

RESEARCH ARTICLE

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SALINITY LESSENS THE IMPACT OF HIGH STOCKING DENSITIES AND METABOLIC COST IN WHITE MUSCLE AND LIVER OF JUVENILE NILE TILAPIA, *OREOCHROMIS NILOTICUS***ABSTRACT:**

The effect of salinity on juvenile Nile tilapia, *Oreochromis niloticus* (15 ± 2.4 g) reared in unpleasant levels of high stocking density was studied. The study tries to elucidate the effect of moderate salinity levels (8 psu = 8 g NaCl L⁻¹) on stocking density level. To study the effect of water salinity, the two trials: 0 (T1) and 8 psu (T2) were constructed. In each salinity trial, fish were stocked at three densities: 90 fish m⁻³ (D1), 150 fish m⁻³ (D2) and 300 fish m⁻³ (D3). The experiments were conducted for 42 days at $26 \pm 0.5^\circ\text{C}$. The effect of different stocking density levels on growth performance and white muscle and liver metabolism, at T1 and T2, was recorded. In white muscle and liver, the activity of phosphofructokinase (PFK), cytosolic AST and ALT and mitochondrial enzyme glutamate dehydrogenase (GDH), aspartate- and alanine- aminotransferases (AST and ALT) was significantly increased in D2 and D3 at the trial T1 and D3 at the trial T2 in comparison with D1 of each trial. This increase in enzymes activity indicates an increased rate of energy expenditure. The unpleasant effect of increased stocking density level on growth rate recorded in D2 at T1 was alleviated in D2 at T2. This reflects an increased tolerance to an increased level of stocking density when fish subjected to the salinity level, 8 psu.

Key words:

Stress, phosphofructokinase (PFK), glutamate dehydrogenase (GDH), cytosolic- and mitochondrial aspartate- alanine- aminotransferases (AST and ALT)

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INTRODUCTION:

The tilapia intensive aquaculture farming is easy and profitable and progressively increased in the tropical countries (FAO, 2007). Growth performance of the fish is governed not only by its genetic potential but also by its immediate environmental conditions. Considerable progress has been made towards increasing the growth rate and food conversion efficiency of Nile tilapia reared in intensive aquaculture (D'Silva and Maughan, 1995; Yi *et al.*, 1996; Bailey *et al.*, 2000; El-Sayed, 2002; Ouattara *et al.*, 2003; Diana *et al.*, 2004; Ridha, 2006; Muangkeow *et al.*, 2007; Nguyen *et al.*, 2010).

In fish intensive aquaculture farming, stocking density (rearing density) is considered to be one of the important factors that govern fish growth. It has been established that rearing at inappropriate high stocking densities may impair the growth. This effect appear to be species-specific and to be mainly dependent on the sensitivity of fish to the deterioration of water quality at high stocking density and the increase of social interactions at very low and/or very high stocking densities (Di Marco *et al.*, 2008). To enhance production, farmers often increase rearing densities beyond system capacities. To improve the profitability of the fish farm in intensive culture maximum fish production, the density of fish in aquaculture must be evaluated. Therefore, studies on the effect of stocking density are still a fertile area of research. In tilapia, experiments on the effect of stocking density have been conducted on different fish sizes, including fry and juvenile (El-Sayed, 2002), sub-adults (D'Silva and Maughan, 1995) and large tilapia (Yi *et al.*, 1996). Studies were also conducted using different culture systems such as tanks (Bailey *et al.*, 2000; Ridha, 2006), ponds (Diana *et al.*, 2004) and net cages (Cruz and Ridha 1991; Yi *et al.*, 1996; Ouattara *et al.*, 2003). The results of these studies generally demonstrated an inverse relationship between stocking density and growth rate. The results also indicated that fish intensification by increasing stocking density is a suitable method to increase fish

yield and to overcome the problem of land shortage and the suitable density is fish species-dependent.

Tilapias are euryhaline fish and can tolerate a wide range of salinity from fresh water to full seawater. This tolerance was differ according fish species (Philippart and Ruwet, 1982). Advantageous effects of salinity have been reported in the avoidance of some diseases in juvenile and adult fish (Barton and Zitzon, 1995; Altinok and Grizzle, 2001). Reports have described the efficacy of salts to alleviate the effects of stress during handling, crowding and transport (Carneiro and Urbinati, 2001) and in post-stress recovery (Tsuzuki *et al.*, 2001).

