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Changes in the physiological parameters, fatty acid metabolism, and SCD activity and expression in juvenile GIFT tilapia (*Oreochromis niloticus*) reared at three different temperatures

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Abstract We evaluated the effects of rearing temperature on the composition of fatty acids and stearoyl-CoA desaturase (SCD) activity and gene expression in GIFT (genetically improved farmed tilapia) tilapia. Three triplicate groups of fish were reared for 40 days at 22, 28, or 34 °C. At the end of the trial, the final body weight of juveniles reared at 28 °C was higher than that of fish reared at 22 or 34 °C. Feed intake, feed efficiency, and the protein efficiency ratio were also higher at 28 °C. The fatty acid composition of muscle tissue differed significantly ($P < 0.05$) among the treatment groups. The content of SFA decreased with decreasing temperature, whereas the UFA content increased. We observed high levels of PUFA, particularly n-3 PUFAs, in fish reared at the lower temperature. Rearing at low temperature significantly ($P < 0.05$) increased the expression and activity of the SCD gene. Increased SCD activity and gene expression can increase the biosynthesis of MUFAs in GIFT tilapia muscle. Additionally, cold acclimation can decrease the content of TC and TG in GIFT tilapia, which can help increase cold tolerance.

Introduction

Fatty acids are the fundamental structural components of almost all forms of lipids, acting as precursors of bioactive molecules. Fatty acids have structural and functional roles and influence processes such as reproduction, osmoregulation, and the response to stress (Makoto et al. 1989; Patricia et al. 2014). Increased understanding of the nutritional importance of fatty acids has led to increased research into their use in animal nutrition (Guerreiro et al. 2012). As with other organisms, fatty acids play a key role in growth and reproduction in fish, promoting fat-soluble vitamin absorption and transportation in the body and supplying essential fatty acids (EFAs) (Yue et al. 2011). Some long-chain polyunsaturated fatty acids (LC-PUFAs) are precursors to class eicosane active substances and play an important role in nerve conduction and information delivery. Additionally, other LC-PUFAs are essential for fish growth and development, especially in marine fish and fish larvae (Yue et al. 2011; Mu and Wang 2003). Fish represent a

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valuable source of n-3 PUFAs for humans. Eilander et al. (2007) reported that n-3 PUFAs, such as DHA and EPA, play a significant role in preventing human cardiovascular disease and improving the development of the body's immune function and brain nervous system.

Environmental factors such as salinity, temperature, and seasons influence fatty acid regulation in fish (Douglas et al. 1994; Michel et al. 2002; Akhtara et al. 2014). Among these factors, water temperature plays a particularly important role in influencing fatty acid metabolism in fish. Because fish are ectothermic vertebrates, water temperature has an immense influence on fish growth, development, sex differentiation, metabolism, and immune responses (Logue et al. 2000). Optimal water temperatures facilitate the increased efficiency of physiological processes and biochemical reactions in order to help fish to adapt environmental temperature variations. The fatty acid composition of animal tissues can be modified by a change in environmental temperature. For example, exposure of fish to a short-term temperature stress, over a period of days or a few weeks, alters the content of saturated fatty acids (SFAs), triglycerides, neutral fats, and unsaturated fatty acids (UFAs) (Logue et al. 2000; Akhtara et al. 2014). Tibor and Istvan (1976) noted that carp (Cyprinus carpio L.) adjust their fatty acid metabolism to the prevailing temperature in such a manner that a decrease in the environmental temperature results in the accumulation of LC-PUFA and a decrease in SFAs. In common carp (Wodtke and Cossins 1991), rainbow trout Salmo gairdneri (Bowden et al. 1996), and milkfish Chanos chanos (Hsieh et al. 2002, 2003), UFA levels increase substantially in response to cold stress. Taken together, these observations suggest that UFAs play a significant role in the thermal adaptation of fish to fluctuating environmental conditions.

Fish regulate fatty acid metabolism and composition through a series of enzymatic cascades (Cheng et al. 2006). These enzymes include three types of fatty acid desaturases, Acyl-CoA, Acyl-lipid, and Acyl-ACP (Hsieh et al. 2001), which are known for their roles in the physiological functioning of cell membranes and fatty acid metabolism. Stearoyl-CoA desaturase (SCD, EC 1.14.99.5), an acyl-CoA desaturase (Hsieh et al. 2001), is a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs). This enzyme plays a central role in the regulation of fatty acid metabolism (Heinemann and Ozols 2003). Hsieh et al. (2003) demonstrated that the SCD and fatty acid composition of hepatic membranes changed significantly during cold acclimation in milkfish (C. chanos), a warm-water teleost fish. Additionally, the changes in MUFA levels were highly correlated with changes in SCD activity in milkfish hepatic microsomes. The activity and gene expression of SCD have been reported in a number of aquatic animals, including grass carp Ctenopharyngodon idella (Hsieh and Kuo 2005; Chang et al. 2001), common carp (Tiku et al. 1996), common octopus Octopus vulgaris (Monroig et al. 2012), hybrid tilapia Oreochromis niloticus × O. aureus (Hsieh et al. 2007), and the sea urchin Strongylocentrotus intermedius (Ding et al. 2012). SCD gene cloning and analysis in tilapia were first performed by Hsieh et al. (2004), who also studied hormonal effects on SCD activity and fatty acid composition in tilapia. Subsequently, the authors reported the influence of dietary lipids on the fatty acid composition and stearoyl-CoA desaturase expression in hybrid tilapia exposed to cold shock (Hsieh et al. 2007).

