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Increased Lipogenesis in the Liver of Seawater-Acclimated Nile Tilapia, *Oreochromis niloticus*

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Abstract

Seawater (SW)-acclimatized *Oreochromis niloticus* (in 27 - 30 ppt) has been generated for more than 10 generations in a private farm in Thailand, and it was noted that the muscle of the fish was softer than that of the fish reared under freshwater (FW) (in 0 - 3 ppt). We hypothesized that high lipid content in the muscle might be the cause. Proximate analysis of the muscle from FW and *O. niloticus* reared in SW revealed significantly higher ($P < 0.05$) lipid content in the SW fish. The SW fish also had significantly higher hepatosomatic index (HIS), mesenteric adipose tissue somatic index, and lipid content in the liver (all, $P < 0.05$), compared to the FW fish. Liver histology of the 2 groups revealed hypertrophy of the hepatocytes and a significant increase in the percentage of apoptosis of the hepatocytes, as well as an increasing size of lipid droplets observed by Oil Red-O staining. In the liver, the genes *accl*, *accα*, and *fas*, which are related to lipogenesis, were expressed at significantly higher levels in the SW fish, compared to the FW fish. The genes *dgat2*, targeting the enzyme catalyzing the last step of triglyceride synthesis, and *cd36*, targeting a glycoprotein for the transportation of long-chain fatty acids across cell membrane, were also expressed at significantly higher levels in the SW fish. The only gene tested that did not show a difference between the FW and SW fish was *scd*, which targets the enzyme catalyzing saturated fatty acid to monounsaturated fatty acid. On the contrary, the gene *gys1*, targeting glycogen synthase, was down-regulated. All the data indicate that *O. niloticus* reared in SW adapted, or re-differentiated, themselves to cope with a high-salinity environment by increasing liver biosynthesis and storage of lipid.

Keywords: Tilapia, High salinity, Lipogenesis, Lipid storage

Introduction

Nile Tilapia (*Oreochromis niloticus*) is the 4th highest-produced fish worldwide [1]. This species has several benefits for commercial cultivation, such as fast growth, ability to withstand stress, adaptability to environmental changes, and successful reproduction in captivity. It plays a significant role in providing economic and nutritional support to small communities in low- and middle-income countries globally [2].

Following reports that *O. niloticus* could tolerate high salinity [3,4], a selective breeding program for growth and survival rate of the fish reared under SW, 25 - 30 ppt, has been continuously carried out in reservoirs of a private shrimp farm (AquaAcademy Farm) in Thailand. Natural breeding of the fish under SW, however, has faced a limitation since embryogenesis of the fish could not progress to full-term larvae without additional manipulation, either by re-acclimatizing the fish to FW environment before the breeding program or by applying step-wise increase in salinity during the progression of embryogenesis [5]. Most SW teleost need to adjust their osmoregulatory physiology in order to survive under elevated salinities by ingesting high amount of SW, retaining water in their bodies, and eliminating excessive ions from their blood, the processes being carried out through functions of gills, kidney, intestine, and pituitary gland [6,7]. These activities require energy, in which most fish depend on their lipid, more than carbohydrate, as a source of energy [8,9].

Fish lipids are well-known for containing high levels of healthy fats, i.e., long-chain omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Since humans cannot produce them, they must obtain them from their diet [10]. It has been reported that a high-salinity environment increases lipid composition in the liver and muscle of rainbow trout (*Oncorhynchus mykiss*) [11]. In our study, we observed that the muscle of marketable-sized *O. niloticus* reared in SW has a softer texture than that of the fish reared under FW. This difference may be due to a higher lipid and/or water content in their muscle. We hypothesize that high salinity culture may increase the lipogenesis in *O. niloticus* reared in SW. Additionally, the lipid metabolism in several organs in the *O. niloticus* reared in SW may differ from that of the FW ones. Among others, the organs that play roles in lipid metabolism are the liver and mesenteric adipose tissue.

Fatty acids are synthesized from acetyl-CoA originally in the mitochondria and bound to oxaloacetate to produce citrate, which can enter the cytoplasm and is subsequently reconverted to oxaloacetate and acetyl-CoA catalyzed by ATP citrate lyase (ACLY) [12]. Acetyl-CoA is synthesized into malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACC) [13,14]. Acetyl-CoA is extended 7 times by the addition of malonyl-CoA to the carboxyl terminus to produce palmityl-acyl carrier protein (ACP), through a process catalyzed by fatty acid synthase (FASN) [15]. Palmityl-ACP is further catalyzed by palmityl ACP thioesterase to release palmitic acid, which completes the entire fatty acid biosynthesis process [16].

In this study, we investigated the nutritional components of the muscle, using proximate analysis, determined the size and lipid content of the liver, examined the liver histology, and determined relative expressions of genes related to lipogenesis of the liver, comparing between the FW and *O. niloticus* reared in SW.

Materials and methods

Experimental animals

Following the breeding program [4], a batch of *O. niloticus* juveniles (30 - 50 g body weight) were reared in FW (0 - 3 ppt) and SW (25 - 30 ppt) environment in 28-ton round canvas ponds at the density of 10 individuals/m^3 of water. They were provided with floating commercial pellets (CPF, Thailand). Water quality was monitored according to the standard practices to assure optimum conditions for the fish, i.e., total ammonia nitrogen and total nitrite, < 0.5 ppm, water dissolved oxygen > 4 ppm, pH 7.0 - 8.0, and alkalinity 50 - 80 ppm (for the FW fish) and 140 - 180 ppm (for the SW fish). After 6 months of rearing, 20 individuals of the *O. niloticus* were randomly sampled from each of FW and SW ponds for the study. The animals and tissue samples used in this study were obtained in accordance with the Thai national guidelines on the care and use of animals for scientific purposes [17]. The study was conducted under the approved protocol number AQ062/2023 by the Institutional Animal Care and Use Committee at Prince of Songkla University.