Stress in aquaculture results from routine aquaculture management practices, such as high stocking levels or overcrowding, water temperature, poor water quality, physical disturbance, can act as powerful environmental stressors, thereby reducing the growth performance of the fish (Barton and Iwama, 1991; Schreck *et al.*, 2001; Hastein *et al.*, 2005; Kassahn *et al.*, 2007). The chief stress response of fish occurred in the activation of the brain-sympathetic-chromaffin cell axis and the brain-pituitary-interrenal axis (Balm *et al.*, 1995), leading to the swift discharge of catecholamines and cortisol into the blood. Cortisol is the principal corticosteroid in teleost fish and its concentration in blood rises dramatically during stress (Mommsen *et al.*, 1999). Cortisol are known to increase the activity of amino acid metabolizing enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glutamate dehydrogenase (GDH) in the different animal tissues (Mommsen *et al.*, 1999).

The aim of the present study is to evaluate the effect of salinity (8 g NaCl/L = 8 psu) on stocking density level and growth rate of Nile tilapia juveniles. To assess this, the activities of the key enzymes, PFK, AST, ALT, GDH in white muscle and liver of Nile tilapia were assayed.

MATERIAL AND METHODS:

Fish husbandry:

Juvenile Nile tilapia, *Oreochromis niloticus* were obtained from the fish hatching pond in Fowa city (Kafer El-Sheikh Governorate). The fish were transported in oxygenated cellophane bags at mid December, 2009 when the ambient water temperature was $15 \pm 2^\circ\text{C}$. Fishes of initial weight 15 ± 2.4 g were distributed in 36 glass aquaria of 33 L size. Each aquarium was equipped with a continuous air flow via a sponge biological filter. The filter was washed and cleaned thoroughly every day.

Dissolved oxygen level in the aquaria was maintained at $6.8\text{--}7.1\text{ mg L}^{-1}$. The daily ration was 3% of fish weight, and the fish were fed commercially available pellets (25.2% protein, lipids, carbohydrate, and fibers with total energy, 2505 kcal kg^{-1}) once daily at 10:00 a.m.

The fish were acclimated to the laboratory conditions for one week. Each aquarium was equipped with high precision-thermostat-controlled heater. The temperature was steadily raised from 15°C during 10 days by 2°C every two days to reach $26 \pm 0.5^\circ\text{C}$ which is the optimum temperature for maximal growth of Nile tilapia (Hegazi *et al.*, 2010). The fish were acclimated to this temperature for four weeks.

The experimental design:

To study the effect of water salinity, the two trials: 0 (T1) and 8 g NaCl L^{-1} (T2) were constructed. Maximum growth of adult Nile tilapia at high stocking density was produced at this salinity level (Chowdhury *et al.*, 2006).

The 36 fish aquaria were divided to two of equal categories to construct two salinity trials. Water salinity in the first (T1) and the second (T2) trials was 0 and 8 g NaCl L^{-1} (8 psu), respectively. The fish were stocked at three densities: 90 fish m^{-3} (D1) (3 fish aquarium $^{-1}$), 150 fish m^{-3} (D2) (5 fish aquarium $^{-1}$) and 300 fish m^{-3} (D3) (10 fish aquarium $^{-1}$). This attained six replicates from each density treatment for each salinity trial. The experiment was conducted for 42 days. Average fish growth (final weight – initial weight) was recorded at the end of the experiment in each aquarium. Fifty percent of the water aquarium was daily replaced by water which has the corresponding salinity for each trial, and previously heated to 26°C .

Tissue sampling:

At the end of the experiment, fish were killed by a rapid blow on the head and weighed. Small pieces of white muscle and liver were carefully excised on ice, washed in ice-cold isotonic NaCl saline, blotted and weighed.

The cytosolic and mitochondrial enzyme fractions were prepared according to the procedure described by Moon and Ouellet (1979) as modified by Hegazi *et al.* (2010). The buffer B1 contained 20 mM HEPES, 1 mM EDTA, 30 mM β -mercaptoethanol, 225 mM mannitol, 50 mM sucrose pH 7.6. While buffer B2 contained 20 mM HEPES, pH 7.6, 1 mM EDTA, 1% triton X-100, and 30 mM β -mercaptoethanol. Buffer B1 was used for the extraction of the cytosolic enzyme fraction, while the buffer B2 was used for the extraction of the mitochondrial enzyme fraction.