GIFT tilapia, a tropical species, are suitable for culture in warm waters. However, exposure to cold stress can cause mass mortality and, subsequently, economic loss (Huang. 2008). SCD can influence the saturation of fatty acids at different temperatures, thereby maintaining cell membrane fluidity and cell function (Zhang et al. 2013). Increased understanding of the properties of SCD may lead to methods to improve the cold tolerance of GIFT tilapia. To the best of our knowledge, there have been no studies on the effects of temperature on the regulation of SCD in GIFT tilapia. Our objective was to evaluate the effect of three different water temperatures (22, 28, and 34 °C) on the growth performance, blood parameters, fatty acid composition, and SCD activity and gene expression in GIFT tilapia juveniles. Our results may also provide a theoretical and experimental basis for feeding GIFT tilapia that result in increased health benefits to humans.

Materials and methods

Source of experimental fish

Two hundred healthy juvenile GIFT tilapia were obtained from Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences, Yixing.

Prior to the experiments, the fish were acclimated for 10 days in indoor plastic containers at 28 °C water temperature. During acclimation, the photoperiod was set at a 12-h light/dark cycle, and dissolved oxygen was kept near saturation. Additionally, the fish were conditioned to accept floating feed (crude protein 28 %; crude lipid 6 %).

Experimental management

The experiment began in May and lasted for 40 days. At the beginning of the experiment, each of the nine plastic 480-L tanks was filled with 450 L tap water and aerated for three consecutive days. Each tank was equipped with a heating device (Zhongkehai Recirculation Aquaculture Systems Co., Ltd, Qingdao, China) to maintain the preselected water temperature (22 ± 0.3, 28 ± 0.3, or 34 ± 0.3 °C). After 10 days acclimation, healthy GIFT tilapia fish (mean weight of 33.57 ± 0.11 g) were randomly assigned into one of three groups (22, 28, or 34 °C) and stocked into one of the nine tanks (three tanks/treatment group) at a density of 18 fish/480-L plastic tank. There was no significant difference in initial fish weight between the various experimental groups or between all replicates (ANOVA; $P > 0.05$). During the entire experiment, the dissolved oxygen was kept near saturation and feces on the bottom of the plastic tanks were siphoned off daily. Additionally, one-third of the water volume was replaced every 3 days, resulting in a temperature change of < 0.3 °C. During the experiment, water temperature and pH were monitored daily, while dissolved oxygen, ammonia-N, and nitrite were measured weekly. The pH ranged from 7.4 to 7.8, and ammonia-N and nitrite were both maintained at concentrations lower than 0.01 mg L^{-1}. The photoperiod was maintained on a 12-h light/dark cycle.

During the trial, fish were hand-fed twice a day (08:00 and 16:00 h) with floating feed (NingboTech-Bank Co., Ltd, Yuyao, China; crude protein 28 %; crude lipid 6 %; crude fiber 15 %; crude ash 18 %; moisture 12 %; calcium 1 %; total phosphorus 1 %; NaCl 3 %; lysine 1.3 %). The fish were fed 6 % of their body weight daily. During the experiment, care was taken to avoid feed losses. Prior to sampling, fish were deprived of food for 1 day. At the end of the experiment, all fish were weighed and three fish from each tank were randomly sampled to determine the hepatosomatic indices. To minimize the stress associated with handling, another three fish from each tank were sedated with an overdose of tricaine methanesulfonate (2 %; MS-222; Suzhou Sciyoung BioMedicine Technology Co., Ltd, Suzhou, China) within 1 min after capture. The livers were dissected from these fish and frozen at −80 °C until analysis for enzymatic activity and RNA. The liver tissue from each individual was divided into two subsamples. One subsample was used for the determination of enzymes and liver glycogen. The second subsample was later used to measure SCD gene expression. Additionally, we collected a sample of muscle tissue from three fish from each treatment group. The muscle tissue was divided into two portions: One was blended for fatty acid composition and the other was used to determine crude fat and crude protein. We collected a caudal vein blood sample from four fish per tank using a heparinized syringe (Changzhou Yuekang Medical Appliance Co., Ltd, Changzhou, China) and divided the sample into two aliquots. The first was immediately centrifuged and the serum frozen at −20 °C until analysis. The second was used to test blood parameters.

Analytical methods

Growth analysis and determination of muscle composition

We calculated a variety of growth indices, including growth rate, feed conversion rate (FCR), protein efficiency ratio (PER), specific growth rate (SGR), hepatopancreas somatic index (HSI), and visceral somatic index (VSI) using the following formulae:

Growth rate (%) = 100 × (final weight − initial weight)/(initial weight);

FCR = (Fish feed intake)/(final weight − initial weight);

PER = (final weight − initial weight)/(feed protein intake);

SGR = 100 × [(In (final weight) − In (initial weight))/days];

HSI(%) = (liver weight × 100)/(final weight);

VSI(%) = (visceral weight × 100)/(final weight).

Analysis of blood sample collection

Blood cell counts were calculated using the methods described in Qiang et al. (2013). To determine the total

white blood cell (WBC) count, a 1 in 100 dilution of the blood was made in phosphate saline buffer (PBS, 0.02 M, pH 7.3). Similarly, the blood was diluted 1 in 1000 to count the red blood cells (RBC). Counts were carried out using a Neubauer haemocytometer (Hawksley & Son Ltd, England) and were expressed as cells μL^{-1}. The concentration of hemoglobin (Hb) was determined colorimetrically by measuring the formation of cyanomethemoglobin following the method of Van Kampen and Zijlstra (1961).

The concentration of total protein (TP), triglyceride (TG), total cholesterol (TC), glucose (GLU), and high-density lipoprotein (HDL-G) and the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed in serum obtained by centrifuging the blood sample (5000 rpm, 15 min). These indices were all measured using an automatic biochemical analyzer (Mindray, BS-400, Shenzhen, China). Test kits were all purchased from Shenzhen Mindray Bio-Medical Electronics Co. Ltd (Shenzhen, China).