Experimental procedure

O. niloticus reared in FW and SW, 20 of each, were anesthetized with 2-phenoxyethanol (200 mg/L), weighed, and individually weighed. The entire skin-free muscle from both sides of the fish was isolated and kept at –80 °C for proximate analysis. Since approximately 200 g of the muscle was required for each determination and the muscle isolated from individual fish was approximately 100 g, the muscles of 2 fish from the same group were combined for 1 determination. Before freezing, a piece (5 g) of the dorsal muscle from both groups of fish (N = 10 per group) was isolated and kept under –80 °C for the determination of gene expressions related to lipogenesis. The liver and mesenteric adipose tissue were isolated and weighed, and the relative weight of the liver (HSI) and mesenteric adipose tissue somatic index (ASI) were determined. Small pieces of the liver (10 - 20 g) from the individual fish of both groups (N = 10 each) were immediately isolated and fixed in 10 % neutral formalin for paraffin sectioning or in 4 % paraformaldehyde in phosphate-buffered saline (PBS) for cryotome sectioning. The rest of the liver was divided into 2 pieces (approx. 2.5 g each), 1 for the determination of total lipid content and another for the analysis of gene expressions related to lipogenesis; the samples were immediately kept at –80 °C until use.

Proximate analysis

Moisture, crude protein, crude lipid, and ash of the fish muscle were determined using standard methods [18]. Moisture content was determined by oven drying at 105 °C until constant weight and expressed as a percentage. The Kjeldahl method was used to analyze crude protein (nitrogen×6.25) with the Auto Kjeldahl system (FOSS KT260, Switzerland). This system involves 3 main steps: Digestion, distillation, and titration. An Ankom XT15 ether extraction system (USA) was utilized to determine the crude fat content, while the ash content was measured by burning 1 g samples in a GALLENKAMP size 2 muffle furnace (UK) at 600 °C overnight. The crude fiber content was calculated by finding the difference between the weight before ashing and the weight after ashing. The carbohydrate content was determined by subtracting the sum of protein, lipid, fiber, and ash content from 100 %. Crude protein, crude lipid, ash, calculated fiber, and calculated carbohydrate were all expressed as % dry weight.

Determination of total lipid content in the liver

The lipid content in the liver samples was determined using the method described by Bligh and Dyer [19], which was modified by Breil *et al.* [20]. Approximately 0.6 g of frozen liver tissues were homogenized in 1 mL of distilled water and transferred to glass test tubes. A mixture of 1.25 mL of chloroform and 2.5 mL of methanol was combined in a glass tube to achieve a chloroform: methanol: water ratio of 1:2:0.8 (v/v/v). After incubating the mixture at room temperature for 1 h, 1.25 mL of chloroform and 1.25 mL of water were added to achieve a new chloroform: methanol: water ratio of 1:1:0.9 (v/v/v). The solution was thoroughly mixed and then incubated at 4 °C overnight for settling. Subsequently, the solution was centrifuged at 1,500 g for 15 min at 4 °C until the 3 phases of the solution appeared. The lower layer, which includes lipids and chloroform, was gathered and moved to a new glass tube that had been weighed beforehand using a glass Pasteur pipette. The sample in the glass tube was then heated to 40 °C, and the lipids were dried using a nitrogen stream in a fume hood and stored in a –20 °C freezer until needed. The weight of the dried lipid was determined by subtracting the weight of the glass test tube. The total lipid content of the sample was calculated as a percentage of the lipid dry weight in the frozen tissue.

Histological examination

The liver and muscle tissue samples from both groups of fish were processed through routine paraffin sectioning method by dehydration under a series of ethanol concentrations, embedding in paraffin, sectioning at 4 μm, and staining with hematoxylin and eosin (H & E). For light microscopic (LM) examination, details of all parts of the microscopic features were examined and compared between the 2 groups.

The size of hepatocytes was determined indirectly by counting the number of hepatocytic nuclei within the randomly sampled area of 0.01 mm^2 in 20 different histological sections from individual fish. The numbers of the nuclei from each area examined were averaged and presented as hepatocytic density (number of nuclei/0.01 mm^2). The observation of the hepatocytic nuclei included those with dark-staining and fragmented ones, which were apoptotic hepatocytes [21]. The number of apoptotic hepatocytes was counted, and its percentage was determined from 800 - 1,000 hepatocytes per histological section; 3 sections were randomly sampled from the individual fish, and the percentage from these sections was averaged to represent the percentage of apoptotic hepatocytes of the individual fish sample.

Another histological preparation was aimed at a visualization of lipid droplets in the liver, using Oil Red-O (ORO) staining [22]. The liver tissues were fixed in a freshly prepared 4 % paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C overnight at a ratio of 1:20 (w/v), rinsed with PBS a few times and kept in PBS at 4 °C. The samples were transferred into cryomolds, acclimated in an optimal cutting temperature medium, and frozen in liquid nitrogen. The frozen tissue samples were cut at a thickness of 10 µm using a cryostat microtome and placed on glass slides. The slides were kept at –80 °C until used. The tissue sections were stained using ORO solution for 15 min, washed with 60 % propanol, and counter-stained with hematoxylin for 10 s. The sections were washed again with distilled water and mounted in glycerol buffer for LM examination.