Tissue was homogenized (1: 2 w:v) in cold buffer B1 (4°C) using a glass manual homogenizer immersed in ice. About 200 µL of the resulting homogenate was delivered to a centrifuge tube and diluted using the B1 buffer to bring the dilution to 1:10 w:v. To another centrifuge tube, an equal volume from the resulting homogenate was also delivered and diluted using the B2 buffer to bring the dilution to 1:10 w:v. The contents of each tube were mixed thoroughly. The homogenate of B1 buffer was centrifuged using a cooling centrifuge at 12,000 xg for 15 min at 4 °C. The yielded high-speed supernatant of B1 was decanted and used immediately to examine PFK and the cytosolic fraction of ALT, and AST. The homogenate of B2 buffer was frozen-thawed three times for the complete disruption of mitochondria (Salach, 1978). The homogenate was then centrifuged using a cooling centrifuge at 6000 x g for 15 min at 4°C. The B2 fraction contained the enzymes GDH, total cytosolic- and mitochondrial- ALT, and AST. The supernatants were carefully collected, avoiding contamination with the upper lipid layer at the top of the tubes. The mitochondrial ALT and AST activities represent the difference between the enzyme activities in the two fractions. To test the mitochondrial-residue contaminations in the cytosolic fraction of B1, the activity of GDH was assayed in the cytosolic fraction.

Enzymes assays:

The reaction rate for each enzyme assayed was linear with time and protein content. Enzymes were assayed by following the rate of NADH oxidation at 340 nm ($E_{\text{ext}} = 6.22 \text{ cm}^2 \mu\text{M}^{-1}$) with an UV/vis Spectrophotometer (JENWAY 6505, UK). Assay conditions were selected to give maximal activity for enzymes and suitable controls were run to substrate non-specific activity in the supernatant. All enzyme measurements were done in duplicate. Assays were carried out in 80 mM imidazole-HCl buffer (pH 7.4) in a final volume of 1 ml. All enzyme activities were measured at 25°C in the bases of methods used by Moon (1983):

1. Phosphofructokinase (PFK, EC 2. 7. 1. 11): 6 mM fructose 6-phosphate, 0.15 mM NADH, 2mM ATP, 100 mM KCl, 10 mM MgCl_2 and excess triose phosphate isomerase (TPI, EC 5.3.1.1), glycerol 3-phosphate dehydrogenase (G3PDH EC, 1.1.1.8) and aldolase (Ald, EC 4.1.2.7).

2. Aspartate aminotransferase (AST, EC 2.6. 1.1): 40 mM aspartate, 7 mM α -ketoglutarate, 0.15 mM, NADH, 0.025 mM pyridoxal phosphate and excess malate dehydrogenase (MDH, EC 1.1.1.37).

3. Alanine aminotransferase (ALT, EC 2.6.1.2): 200 mM alanine, 10.5 mM

α -ketoglutarate 0.15 mM NADH, 0.025 mM pyridoxal phosphate and excess lactate dehydrogenase (LDH, EC 1.1.1.27).

4. Glutamate dehydrogenase (γ -GDH, EC 1.4.1.2): 10 mM α -ketoglutarate, 150 mM ammonium acetate, 1mM ADP and 0.15 mM NADH.

Statistical analyses:

Results are presented as means \pm standard deviation ($X \pm SD$). The statistical evaluation of all data was done using two-way analysis of variance (ANOVA) followed by Dunnett's test (D2 and D3 vs. D1, the respective controls of each trial). P values ≤ 0.05 were regarded as statistically significant. GraphPad 4 statistics program was used.

RESULTS:

There was a significant interaction ($P \leq 0.05$) between the effect of salinity and stocking density as indicated by two-way ANOVA. Growth performance proceeded normally in fish reared at D1 at T1 and D1 and D2 at T2 (table 1). A notable effect had been occurred in enzyme activities of Nile tilapia reared at higher stocking density D2 and D3 at T1 and D3 at T2 (Figs 1-12). Water salinity 8 g NaCl improves the fish tolerances to the high stocking level of D2 (150 fish m^{-3}) with a notable effect on enzyme activity occurred only in D3 (300 fish m^{-3}). There was significant ($P \leq 0.05$) increase in PFK and GDH activities in white muscle and liver of fish of higher levels of stocking densities D2 and D3 at T1 and D3 at T2. The activity of the cytosolic and mitochondrial AST, ALT of white muscle and liver of fish of stocking densities D2 and D3 at T1 and D3 at T2 showed also significant ($P \leq 0.05$) increase.