Lipid extraction and fatty acid analysis

Total lipid was extracted following the method of Folch et al. (1957) using a chloroform/methanol mixture (2:1). Samples were stored at −80 °C prior to analysis. Three aliquots from each sample were used for fatty acid analysis. The fatty acid methyl esters (FAMEs) were prepared according to Ren et al. (2012) by methylating in 1 % sulfuric acid in methanol at 70 °C for 3 h to prepare fatty acid methyl esters (FAMEs). FAMEs were extracted in heptanes and analyzed using a gas chromatograph (GC-2010, Shimadzu, Japan) equipped with an auto-sampler and a hydrogen flame ionization detector. The GC was fitted with a 30 m × 0.25 mm × 0.25 μm capillary column (VF-23 ms, Varian, Salt Lake City, Utah, USA). The carrier gas was N_2 and the combustion-supporting gases were air and H_2. The injector and detector temperatures were both 250 °C. The column temperature was initially held at 120 °C for 3 min,

followed by an increase at a rate of 10 °C/min to 190 °C. The rate then increased to 2 °C/min to a final temperature of 220 °C, which was maintained for 15 min. Individual fatty acids were identified by comparison against commercial standards (Sigma, USA) and quantified using the CLASS-GC10 GC workstation (Shimadzu, Japan). All lipids were extracted from muscle tissue.

Hepatic glycogen and SCD activity

To determine the hepatic glycogen concentration and SCD activity, one portion of the liver sample from each individual was homogenized (dilution 1/9) in ice-cold buffer (0.02 M Tris; 0.25 M sucrose; 2 mM ethylenediaminetetraacetic acid (EDTA)) and centrifuged for 15 min (12,000 rpm, 4 °C). The supernatant was then removed and used for analysis. All test kits were purchased from Shanghai Lengton Bio-science Co., Ltd (Shanghai, China). Hepatic glycogen content was determined as described by Plummer (1987). The SCD content was determined by enzyme-linked immunosorbent assay (ELISA) (BioTek Eon, Vemont, USA).

Real-time quantitative PCR

Gene expression levels were determined by real-time quantitative RT-PCR. Primers for RT-PCR were designed with reference to the known sequences for tilapia (Table 1). All primers were synthesized by Shanghai Genecore, BioScience & Technology Company (Shanghai, China). The PCR products were 100–110 bp long.

Total RNA was extracted from the liver using Trizol reagent (Dalian Takara Co. Ltd, Dalian, China). RNA samples were treated with diethyl pyrocarbonate (DEPC)-treated water (Dalian Takara Co. Ltd). cDNA was generated from 350 ng RNA using a Prime-Script RT reagent kit (Dalian Takara Co. Ltd), a PCR system (ABI, Foster City, California, USA), and a SYBR

Table 1 Primer sequences	Target mRNA	Sequence	NCBI GenBank accession no
	SCD	F: 5′–ACAAGCTCTCCGTGCTGGTCAT–3′	AY150696.1
		R: 3′–GCAGAGTTGGGACGAAGTAGGC–5′	
	β-actin	F: 5′–CCACACAGTGCCCATCTACGA–3′	EU887951.1
SCD stearoyl-CoA desaturase		R: 3′–CCACGCTCTGTCAGGATCTTCA–5′	

Green PCR Master Mix (ABI), following the manufacturer's instruction. RT-PCR was performed as follows: denaturation at 95 °C for 5 min; 40 cycles of denaturation at 95 °C for 15 s; annealing at 59 °C for 60 s. The relative quantification of the target gene transcript SCD was calculated with a chosen reference gene transcript (β-actin) using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). This mathematical algorithm computes an expression ratio based on real-time PCR efficiency and the crossing point deviation of the sample versus a control. PCR efficiency was measured by constructing a standard curve using a serial dilution of cDNA. No-template control (NTC) and no-reverse transcriptase control (NRT) were used as controls for template and genomic contamination, respectively. Values for SCD mRNA were detected using a homogenization procedure and assigned the maximum value of 1.

Statistical analysis

Differences between groups were analyzed using one-way ANOVA followed by Duncan's multiple comparison test. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Effect of water temperature on growth and muscle composition

No mortality was observed throughout the experiment. At the end of the experiment, the biological indices differed between the three temperature treatment groups (Table 2). Final body weights were significantly ($P < 0.05$) greater in fish reared at 28 and 34 °C than at 22 °C; final body weight was higher at 28 °C than at 34 °C. The differences in SGR between groups were similar to those for final body weight. Rearing temperature also had a significant effect ($P < 0.05$) on FCR, PER, HSI, and VSI. The FCR was significantly ($P < 0.05$) lower at 28 °C than at 22 and 34 °C, and the FCR was higher at 34 °C than at 22 °C. The pattern of differences in PER was opposite to that of FCR. PER was significantly ($P < 0.05$) higher at 28 °C than at 22 and 34 °C and was higher at 22 °C than at 34 °C. The HSI was significantly lower at 28 °C than at 22 and 34 °C. The VSI was significantly ($P < 0.05$) higher at 28 °C than at 34 °C, but not 22 °C.