Total RNA extraction, cDNA synthesis, and quantitative PCR (qPCR) analysis

Total RNA from the liver and muscle of *O. niloticus* were extracted using Tri Reagent® (Molecular Research Center, Inc) [23]. The quality and quantity of RNA were assessed by measuring their absorbance at 260 and 280 nm using Nanodrop One (Thermo Scientific). One µg of total DNase I treated RNA of each sample was applied to cDNA synthesis with Random Hexamers (Invitrogen, USA) using a SuperScript® III Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's protocol. The completed cDNA solution was stored at –20 °C for further analysis.

The cDNA from the liver was used as a template for qPCR, and relative expressions of the transcripts relating to lipogenesis were assessed using specific primers as shown in **Table 1**. The transcripts *acly*, *accα*, *fas*, and *scd* encode for the enzymes ACLY, ACC α subunit, FASN, and stearoyl-CoA desaturase (SCD), which catalyze the biosynthesis of acetyl-CoA, malonyl-CoA, palmitic acid, and mono-unsaturated fatty acids (MUFAs), respectively [12,24-27]. The transcript *dgat2* encodes the enzyme diacylglycerol O-acyltransferase 2 (DGAT2), which catalyzes the final reaction in the synthesis of triglycerides [28]. The transcript *cd36* encodes a membrane glycoprotein cluster determinant 36 (CD36), which facilitates the entry of long-chained fatty acids into cytoplasm [29]. Lastly, the transcript *gys1* encodes the enzyme glycogen synthase 1 (GYS1), which helps link together glucose molecules to form glycogen [30]. All these 7 transcripts were determined for their relative expressions against *ef1α*, which encodes the elongation factor 1α [31].

The qPCR was carried out using a QIAquant 96, 5 plex (Qiagen). The amplification was performed in a 96-well plate in a 20 µL reaction volume that contained 1 µL of cDNA from each tissue, 10 µL of SensiFASNT™ SYBR® No-ROX kit (Bioline), 0.4 µL of each primer (10 µM/µL) and 8.2 µL sterile dH_2O. The thermal qPCR cycle profile was performed according to the manufacturer's protocol. Briefly, 1 cycle of 95 °C for 2 min for enzyme activation, followed by 40 cycles of 95 °C for 5 s for denaturation, 60 °C for 20 s for annealing/extension reaction. A reaction using sterile distilled water instead of a cDNA template was applied as the negative control, and amplification of *ef1α* was done as an internal control. The comparative CT method ($2^{-\triangle\triangle CT}$ method) was used to analyze the expression level of the examined genes [32]. All samples were analyzed in duplicate, and the results were expressed as relative folds of 1 sample as mean ± SD.

Table 1 Lists of primers used for qPCR to examine the relative expressions of lipid and glycogen biosynthesis genes.

Primer name	Sequence 5’ → 3’	Product size (bp)	References
acly	F: GCCAGCCTGAAACTCACTCT R: AGCTCGTTAACCCCACCAAG	119	XM_003442027.5 (This study)
accα	F: TAGCTGAAGAGGAGGGTGCAAGA R: AACCTCTGGATTGGCTTGAACA	110	XM_025910659.1 [33]
fasn	F: TGAAACTGAAGCCTTGTGTGCC R: TCCCTGTGAGCGGAGGTGATTA	141	XM_003454056.5 [34]
scd	F: ACAAGCTCTCCGTGCTGGTCAT R: GCAGAGTTGGGACGAAGTAGGC	102	XM_005471382.2 [33]
dgat2	F: AGGACCTCCCCTCAGGGTTA R: GCCAGCAGTCAGTGCAGAAG	149	XM_003458972.5 [34]
cd36	F: GGGCATCTTCAGAGATCGCA R: GGGTTGAATGTGACGTTCGC	160	XM_003452029.5 [33]
gys1	F: CCTCACTCTGCGCTGTTATTC R: CAGCGGCATGCCTTCAGTTT	99	XM_013276796.3 [35]
ef1α	F: GCACGCTCTGCTGGCCTTT R: GCGCTCAATCTTCCATCCC	250	XM_019350343.1 [33]

Statistical analysis

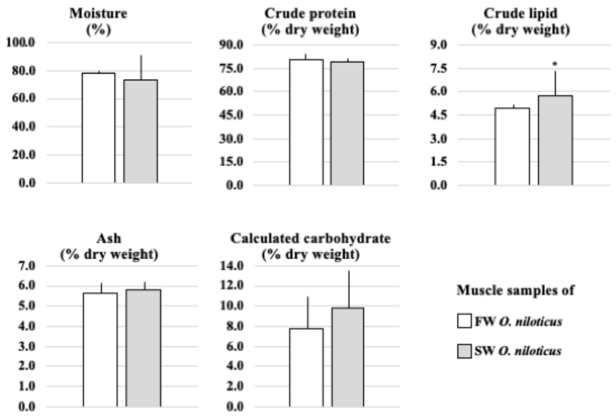
Values were expressed as means ± SD (N), and statistical analysis between the 2 groups was analyzed by student t-test. A significant difference was noted when $P < 0.05$.

