilapia indicate that they suffer mortality at 13–10 °C (Lahav, Ra'anana, 1998), 11–9 °C (Khater and Smitherman, 1988) and 11.7–7.5 °C (Charo-Karisa et al., 2005). Slightly better cold tolerance of 11–7.4 °C has been recorded for the Egyptian Nile tilapia strain reared in China (Sifa et al., 2002). This might be due to the fact that the further the geographic location from the equator, the more tolerant is Nile tilapia strains to cold (Sifa et al., 2002). Sifa et al. (2002) attributed this phenomenon to 'natural selection' processes.

Fish fed on coconut oil-based diet in the present study showed the lowest cold tolerance, presumably because coconut oil contains high levels of 12- and 14-carbon SFA (33%) and total SFA (58%). This may have led to membranes solidification; since lipids containing SFA solidify at higher temperatures than those containing UFA. Therefore, the high proportion of UFA increases the fluidity of the cell membranes (Steponkus et al., 1993). The present finding is consistent with the results of Kelly and Kohler (1999), who found that striped bass, white bass, and their hybrids fed on SFA-rich diets, followed by exposure to cold stress, displayed high mortality (50–90%), whereas no mortality was recorded in the groups fed on UFA-rich diets. Similar results were

also reported on red drum (*Sciaenops ocellatus*) juveniles (Craig et al., 1995). These studies demonstrated the role of dietary UFA sources in the induction of muscle fatty acid composition, and in turn, in the improvement of cold tolerance and lower lethal temperature, compared to SFA sources.

In another supportive study, juvenile Nile tilapia were fed five dietary lipid sources (fish, linseed, sunflower, olive and coconut oils) at optimal (28 °C) and suboptimal (22 °C) water temperatures (Corrêa et al., 2017). Feed efficiency and apparent net protein utilization increased as dietary unsaturated fatty acids, especially n-3 PUFA, increased. Fish fed on coconut oil exhibited the worst growth rates. At all dietary lipid sources, body PUFA concentration increased as water temperature decreased. The authors suggested that dietary lipid sources containing high PUFA and low SFA levels are necessary for the improvement of the growth and feed efficiency of Nile tilapia reared at suboptimal water temperatures.

Despite the significant effects of dietary lipid source on the response of Nile tilapia to cold stress in the present study, some other studies reported no effects of such lipid sources on cold tolerance of tilapia. For example, Atwood et al. (2003) found that when Nile tilapia were fed diets containing either menhaden oil (high in UFA) or coconut oil (high in SFA), their cold tolerance was not significantly affected. Similarly, cold tolerance and survival of hybrid tilapia (*O. niloticus* × *O. aureus*) were not significantly affected by dietary lipid sources (fish oil, palmitoleic oil, coconut oil or a mixture of fish oil and corn oil) (Hsieh et al., 2007). Results on another freshwater angelfish (*Pterophyllum scalare*) indicated that dietary plant oil sources did not induce cold resistance (Ikeda et al., 2011). These discrepancies could be attributed to fish species and size, dietary lipid levels and cold challenge protocol and duration.

The information on fatty acid requirements of tilapia is contradictory. Several studies indicated that tilapia require n-6 fatty acids rather than n-3 fatty acids for optimum growth and reproductive performance (Stickney et al., 1982; NRC, 1993; Lim et al., 2009). For example, the growth of Nile tilapia fed on a fish oil-containing diet (rich in n-3 fatty acids) was significantly reduced as compared with those fed soybean oil or corn oil diets (rich in n-6 fatty acids) (Takeuchi et al., 1983). Santiago and Reyes (1993) found also that Nile tilapia broodstock fed on fish oil-based diets had significantly lower reproductive performance than those fed soybean oil diets.

On the other hand, many other studies indicated that tilapia require both n-3 and n-6 fatty acids (Stickney and McGeachin, 1983; Chou and Shiau, 1999; Yildirim-Aksoy et al., 2007). In the present study, fish oil and fish oil: corn oil mix (1:1) produced the best performance. Similar results were reported by Yones et al. (2013), where fish oil and a mixture of fish oil, sunflower seed oil and cotton seed oil resulted in the best growth rates of Nile tilapia. The importance of dietary n-3 PUFA (from cod liver oil) for optimum growth of tilapia, at both optimal and suboptimal temperatures, was emphasized by Corrêa et al. (2018). These authors also highlighted the important role of dietary n-6 PUFA (from sunflower oil) at cold temperatures. They concluded that Nile tilapia requires dietary n-6 PUFA alone or a combination of n-6 and n-3 PUFA for optimum performance. It is evident from the above discussion that the requirements of tilapias for fatty acids are species specific, and more work is needed to quantify the fatty acids requirements of different tilapias under different culture systems and different water environments.