We measured the levels of crude lipid and crude protein in tilapia that were reared at three different

Table 2 Growth performance and the indices of hepatosomatic and viscera of juvenile GIFT tilapia

Temperature	22 °C	28 °C	34 °C
Initial body weight (IBW) (g)	33.63 ± 0.10	33.50 ± 0.13	33.56 ± 0.11
Final body weight (FBW) (g)	75.04 ± 0.46[a]	88.43 ± 3.15[b]	87.70 ± 1.85[b]
SGR (%/d)[a]	2.01 ± 0.01[a]	2.42 ± 0.10[b]	2.40 ± 0.06[b]
FCR[b]	1.08 ± 0.06[a,b]	1.06 ± 0.03[a]	1.16 ± 0.04[b]
PER[c]	3.29 ± 0.17[a,b]	3.36 ± 0.10[b]	3.06 ± 0.09[a]
HSI (%)[d]	1.68 ± 0.55[a]	1.52 ± 0.56[b]	1.65 ± 0.51[a]
VSI (%)[e]	11.87 ± 2.34[a,b]	12.39 ± 1.43[b]	10.82 ± 1.96[a]
Liver glucose	39.09 ± 14.47	41.56 ± 8.37	34.77 ± 9.65
Muscle composition (%)			
Crude fat	1.07 ± 0.13	1.14 ± 0.17	1.05 ± 0.14
Crude protein	18.26 ± 2.01[b]	19.32 ± 1.75[b]	15.54 ± 1.62[a]

Means in the same column which have different letters on the top right have significant difference ($P < 0.05$). All data are presented as means ± standard error

[a] SGR specific growth rate: $100 \times [(\text{In (final weight)} - \text{In (initial weight)})/\text{days}]$

[b] FCR feed conversion rate: (fish feed intake)/(final weight − initial weight)

[c] PER protein efficiency ratio: (final weight − initial weight)/(feed protein intake)

[d] HSI hepatopancreas somatic index: (liver weight × 100)/(final weight)

[e] VSI visceral somatic index: (visceral weight × 100)/(final weight)

temperatures. Crude protein (n × 6.25) was measured by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganas, Sweden). Crude lipid was measured using the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). There was no significant ($P < 0.05$) difference in the content of crude lipid between the three treatment groups. Conversely, there was a significant ($P < 0.05$) difference in the content of crude protein. The content of crude protein was significantly lower in fish reared at 22 °C ($P < 0.05$) than at the two higher temperatures (Table 2).

Effect of water temperature on blood parameters of GIFT tilapia juveniles

The RBC, WBC, Hb, and Hct values in GIFT tilapia juveniles from the three treatment groups are given in Table 3. The RBC and WBC counts were significantly ($P < 0.05$) higher at 28 and 34 °C than at 22 °C. RBC counts peaked at 28 °C (2.09×10^6 μL^{-1}) and were lowest at 22 °C (1.89×10^6 μL^{-1}). The WBC counts increased with increasing temperature and were highest at 34 °C. The pattern of differences in Hct between groups was similar to that in RBC, but the difference was not significant between the groups reared at 22 and 34 °C. The Hb was significantly higher ($P < 0.01$) at 28 and 34 °C than at 22 °C and was higher at 34 °C than at 28 °C.

Effect of water temperature on biochemical parameters in serum

There was no significant difference in TG ($P > 0.05$) between the three treatment groups, though levels did increase with increasing temperature. The pattern of differences in TP and TC was similar to that in TG,

though TC levels were significantly higher at 34 °C ($P < 0.05$) than at the other two temperatures. The levels of ALT, AST, and GLU were lowest at 28 °C and were significantly ($P < 0.05$) higher at 34 °C.

Effect of water temperature on fatty acid composition

We documented 21 fatty acids in the muscle of GIFT tilapia, of which there were six SFAs, three MUFAs, and 12 PUFAs. There was a significant difference ($P < 0.05$) in muscle fatty acid composition between the three treatment groups (Table 5). The most common fatty acids in all groups were C16:0, C18:0, C18:1, C18:2, C20:4, and C20:6. There was no significant difference between groups in total MUFA, the ratio of MUFA/SFA, or the ratio of n-3 to n-6 PUFA. However, the total SFA and PUFA were significantly higher ($P < 0.05$) at 34 °C than at 22 and 28 °C.

The total fatty acid extracted from the muscle of cold-adapted fish contained more arachidonic acid (ArA, 20:4n-6), eicosapentaenoic acid (EPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) than from the other two treatment groups. However, there was no difference in the levels of stearic acid (18:0) and oleic acid (18:1) between the three groups. The concentration of stearic acid tended to decrease with increasing temperature, and there was a negative correlation between the levels of C18:1 and C18:0. The level of palmitic acid (16:0) and palmitoleic acid increased with increasing temperature and was significantly ($P < 0.05$) higher at 34 °C than at the other temperatures. The concentration of n-3 PUFAs decreased as temperature increased, whereas there was no obvious difference in the levels of n-6 PUFAs between groups (Table 5). The EFA content decreased as temperature increased.

Table 3 Blood parameters of GIFT tilapia juveniles in different temperatures

Temperature	22 °C	28 °C	34 °C
RBC (× 10^{12}/L)	189.45 ± 13.67[a]	209.29 ± 22.33[b]	206.33 ± 19.41[b]
WBC (× 10^9/L)	2.02 ± 0.20[a]	2.34 ± 0.36[b]	2.35 ± 0.45[b]
Hb (g/L)	95.17 ± 11.34[a]	107.58 ± 15.91[b]	115.19 ± 10.93[b]
Hct (%)	37.90 ± 7.73[a]	48.49 ± 8.70[b]	45.15 ± 10.39[ab]

Means in the same column which have different letters on the top right have significant difference ($P < 0.05$). All data are presented as means ± standard error

Effect of water temperature on the activity and mRNA levels of SCD

SCD activity and mRNA levels were significantly ($P < 0.05$) influenced by water temperature, both decreasing as temperature increased. SCD activity was higher in fish reared at 22 °C than at 28 or 34 °C. The change in SCD activity mirrored the changes in SCD mRNA expression. mRNA expression was significantly ($P < 0.05$) lower in fish reared at 34 °C than at the other two temperatures and increased as temperature decreased.