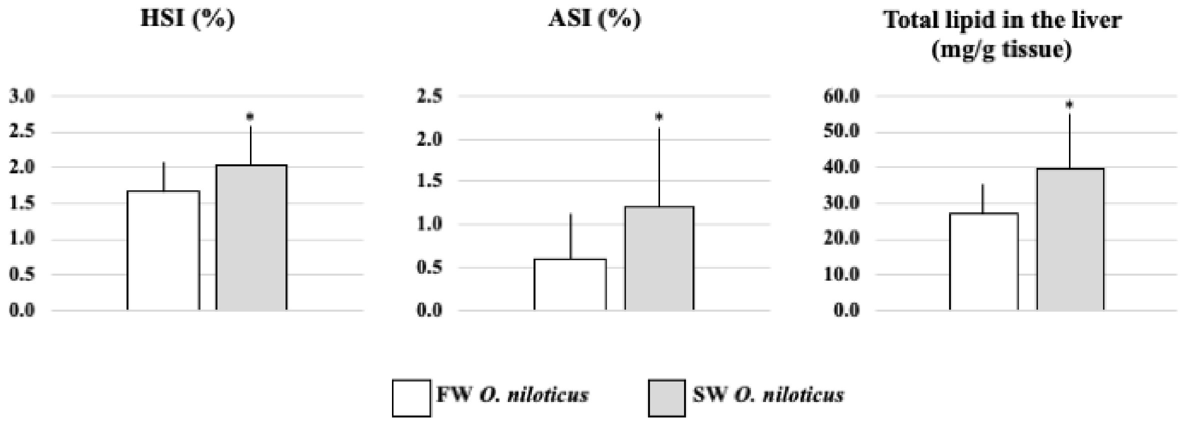
Results and discussion

Results

Proximate analysis, organ weights, and total lipid content in the liver

The body weights of the fish reared in FW and SW after 6 months of rearing were 318.6 ± 56.1 g (N = 20) and 365.0 ± 99.1 g (N = 20), respectively; the difference was statistically non-significant. The proximate analysis revealed that the levels of moisture, crude protein, ash, and calculated carbohydrate were not statistically different between the 2 groups (**Figure 1**). The levels of crude lipid of the fish reared in SW were highly variable among individuals, but the average value was significantly higher ($P < 0.05$) than that of the fish reared in FW. The HSI, ASI, and total lipid content in the liver of the fish reared in SW were significantly higher ($P < 0.05$) than those of the fish reared in FW (**Figure 2**).





Histology

The histology of the liver was mainly composed of hepatocytes, which were polygonal in shape, with a width of 15 - 25 µm (**Figures 3(a)** and **3(b)**). Each hepatocyte had a spherical central or subcentral nucleus (5 - 6 µm) with a central single nucleolus; the nucleus was surrounded by a large area of clear cytoplasm. Interspersed among the hepatic parenchyma were narrow sinusoids containing variable amounts of red blood cells (RBCs) in their lumina. Many of the hepatocytic nuclei, especially those of the SW fish, appeared apoptotic (**Figure 3(b)**, inset). The intrahepatic exocrine pancreas (IEP) surrounding the hepatic portal vein was frequently observed (**Figures 3(a)**, **3(b)**, and with higher magnification in **3(c)**). The IEP comprised cells with basophilic nuclei and eosinophilic zymogen granules in the cytoplasm.

Groups of brownish pleomorphic structures were often observed within IEP (**Figures 3(b)** and **3(c)**). These structures, melano-macrophage centers (MMCs), have been described as clusters of phagocytic cells containing melanin, hemosiderin, and lipofuscins [36]. In this study, they were observed mostly within the IEP and rarely within the hepatic parenchyma (**Figure 3(c)**, inset).

Hepatic venules containing variable amounts of RBCs, or occasionally being empty, were observed throughout the hepatic parenchyma, with several connected sinusoids (**Figure 3(d)**). The hepatic arterioles characterized by thick connective tissue wall (**Figure 3(e)**) and the biliary ductules surrounded by cuboidal epithelial wall (**Figure 3(f)**), both were rarely observed.

The histological features of the liver described did not reveal distinctive differences between the FW and SW fish. However, when the densities of the hepatocytic nuclei were compared, it was found that the density of the SW fish was significantly lower ($P < 0.05$) than that of the FW fish (**Figure 4**), indicating that the size of the hepatocytes of the SW fish was significantly larger than that of the FW fish. Another feature that revealed the difference between the 2 groups was the percentage of the apoptotic nuclei of the hepatocytes, which was significantly higher ($P < 0.05$) in the SW fish, compared to that of the FW fish.

Histological sections of the liver with ORO staining for lipid droplets revealed red droplets representing neutral lipid within the hepatocytes of both FW and SW fish. The sections from the SW fish clearly showed higher amount of lipid droplets in the SW fish, compared to that of FW fish (**Figure 5**).