In conclusion, the present study indicated that fish oil- and oil mix-based diets resulted in the best growth performance and feed utilization of Nile tilapia. The best cold tolerance was achieved in fish fed plant oil diets (corn oil and oil mix). This study suggests that dietary plant oil or fish oil/plant oil mixture should be provided to Nile tilapia before overwintering.

## References

- AOAC (Association of Official Analytical Chemists), 1990. Official Methods of Analysis. AOAC, Arlington, Virginia.
- Atwood, H.L., Tomasso, J.R., Webb, K., Gatlin, D.M., 2003. Low-temperature tolerance of Nile tilapia (*Oreochromis niloticus*): effects of environmental and dietary factors. *Aquaculture* 34, 241–251.
- Bendiksen, E.Å., 2003. Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt (Ph.D. thesis). Norwegian University of Science and Technology.
- Cao-Hoang, L., Dumont, F., Marechal, P.-A., Gervais, P., 2010. Inactivation of *Escherichia coli* and *Lactobacillus plantarum* in relation to membrane permeabilization due to rapid chilling followed by cold storage. *Arch. Microbiol.* 192, 299–305.
- Charo-Karisa, H., Rezk, M.A., Bovenhuis, H., Komen, H., 2005. Heritability of cold tolerance in Nile tilapia (*Oreochromis niloticus*) juveniles. *Aquaculture* 249, 115–123.
- Chou, B.S., Shiau, S.Y., 1999. Both n-6 and n-3 fatty acids are required for maximal growth of juvenile hybrid tilapia. *Aquaculture* 61, 13–20.
- Craig, S.R., Neill, W.H., Gatlin, D.M., 1995. Effects of dietary lipid and environmental salinity on growth, body composition, and cold tolerance of juvenile red drum (*Sciaenops ocellatus*). *J. Fish. Physiol. Biochem.* 14, 49–61.
- Corrêa, 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, 1016–1026.
- Corrêa, 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.
- FAO (Food and Agriculture Organization of the United Nations), 2018. Global Aquaculture Production 1950-2016. <<http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en>>.
- Farkas, T., Csengeri, I., 1976. Biosynthesis of fatty acids by the carp, *Cyprinus carpio* L., in relation to environmental temperature. *J. Lipids* 11, 401–407.
- Hassan, B., El-Salhia, M., Khalifa, A., Assem, H., Al Basomy, A., El-Sayed, M., 2013. Environmental isotonicity improves cold tolerance of Nile tilapia (*Oreochromis niloticus*) in Egypt. *Egypt. J. Aquat. Res.* 39, 59–65.
- Hazel, J.R., Williams, E.E., 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* 29, 167–227.
- Hsieh, S.L., Kuo, C.M., 2005. Stearoyl-CoA desaturase expression and fatty acid composition between milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon idella*) during cold acclimation. *Comp. Biochem. Physiol. Part B.* 141, 95–101.
- Hsieh, S., Hu, C., Hsu, Y., Hsieh, T., 2007. Influence of dietary lipids on the fatty acid composition and stearyl-CoA desaturase expression in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) under cold shock. *Comp. Biochem. Physiol. Part B.* 147, 438–444.
- Ikeda, A.K., Zuanon, J.A.S., Salario, A.L., Freitas, M.B.D., Pontes, M.D., Souza, L.S., Santos, M.V., 2011. Vegetable oil sources in diets for freshwater angelfish (*Pterophyllum scalare*, Cichlidae): growth and thermal tolerance. *J. Arq. Bras. De. Med. Veter.- e Zootec.* 63, 670–677.
- Kelly, A.M., Kohler, C.C., 1999. Cold tolerance and fatty acid composition of striped bass, white bass, and their hybrids. *North Am. J. Aquac.* 61, 278–285.
- Khater, A.A., Smitherman, R.O., 1988. Cold tolerance and growth of three strains of (*Oreochromis niloticus*). In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K., Maclean, J.L. (Eds.), The Second International Symposium on Tilapia in Aquaculture. ICLARM, Manila, Philippines, pp. 215–218.
- Lahav, E., Ra'anan, Z., 1998. Cold tolerance of genetically produced all-male tilapia hybrids (*Oreochromis aureus*). In: Fitzimmons, K. (Ed.), Tilapia Aquaculture, Proceedings from the Fourth International Symposium on Tilapia Aquaculture. Northeast Regional Agricultural Engineering Service (NRAES), Ithaca, NY, USA, pp. 662–670.