Discussion

Effect of water temperature on growth and muscle composition

Changes in temperature resulted in significantly different growth rates, as is expected in poikilothermic organisms. Previous research suggests that the optimum temperature for growth in GIFT tilapia juveniles is 28 °C (Qiang et al. 2012). This is consistent with our observation of highest growth in fish reared at 28 °C. A similar effect of temperature on the growth of this species was also observed by Likongwe et al. (1996) and Azaza et al. (2008). Elliot (1972) speculated that higher temperatures promote gastric evacuation, thus leading to poor feed utilization and inhibited growth. In the present experiment, we observed maximum growth in GIFT tilapia at 28 °C corresponding with maximum SGR and PER and lower FCR (Table 2). Musuka et al. (2009) concluded that higher temperatures can lead to increased food intake and feed utilization and, in turn, to more efficient intestinal digestion and absorption. In our study tilapia, the FCR was lowest at 28 °C and highest at 34 °C, whereas SGR was highest at 28 °C. This is consistent with the efficient use of energy at lower temperatures (Jorge et al. 2013). Azaza et al. (2008) confirmed that such a depression of growth is because of the higher energy cost for maintenance metabolism and occurs primarily because of a loss of appetite when the experimental temperature reaches the upper extreme of the tolerance range. Additionally, the significant reduction we observed in muscle crude lipid content at high temperatures is consistent with previous research into the effect of stocking density, whereby there is high lipid utilization at high stocking densities to satisfy energy demands (Li et al. 2010).

The HSI and VSI can be used to indicate the health status and quality of fish. A smaller VSI is associated with an increase in the edible proportion of an individual (Wei et al. 2014). However, the VSI was significantly higher at 28 °C ($P < 0.05$) than at the other two temperatures. Unfortunately, our experiment was conducted during the breeding season. Thus, the increase in VSI was likely due to development of their gonads, particularly in tilapia that were reared at the optimum water temperature (Dzikowski et al. 2001). Water temperature can significantly affect the HSI in fish. In our study, the HSI was significantly ($P < 0.05$) lower at 28 °C than at 22 and 34 °C. Deviating from the optimum water temperature can result in high liver load conditions for fish (Du et al. 2002).

Effect of temperature on blood parameters of GIFT tilapia juveniles

The composition of fish blood is influenced by metabolism, nutritional status, and diseases (He et al. 2007). Changes in the levels of blood parameters such as RBC, WBC, Hb, and Hct give an insight into the health status of an individual (Harikrishnan et al. 2011). RBC affect transportation of O_2 and CO_2. Hb is a protein that is responsible for carrying oxygen in the fish body, so its concentration is closely correlated with RBC counts (Clark et al. 1979). In this study, RBC counts and Hct were highest in fish reared at 28 °C. Conversely, the concentration of Hb tended to increase as temperature increased. Both the PER and SGR were higher at 28 °C than at the other two temperatures. We speculate that at the optimum temperature, food intake is increased and the metabolic capacity is higher. Thus, the oxygen demand increases, resulting in an increase in the RBC (Zhi et al. 2008). This is consistent with Zhang (1991a, b), who reports that Hb concentrations increased with an increase in temperature. In contrast, Qiang et al. (2013) reported that RBC counts and Hb concentration peaked at 28 °C and decreased thereafter. In the present study, the concentration of Hb was highest at 34 °C. This difference may be a function of other factors such as size and nutritional status. WBC play a role in the immune system. Thus, changes in WBC counts can be used to detect diseases and/or injury in

Table 4 Indices of serum biochemistry in different temperatures

	22 °C	28 °C	34 °C
ALT (U/L)	16.45 ± 13.18^a	11.22 ± 4.46^a	28.86 ± 13.18^b
AST (U/L)	25.84 ± 13.89^a	23.66 ± 11.66^a	86.82 ± 23.83^b
TP (g/L)	35.68 ± 5.06	37.13 ± 5.67	38.46 ± 4.12
TC (g/L)	4.74 ± 1.37^a	4.92 ± 1.40^a	7.69 ± 1.63^b
TG (g/L)	1.71 ± 1.13	2.09 ± 2.44	2.78 ± 1.17
HDL-G (mmol/L)	2.64 ± 0.45^a	2.71 ± 0.40^a	3.18 ± 0.30^b
GLU (mmol/L)	3.19 ± 1.29^a	2.98 ± 0.92^a	6.99 ± 1.05^b

Means in the same column which have different letters on the top right have significant difference ($P < 0.05$). All data are presented as means ± standard error. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *TP* total protein, *TC* total cholesterol, *TG* triglyceride, *HDL-G* high-density lipoprotein, *GLU* glucose

fish (Ellis 1981). The WBC count was highest at 28 °C, which is consistent with observations by Qiang et al. (2013) and Slater and Schreck (1998). The increase in WBC at this temperature may explain the increased disease resistance in tilapia that are reared at the optimal temperature. Taken together, these observations are further evidence that the optimum temperature for GIFT tilapia is 28 °C because both growth and immunity are maximized.