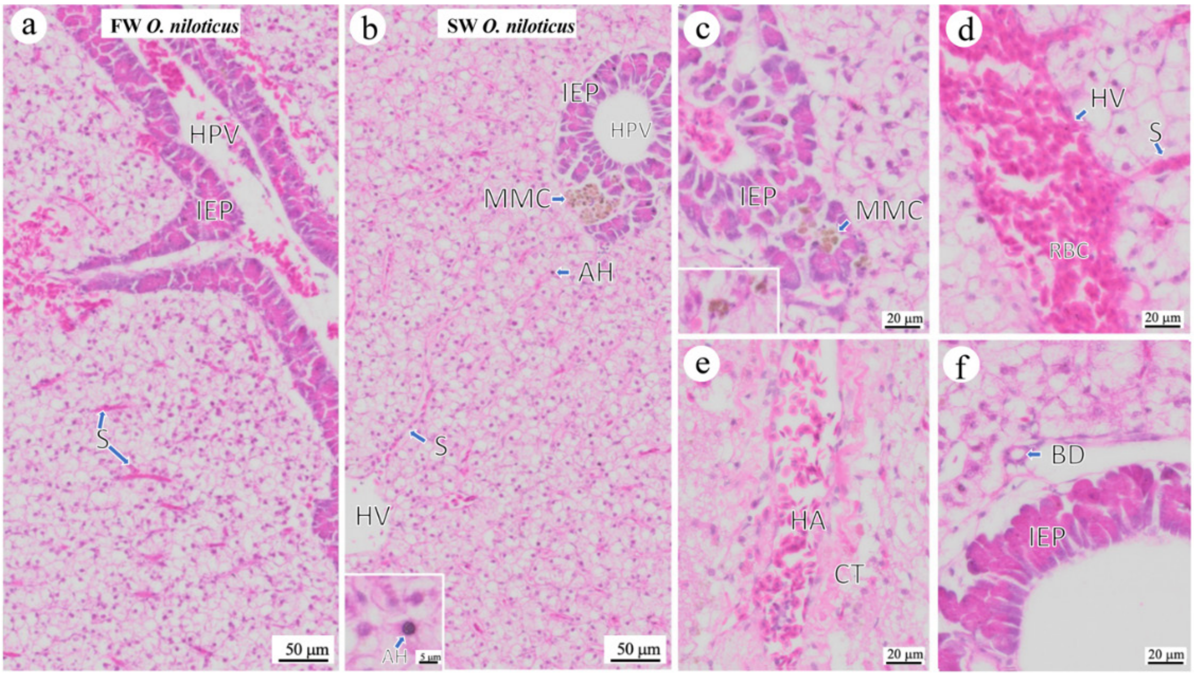


Figure 3 (a) and (b) Liver histology of *O. niloticus* reared in FW and SW showing similar features. The features consisted of hepatic parenchyma, IEP, hepatic portal vein, MMCs, sinusoid, hepatic vein, and apoptotic nuclei of hepatocyte (b), inset). The hepatocytic parenchyma of the SW fish showed lower density of the nuclei, indicating hepatocytic hypertrophy. (c) High magnification of IEP and frequently observed melanin-macrophage center within its tissue, but was rarely observed in the hepatocytic parenchyma (inset). (d) Higher magnification of the hepatic vein being filled with RBCs; a sinusoid was being connected to its lumen. (e) The hepatic arteriole with thick connective tissue wall. (f) The rarely observed bile duct. The histology shown from c to f were from *O. niloticus* reared in FW, which was indistinguishable from that of the SW fish, except the larger size and high percentage of apoptotic nuclei of the hepatocytes in the SW fish. H & E staining. AH, apoptotic nuclei of hepatocyte; BD, bile duct; CT, connective tissue; HA, hepatic arteriole; HPV, hepatic portal vein; HV, hepatic vein; IEP, intrahepatic exocrine pancreas; MMC, melano-macrophage center; RBC, red blood cells; s, sinusoid.

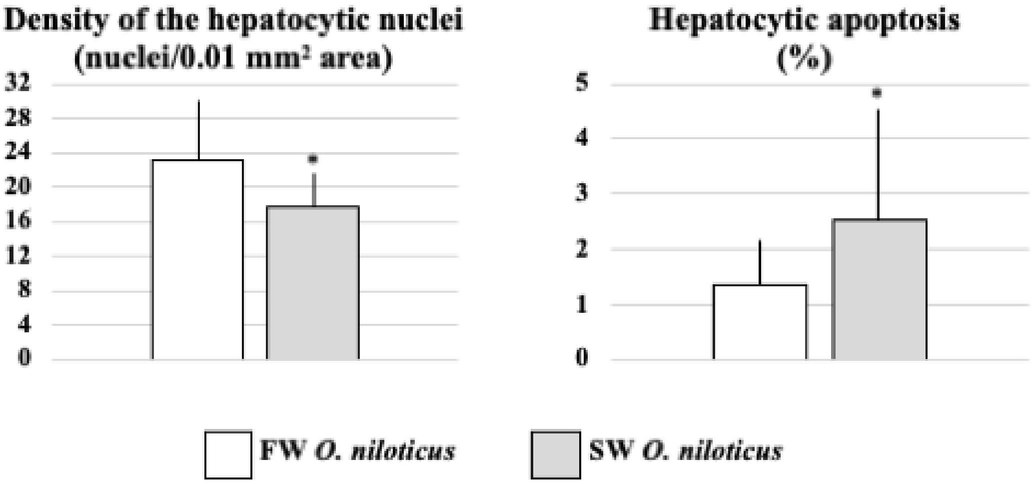


Figure 4 Histogram showing the density of hepatocytic nuclei and the percentage of apoptotic nuclei of hepatocytes of *O. niloticus* reared in FW and SW. *$P < 0.05$.