Table 1. Combined effect of stocking density and salinity on growth performance of Nile tilapia acclimated at 26 °C.

	Salinity 0 g NaCl L ⁻¹			Salinity 8 g NaCl L ⁻¹		
	D1	D2	D3	D1	D2	D3
Initial body mass (g)	15.1 ± 2.4	15.2 ± 2.4	15.1 ± 2.4	15.3 ± 2.4	15.1 ± 2.4	15.2 ± 2.4
Final body mass (g)	26.3 ± 2.8	21.1 $\pm 3.1^*$	18.6 $\pm 3.^*$	26.5 ± 2.8	24.9 ± 2.6	19.1 $\pm 2.6^*$
DGR g day ⁻¹	0.26 ± 0.3	0.16 $\pm 0.02^*$	0.08 $\pm 0.01^*$	0.27 ± 0.3	0.23 ± 0.04	0.09 $\pm 0.01^*$

Each reading represents Mean \pm SD of 10 samples.

* The difference was significant at $P \leq 0.05$.

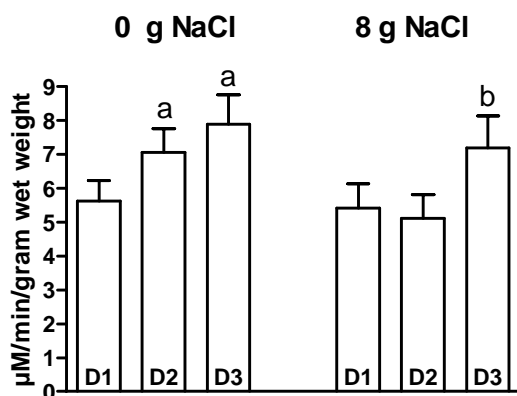


Fig. 1. Effect of stocking density and salinity on PFK activity in white muscle of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1.

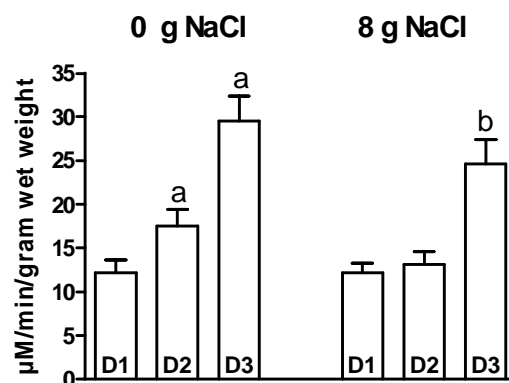


Fig. 4. Effect of stocking density and salinity on m-AST activity in white muscle of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1.

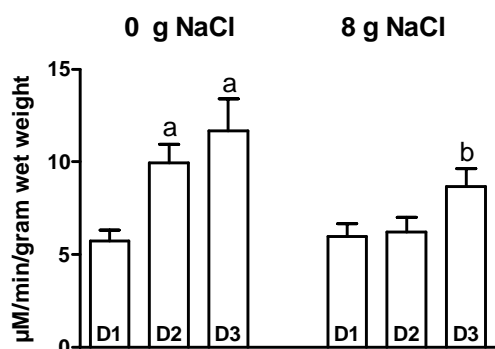


Fig. 2. Effect of stocking density and salinity on GDH activity in white muscle of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1.

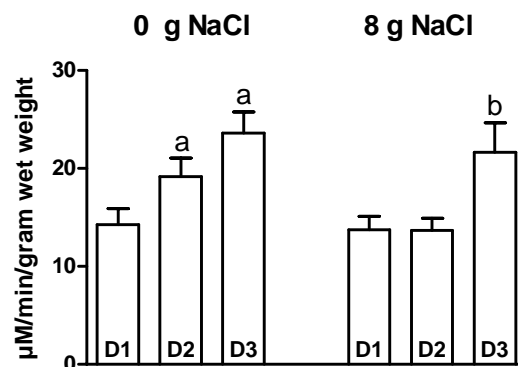


Fig. 5. Effect of stocking density and salinity on c-ALT activity in white muscle of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1.