Lipids are transferred throughout the fish body in the serum. Thus, serum lipid levels reflect the status of body lipid metabolism (Hiraoka et al. 1979). Previous research has indicated that the TC and TG content reflect the absorption of lipid (He et al. 2007). In our study, TC and TG content were highest in GIFT tilapia juveniles reared at 34 °C, suggesting that higher temperatures are not good for the absorption of lipids in tilapia. Instead, lipids are deposited in the body, which can lead to a fatty liver (Cheng et al. 2006). Guerreiro et al. (2012) noted that the lipid content of juvenile Senegalese sole *Solea* increased when the water temperature was raised from 16 to 22 °C. Singh et al. (2009) observed a similar pattern in African catfish, *Clarias batrachus*. The content of TG declined at lower temperatures, potentially because of an increase in oxidation of fatty acids to supply energy (Wu et al. 2000). Some studies have noted that increased TG content is indicative of fat accumulation in the liver and leads to a fatty liver or hypertrophy of the liver (Lu et al. 2010). This explains why the HSI was higher at high temperature. Cholesterol exists in lipoprotein in the blood (Cui et al. 2013). HDL-G can transfer the cholesterol away from artery walls to metabolize in liver (Xiang et al. 2010). The changes in TC and HDL-G were consistent, increasing with the

temperature increase. It is also a possible reason that leads to a higher HSI in high temperature.

Transaminase plays an important role in the metabolism of amino acids and the conversion of proteins, fats, and carbohydrates. ALT and AST are the two primary transaminases (Xiang et al. 2010). Generally, an increase in the activity of AST is indicative of a heart or muscle disorder. If ALT activity is increased in the liver, it may be a sign of injury (Han et al. 2010). Our results suggest the liver was in a high load condition in fish that were reared at the high or low temperature. Both ALT and AST were lowest in fish reared at 28 °C and highest at 34 °C (Table 4). Rearing at high or low temperature may lead to liver tissue injury, resulting in leakage of ALT from injured liver cells to the blood and an increase in serum ALT content (Zhang 1991a, b). GLU is the most important energy substrate in the blood and a direct source of energy for a range of activities in fish. There is a close relationship between GLU levels and liver glycogen catabolism (Zhu and Shen 2002). In the present study, GLU levels were lower at 28 °C than at the higher and lower temperatures, whereas liver glycogen was highest at 28 °C. This indicates that the fish use glycogen to provide energy when reared at low or high temperatures (Carine et al. 2004; Chatzifotis et al. 2010; Table 5).

Effect of temperature on fatty acid composition

Many aquatic organisms are adapted to cope with variation in temperature, allowing physiological processes and biochemical reactions to proceed efficiently at different temperatures (Brett et al. 2006). Ectotherms typically store lipid in the liver and muscle

Table 5 Fatty acid composition of muscle in different temperatures (% of total FAME)

	22 °C	28 °C	34 °C
c14:0	1.21 ± 0.30[a]	1.32 ± 0.25[a]	1.84 ± 0.08[b]
c15:0	0.23 ± 0.01[a]	0.26 ± 0.01[b]	0.25 ± 0.01[a,b]
c16:0	19.43 ± 1.33[a]	19.85 ± 0.48[a]	22.14 ± 0.82[b]
c17:0	0.34 ± 0.04	0.35 ± 0.03	0.32 ± 0.01
c18:0	9.19 ± 0.53	8.61 ± 0.31	8.57 ± 0.37
c20:0	0.21 ± 0.01	0.21 ± 0.02	0.18 ± 0.01
ΣSFA	30.52 ± 1.05[a]	30.59 ± 0.12[a]	33.30 ± 0.87[b]
c16:1	1.76 ± 0.27[a]	1.57 ± 0.16[a]	2.21 ± 0.15[b]
c18:1	17.66 ± 1.40	17.81 ± 1.03	19.79 ± 0.54
c20:1	0.92 ± 0.07	0.84 ± 0.10	0.79 ± 0.07
ΣMUFA	20.34 ± 1.71	20.23 ± 1.24	22.79 ± 0.73
c18:2n-6	20.27 ± 1.26[a]	22.76 ± 0.21[b]	20.08 ± 0.45[a]
c18:3n-3	0.58 ± 0.12	0.51 ± 0.09	0.62 ± 0.04
c18:3n-6	1.60 ± 0.13[a]	1.88 ± 0.13[b]	1.71 ± 0.05[a,b]
c20:2n-6	1.16 ± 0.11	1.14 ± 0.06	0.97 ± 0.08
c20:3n-3	1.07 ± 0.09[a,b]	0.97 ± 0.10[a]	1.23 ± 0.11[b]
c20:4n-6 ArA	4.02 ± 0.92	3.23 ± 0.16	3.54 ± 0.53
c20:5n-3 EPA	0.76 ± 0.09[a,b]	0.78 ± 0.09[b]	0.62 ± 0.02[a]
c22:2n-6	0.31 ± 0.26[a,b]	0.20 ± 0.09[a]	0.84 ± 0.37[b]
c22:3	1.10 ± 0.21[b]	0.80 ± 0.09[a]	0.82 ± 0.10[a,b]
c22:4n-6	1.73 ± 0.39[b]	1.20 ± 0.09[a]	0.94 ± 0.01[a]
c22:5n-3	2.36 ± 0.17[a]	2.14 ± 0.16[a]	1.79 ± 0.04[b]
c22:6n-3 DHA	14.19 ± 2.24[b]	13.59 ± 1.19[a,b]	10.75 ± 0.62[a]
ΣPUFA	49.14 ± 2.71[a]	49.18 ± 1.13[a]	43.91 ± 1.36[b]
ΣUFA	69.48 ± 1.05[b]	68.64 ± 1.31[a,b]	66.70 ± 0.87[a]
ΣMUFA/ΣPUFA	0.42 ± 0.06[a]	0.41 ± 0.03[a]	0.52 ± 0.03[b]
ΣMUFA/ΣSFA	0.67 ± 0.04	0.66 ± 0.04	0.68 ± 0.02
c16:1/c16:0	0.09 ± 0.01[a,b]	0.08 ± 0.01[a]	0.10 ± 0.00[b]
c18:1/c18:0	1.93 ± 0.26	2.07 ± 0.15	2.31 ± 0.15
ΣDHA + EPA	14.95 ± 2.33[b]	14.37 ± 1.28[a,b]	11.37 ± 0.65[a]
Σn-3PUFA	18.96 ± 2.54[b]	17.98 ± 1.39[a,b]	15.01 ± 0.75[a]
Σn-6PUFA	29.08 ± 0.19[b]	30.40 ± 0.36[c]	28.07 ± 0.77[a]
Σn-3/Σn-6PUFA	0.65 ± 0.09	0.59 ± 0.05	0.53 ± 0.02