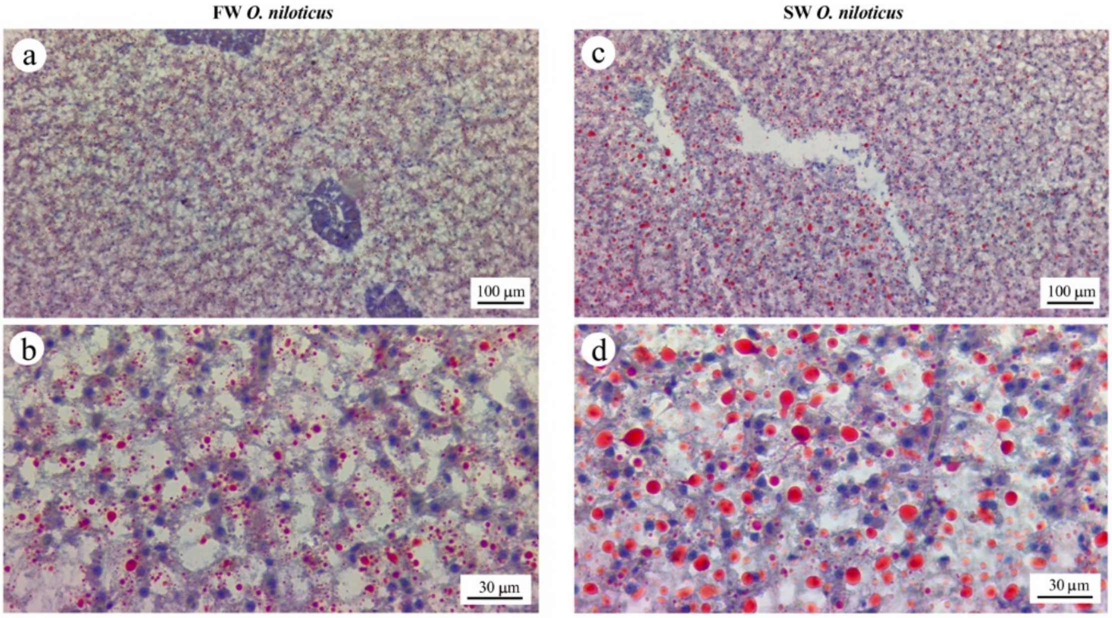


Figure 5 Micrographs showing ORO staining in liver tissues of *O. niloticus* reared in FW (a) and (b) and SW (c) and (d). The red droplets, representing neutral lipids in the hepatocytes, were detected at a much larger size in the liver of *O. niloticus* reared in SW, compared with those reared in FW.

Gene expressions

In the liver, relative expressions of the genes under study revealed significant differences between the FW and SW fish. The 3 lipogenic transcripts: *acly*, *accα*, and *fas* of the SW fish were expressed at significantly higher ($P < 0.05$) levels than those in the FW fish (**Figure 6**). The transcript *scd* of the 2 groups was expressed at a comparable level. The transcripts *dgat2* and *cd36* were expressed at significantly ($P < 0.05$) higher levels in the SW fish, compared to those of the FW fish. On the contrary, the transcript *gys1* was expressed at significantly lower ($P < 0.01$) in the SW fish, compared to that of the FW fish.

In the muscle, the relative expressions of all these transcripts revealed no significant difference between the 2 groups (**Figure 7**).

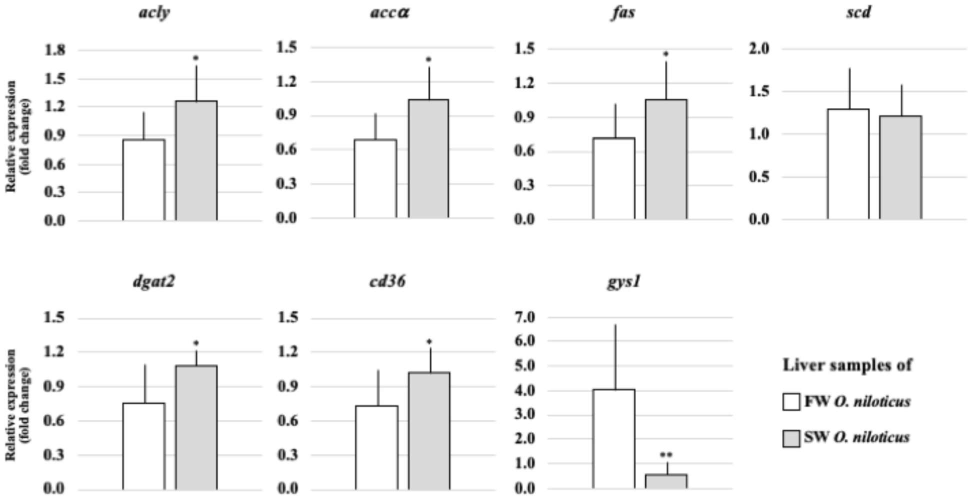


Figure 6 Histogram showing relative expressions of genes related to lipogenesis (*acly*, *accα*, and *fas*), desaturation of fatty acid (*scd*), synthesis of triglyceride (*dgat2*), transportation across cell membrane of fatty acids (*cd36*), and glycogen synthesis (*gys1*) from the liver of *O. niloticus* reared in FW and SW. *$P < 0.05$; **$P < 0.01$.