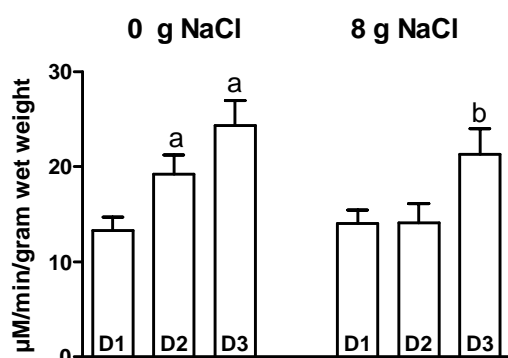


Fig. 3. Effect of stocking density and salinity on c-AST activity in white muscle of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1.

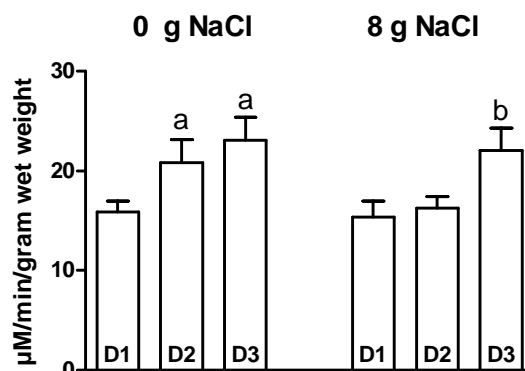


Fig. 6. Effect of stocking density and salinity on m-ALT activity in white muscle of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1.

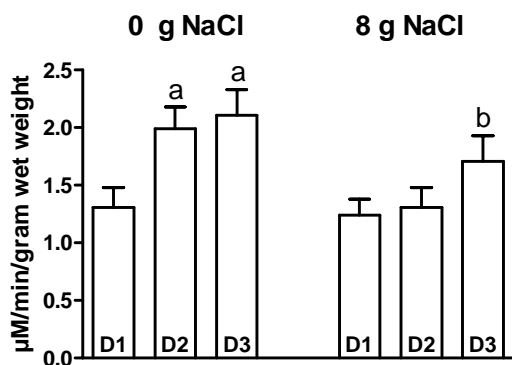


Fig. 7. Effect of stocking density and salinity on PFK activity in liver of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1

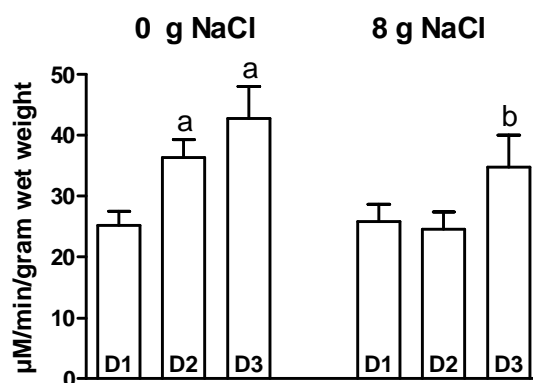


Fig. 10. Effect of stocking density and salinity on m-AST activity in liver of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1

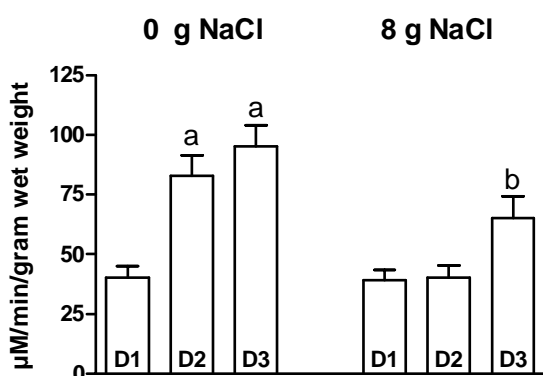


Fig. 8. Effect of stocking density and salinity on GDH activity in liver of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1

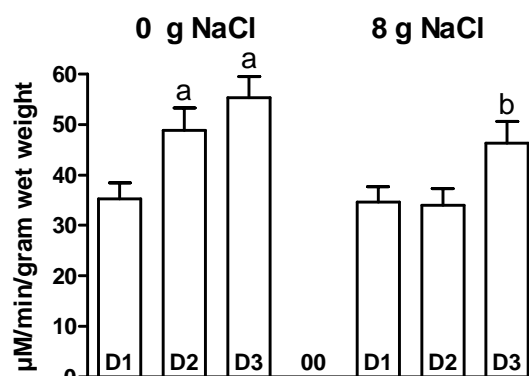


Fig. 11. Effect of stocking density and salinity on c-ALT activity in liver of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1