All above fatty acids presented came from the muscle tissue

Means in the same column which have different letters on the top right have significant difference ($P < 0.05$). All data are presented as means ± standard error. *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *ArA* arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *PUFA* polyunsaturated fatty acid, *UFA* unsaturated fatty acid

in the form of triacylgycerol (Sheridan 1994). The composition of fatty acids changes with changes in temperature (Sargent and Tacon 1999; Cordier et al. 2002). This is likely an adaptation to maintain constant membrane fluidity (Kheriji et al. 2003; Olsen et al. 1999; Nanton and Castell 1999). The temperature-induced variation in fatty acid composition of animal tissues results in changes to the proportion of unsaturated to saturated fatty acids (Hilditch and Williams 1964). An increase in unsaturation in response to decreased environmental temperature has

been documented in many fishes, such as golden mahseer *Tor putitora* (Akhtara et al. 2014), goldfish (Kemp and Smith 1980), and sea bass *Dicentrarchus labrax* (Skalli et al. 2006) and has been implicated in homeoviscous adaptation (Charlotte and Patrick 1994). In our study, we documented a higher proportion of UFAs in fish reared at the low temperature. This is consistent with observations in rainbow trout *Oncorhynchus mykiss* (Calabretti et al. 2003). Additionally, Shen et al. (2005) documented a higher level of UFAs in the oviparous lizard *Phrynocephalus*

przewalskii, a cold-blooded animal, following exposure to lower ambient temperature. The authors speculated that UFA was important for adaptation to the lower temperature environment. However, Olsen et al. (1999) reported that lowering the temperature generally reduced liver SFA and MUFA levels followed by an increase in PUFA, but the changes were inconsistent and nonsignificant in Arctic char *Salvelinus alpinus* (L.) muscle. The authors suggested that the differences between studies may be because of species- and tissue-specific responses, in addition to dietary and other environmental factors.
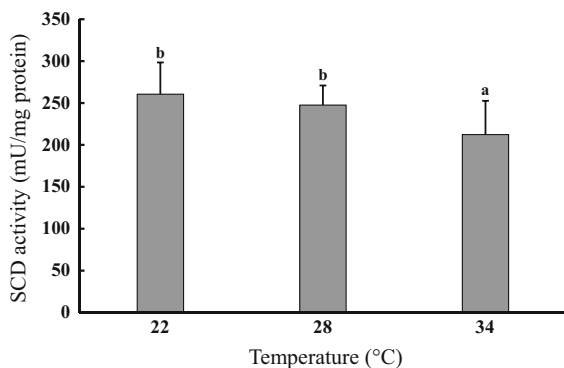
EFAs cannot be synthesized by animals so, instead, must be taken up via their food for growth, development, and maintaining tissue function (Cheng and Li 2004). The physiologically active EFAs in animals include EPA (20:5n-3), DHA (22:6n-3), and ArA (20:4n-6) (Sargent and Tacon 1999). Deficiencies in EFA are associated with decreased growth and feed conversion in fish (Cheng and Li 2004). An inverse relationship between temperature and UFA content, particularly EPA and DHA, has been reported for several poikilotherms (Hazel 1984). In this study, DHA and ArA levels decreased as the temperature increased. Conversely, EPA levels were significantly ($P < 0.05$) lower at 34 °C than at 22 and 28 °C and increased as the temperature decreased. Higher levels of EPA and DHA in European sea bass reared at 22 °C were previously interpreted as a way to maintain membrane fluidity (homeoviscous regulation) (Skalli et al. 2006). Previous studies have reported that UFAs, particularly EPA and DHA, play an important role in reducing the serum TG and TC content (Wang et al. 2012; Zhang et al. 2010). Consistent with this, we observed a decrease in the UFA and EPA + DHA content and an increase in TG and TC levels as temperature increased. These PUFAs have also been linked to species growth, reproductive success, and neural development in both zooplankton and fish (Brett et al. 2006; Perhar et al. 2013). For example, Xu et al. (1994) concluded that levels of n-3 and n-6 PUFAs must be maintained at a suitable ratio for optimal fecundity and hatching rate. In our study, the content of n-3 PUFAs and n-6 PUFAs changed inversely as temperature increased. Dietary n-3 PUFAs are important for human health, and because fish are an important dietary source of n-3 PUFA (Sargent and Tacon 1999), there is likely an economic benefit to manipulating rearing temperatures to