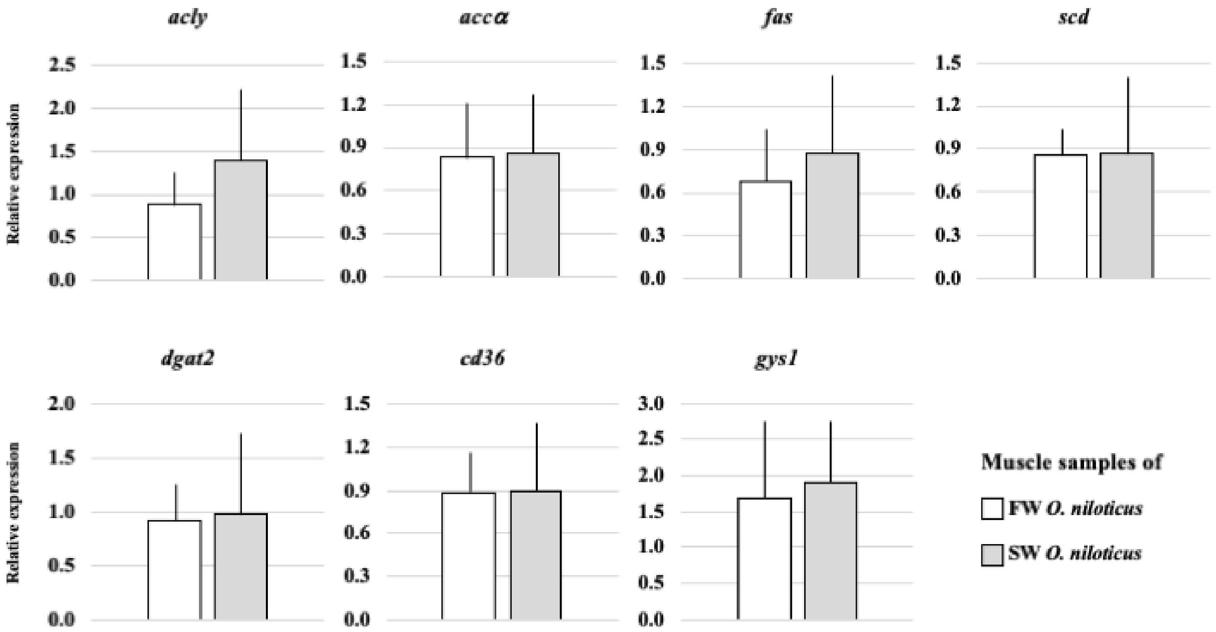


Figure 7 Histogram showing relative expressions of genes related to lipogenesis (*acly*, *accα*, and *fas*), desaturation of fatty acid (*scd*), synthesis of triglyceride (*dgat2*), transportation across cell membrane of fatty acids (*cd36*), and glycogen synthesis (*gys1*) from the muscle of *O. niloticus* reared in FW and SW.

Discussion

The proximate analysis revealed that the muscle of the SW fish contained more lipid than those of the FW fish. The result suggests that higher lipid content in the muscle of the SW fish is a factor making muscle texture of the SW fish softer than that of the FW fish. This finding aligned with a previous report by Li *et al.* [37], who determined the lipid contents in the muscle of approximately 10 fish species and found that fish living in SW had more lipid content in the muscle than those living in FW. In general, both lipid and glycogen are energy reserves of fish, but lipid is the 1st source that provides energy [8,9]. It is likely that SW fish species require more energy to adjust to the hyperosmotic environment than FW species.

In this study, the HSI of the SW fish was significantly higher than that of FW fish and the values of both groups fell within the range of HSI in *O. niloticus* reported by others, who showed that the HSI was affected by dietary treatments [38-41] and ploidy status [42], but no study thus far has reported the relationship between HSI and salinity exposure. The increased HSI in the SW fish in this study was most likely from hepatocytic hypertrophy and the increased de novo biosynthesis of lipid in the liver. The increased size of lipid droplets in the liver of SW fish revealed by ORO staining also supported the above findings.

The ASI of the SW fish was also significantly higher than that of the FW fish. The ASI value at 0.61 % of the FW fish in this study was slightly lower than that reported by others, which were in the range of 1 - 3 % [43-45]. This could be due to the difference in sizes and diet intakes of the fish under study. The higher ASI of the SW fish, compared to that of the FW fish, suggested that the mesenteric adipose tissue is another site of lipogenesis, or lipid storage, or both, of the SW fish.

Besides the hepatocytic hypertrophy and the increased percentage of the apoptotic nuclei of the hepatocytes of the SW fish, other histological features of the liver of the 2 groups were not visibly different. This character has also been mentioned in the previous study [46]. The IEP has been described earlier in *O. niloticus* [47], as well as in other teleosts, and it was suggested that the embryonic origin of IEP migrates with the hepatic portal vein into the liver tissue during embryogenesis [48-51].

An increased number of apoptotic nuclei of the hepatocyte was also recently reported in *O. niloticus* exposed to high salinity, as well as hypothermia [52]. This phenomenon was observed in the fish fed with high-fat or high-starch diet, or being exposed to toxic substances [53-55]. Apoptosis could be induced through extrinsic or intrinsic pathways; the extrinsic pathway is a process induced by extracellular factors such as pathogens, while the intrinsic factor is the process within the cell, such as cell differentiation during embryogenesis [56,57]. The increased incidence of apoptosis in the hepatocytes of the *O. niloticus* reared in SW in this study could be due to either an extrinsic pathway, such as osmotic or oxidative stress, or an intrinsic pathway, such as in adaptive re-differentiation to high salinity. In the latter case, it points to a possibility that hepatocytic apoptosis in the *O. niloticus* reared in SW may be one of the ongoing physiological processes of the fish to cope with a high-salinity environment.