- Lin, Y.H., Shiau, S.Y., 2007. Effects of dietary blend of fish oil with corn oil on growth and non-specific immune responses of grouper, *Epinephelus malabaricus*. *Aquac. Nutr.* 13, 137–144.
- Lim, C., Yildirim-Aksoy, M., Klesius, P., 2009. Lipids and fatty acid requirements of tilapia – Dietary supplementation essential for health, reproduction. *Glob. Aquac. Advocate* 12, 86–87.
- NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC, pp. 114.
- Olsen, Y., 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? In: Arts, M.T., Wainman, B.C. (Eds.), Lipids in Freshwater Ecosystems. Springer-Verlag New York, Inc., New York, NY, pp. 161–202.
- Overgaard, J., MacMillan, H.A., 2017. The integrative physiology of insect chill tolerance. *Annu. Rev. Physiol.* 79, 187–208.
- Pernet, F., Tremblay, R., Comeau, L., Guderley, H., 2007. Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids. *J. Exp. Biol.* 210, 2999–3014.
- Santiago, C.B., Reyes, O., 1993. Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia (*Oreochromis niloticus*) (Linnaeus) broodstock. *J. Appl. Ichthyol.* 9, 33–40.
- Sifa, L., Chenhong, L., Dey, M., Galgalac, F., Dunham, R., 2002. Cold tolerance of three strains of Nile tilapia (*Oreochromis niloticus*) in China. *Aquaculture* 213, 123–129.
- Sinensky, M., 1974. Homoviscous adaptation – a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *PNAS (Proc. Natl. Acad. Sci. USA)* 71, 522–525.
- Skuladottir, G.V., Schiöth, H.B., Gudmundsdottir, E., Richards, B., Gardarsson, F., Jonsson, L., 1990. Fatty acid composition of muscle, heart and liver lipids in Atlantic salmon, *Salmo salar*, at extremely low environmental temperature. *Aquaculture* 84, 71–80.
- Snyder, R.J., Hennessey, T.M., 2004. Cold tolerance and homeoviscous adaptation in freshwater alewives (*Alosa pseudoharengus*). *J. Fish. Physiol. Biochem.* 29, 117–126.
- Steponkus, P.L., Uemura, M., Webb, M.S., 1993. A contrast of the cryostability of the plasma membrane of winter rye and spring oat-two species that widely differ in their freezing tolerance and plasma membrane lipid composition. In: Steponkus, P.L. (Ed.), Advances in Low-Temperature Biology. JAI Press, London, pp. 211–312.
- Stickney R.R. and McGeachin R.B., Effects of dietary lipid quality on growth and food conversion of tilapia. In: Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies, 1983, 37, pp. 352–357.
- Storelli, R., Acierio, R., Maffia, M., 1998. Membrane lipid and protein adaptations in Antarctic fish. In: Portnerand, H.O., Playle, R.C. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge, UK, pp. 166–189.
- Sun, L.T., Chen, G.R., Chang, C.F., 1992. The physiological-responses of tilapia exposed to low temperatures. *J. Therm. Biol.* 17, 149–153.
- Takeuchi, T., Satoh, S., Watanabe, T., 1983. Requirement of *Tilapia nilotica* for essential fatty acids. *Bull. Jpn. Soc. Sci. Fish.* 49, 1127–1134.
- Wallaert, C., Babin, P.J., 1994. Thermal adaptation affects the fatty acid composition of plasma phospholipids in trout. *J. Lipids* 29, 373–376.
- Yones, A.M., El-Saidy, D.M., Abdel-Hakim, N.F., 2013. Effects of fish oil substitution with vegetable oils in diets of juvenile Nile tilapia (*Oreochromis niloticus*) (L.) on growth performance, nutrients utilization and muscle fatty acids contents. *Merit Res. J. Food Sci. Technol.* 1, 9–18.
- Yildirim-Aksoy, M., Allen, L.D., Shelby, D.R., Klesius, P.H., 2007. Influence of dietary lipid sources on the growth performance, immune response and resistance of Nile tilapia (*Oreochromis niloticus*) to *Streptococcus iniae* challenge. *J. Appl. Aquac.* 19, 29–49.
- Zambiasi, R.C., Przybylski, R., Zambiasi, M.W., Mendonca, C.B., 2007. Fatty acid composition of vegetable oils and fats. *CEPPA Bull. Curitiba* 25, 111–120.