enhance the proportion of n-3 PUFA in cultured fish. In the current experiment, tilapia grew best at 28 °C, whereas n-3 PUFA levels were highest at 22 °C. Thus, efforts to regulate the fatty acid content by controlling temperature must also consider other factors, such as growth. Additionally, previous studies have noted that tilapia, like other warm-water fish, are more inclined to requiring greater amounts of n-6 PUFA compared with n-3 PUFA for maximal growth (Hsieh et al. 2007). Huang et al. (1998) reported that high levels of n-3 PUFA depress the growth of tilapia. This is consistent with our observation that tilapia reared at 28 °C had the highest n-6 PUFA levels and highest growth efficiency. However, Chou and Shiau (1999) and Hsieh et al. (2007) pointed out that both n-3 and n-6 PUFAs are essential for maximal growth of hybrid tilapia. Thus, our future research will focus on the effect of different dietary oil sources on tilapia growth performance, fatty acid composition, and other physiological indices. The fatty acid requirements of tilapia remain poorly understood. Despite this, our results suggest optimal rear tilapia may be at 28 °C as this not only is the optimum growth temperature but also results in a satisfactory ratio of n-3 PUFA to n-6 PUFA.
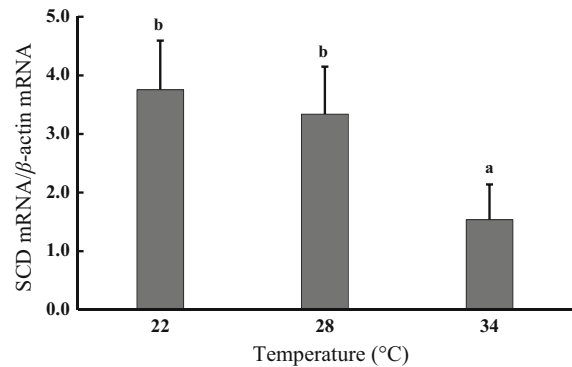
The activity and mRNA levels of SCD

Being a tropical species, GIFT tilapia are well adapted to warm waters, though high mortality of this species has often been reported during winter (Duan et al. 2011). In response to cold stress, fish adapt their fatty acid composition of lipids to tolerate the cold (Hsieh et al. 2007). Fish maintain the fluidity and stability of the cell membrane to resist cold stress by accumulating long-chain UFAs (Farkas 1980). In ectothermic vertebrates, lipid metabolism is regulated by an intracellular lipase and hormone (Sheridan 1994). Temperature has an effect on lipase activity and hormone levels (Shen et al. 2005). Fatty acid desaturases play important roles in the physiological functioning of cell membranes and in fatty acid metabolism (Tiku et al. 1996). SCD, a type of Acyl-CoA desaturase, is a rate-limiting enzyme in the biosynthesis of MUFA and plays a central role in regulating fatty acid metabolism (Heinemann and Ozols 2003). It is responsible for introducing the first double bond between carbons 9 and 10 of palmitoyl (16:0)-CoA and stearoyl (18:0)-CoA to form the

monounsaturated palmitoleic acid (16:1) and oleic acid (18:1), respectively (Jeffcoat et al. 1977). The expression of SCD can be regulated by diet and environmental temperature (Hsieh and Kuo 2005; Waters and Ntambi 1996). We observed a decrease in SCD expression as temperature increased (Figs. 1, 2) and a parallel change in UFA content. These changes are essential for maintaining membrane fluidity during cold acclimation (Hazel and Williams 1990). Hsieh et al. (2007) suggested that tilapia would increase the level of SCD transcription and activity to convert SFA into UFA during acclimation to cold shock. A number of studies have reported a change in the unsaturation index (16:1/16:0; 18:1/18:0) parallel to a change in desaturase activity (Trueman et al. 2000; Hsieh et al. 2002). However, we observed little difference. We presumed that this was because of the time dependence of different acclimation temperatures. Our fish were reared in the preselected water temperature for a longer time because tilapia adjust their metabolism to reach a stable level and therefore gradually adapt to the environmental temperature. Conversely, Trueman et al. (2000) and Hsieh et al. (2002) reared the fish at a given temperature for a short time or subjected the experimental animals to cold stress. Thus, the fish may not have had sufficient time to adjust their metabolic level. The proportion of MUFA was higher at 34 °C than at the lower temperatures. We speculate that this was because the change in SCD activity is an integral part of the response, but as the duration of exposure increases, the activity of monoene-specific elongases becomes more important. A substantial increase in levels of 20:1 (Trueman et al. 2000) is consistent with this hypothesis. In our study, the level of 20:1 was

Fig. 2 SCD mRNA level in juvenile GIFT tilapia reared in three different temperature for 40 days (n = 9)

higher at 22 °C, followed by 28 and 34 °C. Alternatively, the differences could be explained as follows: MUFAs will most likely be elongated to PUFAs (Stoffel and Ach 1964). The biosynthesis of PUFAs involves chain elongation, followed by desaturation, while β-oxidation processes may also be involved in the change in PUFA levels (Sprecher 2000). Additionally, prior research suggests that, compared with SFAs such as 16:0, the MUFAs (e.g., 18:1) are more likely to become the substrate of ACAT (acyl-CoA cholesterol acyltransferase) and DGAT (diacylglycerol acyltransferase) and thus generate TC and TG (Peng et al. 2011). Our results were consistent with this hypothesis. The level of 18:1, TC, and TG increased as the temperature increased. Thus, we found evidence to support the role of SCD in controlling lipid deposition in fish.

In conclusion, our study revealed that there were significant changes and differences in growth, blood parameters, and fatty acid composition in juvenile tilapia reared at different temperatures. Our results confirm that the optimum temperature for GIFT tilapia is 28 °C and provides a theoretical basis for improving the culture of tilapia. For the first time in GIFT tilapia, we documented significant changes in SCD gene expression in fish reared at different temperatures. This gene can affect the saturation of fatty acid and, in turn, affect the capacity for cold resistance. This finding holds promise for improving resistance of tilapia to low temperature.

Fig. 1 SCD activity in juvenile GIFT tilapia reared in three different temperature for 40 days (n = 9)

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