The MMCs observed in this study were previously reported in *O. niloticus* and in other fish species; they were found mainly in the head kidney, liver, and spleen [58-62]. The MMCs are elements of the immune system of fish, including phagocytosis, antigen processing, as well as destruction, detoxification, and recycling of endogenous and exogenous materials [36,59,63]. Increased hemosiderin in splenic MMCs of *O. niloticus* was associated with an inflammatory process triggered by microorganisms or heavy metals [62,64,65].

In this study, the transcripts *acly*, *acc*α, and *fas* that encode for ACLY, ACC, and FASN were significantly higher in the SW fish, compared to those of the FW fish. It was previously reported that the 3 genes were upregulated in *O. niltoicus* with the fast-growth trait [66], and thus it has been suggested that lipogenesis plays a significant role in the growth of the fish. As lipid is the main source of energy expenditure of fish [8,9], both growth and adaptation to high salinity may require the same physiological responses, for instance, enhanced lipogenesis. If increased lipogenesis is essential for growth and salinity adaptation, it is likely that increased lipid consumption may have the same effect as up-regulation of the lipogenic genes. This may be the case since it was found that increased lipid consumption led to the down-regulation of the lipogenic genes in the liver of *O. niloticus* [43].

The transcript *scd* encodes SCD, the enzyme located in the endoplasmic reticulum that converts saturated long-chain fatty acids into MUFAs in mammals [24] and in *O. niloticus* [67,68]. Rearing *O. niloticus* under low temperature (22 °C) induced an increase in *scd* activity and *scd* expression, accompanied by an increased level of polyunsaturated fatty acids in the fish muscle [67]. In this study, the relative expression of *scd* of the FW and SW fish did not differ; therefore, the levels of MUFAs, a class of fatty acids considered beneficial to health [69], of the SW fish may be at the same level as that of the FW fish.

The transcript *dgat2* encodes DGAT2, the lipid mobilization enzyme that adds the 3rd acyl-Co-A to diacyl-glycerol to form triglycerides [28,70]. Like *scd*, the reaction performed by DGAT2 occurs in the endoplasmic reticulum [71]. In *O. niloticus*, *dgat2* was expressed mainly in the liver and muscle [72] and was up-regulated in the fish receiving a high-carbohydrate diet [73] or diet supplemented with α-linolenic acid [74]. The enzyme DGAT2 plays an essential role in mobilizing fatty acids into triglycerides, which is the main energy reserve for the fish. The increased expression of *dgat2* in this study suggests that the SW fish contained more triglycerides in their liver tissue, compared to the FW fish.

The transcript *cd36* was also up-regulated in the SW fish in this study. In mammals, *cd36* is involved in numerous physiological processes, including long-chain fatty acid transportation, glucose metabolism, inflammatory response, cell adhesion, immune response, and as a pattern recognition receptor [75,76]. Being the pattern-recognition receptor, *cd36* also binds pathogens and apoptotic cells, and acts as the scavenger receptor [77]. Up-regulation of *cd36* was observed in *O. niloticus* challenged by *Streptococcus agalactiae* [78], and under exposure to environmental estrogen [79]. In this study, the up-regulation of *cd36* of the SW fish could be due to the need to increase fatty acid level in the hepatocytes by increasing long-chain fatty acid transportation across the membrane. Since the percentage of hepatocytic apoptosis was higher in the SW fish, an alternative explanation for *cd36* up-regulation was a response of the hepatocytes to scavenge apoptotic cell products such as oxidized lipoproteins released from the dead cells.

A significant decrease in *gys1* in the SW fish indicated low biosynthesis of glycogen. Glucose is used to generate ATP in mitochondria and has acetyl Co-A as the end product to fulfill the physiological requirement of lipid synthesis, and this process is mainly performed in the liver [43]. It was found that *O. niloticus* fed with glucose after 36 h of fasting had an up-regulation of *gys1* during the first 8 h following glucose uptake, but down-regulation afterward [80]. In their study, the 3 lipogenic transcripts, *acly*, *acc*α, and *fas*, were up-regulated; and they suggested that lipogenesis is the best way to reduce glucose level in the fish blood. As the SW fish in this study was constantly under a hyperosmotic environment, it is likely that the fish may set up a new physiological standard by constantly suppressing glycogenesis, while lipogenesis is continuously activated.

The transcripts relating to lipogenesis and mobilization in the muscle of the FW and SW fish in this study revealed no significant difference, suggesting that their muscle may play a minor role in the biosynthesis of lipid. These results were similar to a previous report in the muscle of *O. niloticus* fed with different levels of lipid, which showed no changes in the expression levels of lipogenic genes in the tissue [43]. In this study, the finding that the muscle of the SW fish contained a significantly high content of lipid without up-regulation of lipogenic genes suggests that lipid was mobilized and deposited in the muscle as energy reserve rather than being synthesized there.

Conclusions

In SW (27 - 30 ppt), *O. niloticus* has more fat in their muscle and liver compared to FW (0 - 3 ppt) tilapia. There was an increase in de novo biosynthesis of lipid in their liver, indicated by the up-regulation of lipogenic genes. Changes in liver histology suggest an adaptation of the fish to a high-salinity environment. Besides the commercial aspect of SW Nile tilapia, these findings lead to exciting research on the physiology and gene regulations of this highly economic species.

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