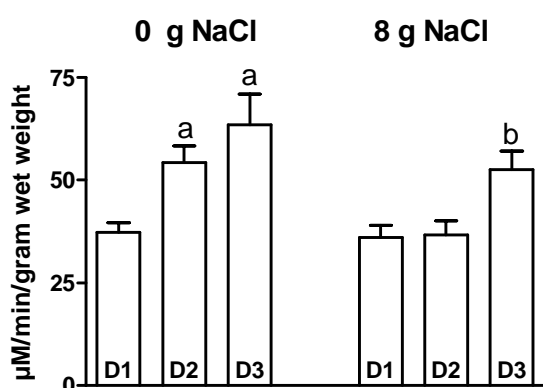


Fig. 9. Effect of stocking density and salinity on c-AST activity in liver of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1

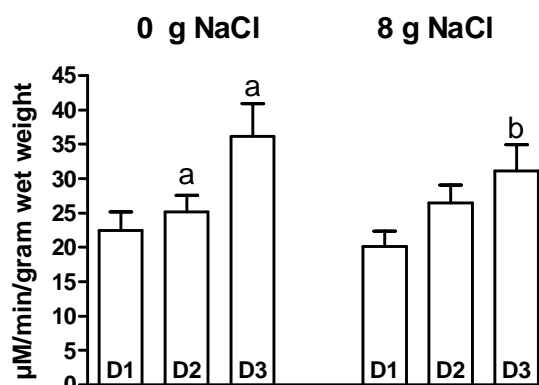


Fig. 12. Effect of stocking density and salinity on m-ALT activity in liver of Nile tilapia acclimated at 26°C. D1 = stocking density 90 fish, D2 = stocking density 150 fish and D3 = stocking density 300 fish. Each reading represents Mean \pm SD of 10 fish. The letter a or b = significantly different at $P \leq 0.05$ in comparison with their respective D1

DISCUSSION:

Salinity (8 psu) used in the current study improves fish growth significantly as reported in the stocking density D2. Better growth and better food efficiency occurred if turbot, *Scophthalmus maximus* reared at intermediate salinities (15–19 psu) in the upper temperature range (>18°C) (Imsland *et al.*, 2001). In European sea bass, *Dicentrarchus labrax* specific growth rate and feed conversion efficiency were affected by salinity (Rubio *et al.*, 2005). Oxygen consumption of 5-days-old staved silversides, *Odontesthes hatcheri* and *Odontesthes bonariensis* was minimal at salinity (0 and 10 psu), respectively. Oxygen consumption rates thereafter increased with increasing salinity, and then abruptly decreased at 30 psu (Tsuzuki *et al.*, 2008). One of the physiological functions clearly influenced by water salinity in fish is growth (Bœuf and Payan, 2001; Engström-Öst *et al.*, 2005). Thus, larval culture at low salinities produces higher growth and survival rates than in freshwater conditions in some freshwater species (Luz and Portella, 2002; Luz *et al.*, 2004). Growth of tilapias species was always affected by the salinity level. In the red hybrid tilapia, *Oreochromis mossambicus* × *Oreochromis urolepis*, early development, and growth were optimal at 18 psu (tested range, 4–36 psu) (Watanabe *et al.*, 1989). A change from 0 to 36.6 psu did not affect growth in the tilapia, *Oreochromis spilurus*, when the salinity was increased progressively over 120 h (Jonassen *et al.*, 1997). Studies in tilapias, using salinities between 0 and 120 psu, determined that certain species or hybrids have surprising growth capacities (Watanabe *et al.*, 1988; Suresh and Lin, 1992; Bœuf and Payan, 2001). Most species grow optimally between 5 and 18 psu, although red tilapia prefer 30–35 psu, food intake, food conversion and growth rates were closely related with increased salinity up to 36 psu. Survival, growth and feed conversion ratio of adult Nile tilapia (144 g) for 88 days were measured at three different salinity levels (8, 15, and 25 psu) and two stocking densities (20 and 40 fish m⁻³) (Chowdhury *et al.*, 2006). Highest net biomass growth was observed in the 40 fish m⁻³ stocking density treatment at 8 psu salinity level.

The activity of the enzyme PFK in white muscle and liver was significantly increased in D2 and D3 of freshwater trial (T1) and D3 in salinity trial (T2). However, in comparison with other fish species, there were significant increase in the activities of liver PFK and no differences in AST, ALT, and GDH in brook charr reared for 30 days at a high stocking density (Vijayan *et al.*, 1990). In fed fish, the oxidation of keto acids (derived from amino acid catabolism) via citric acid cycle may be the main means of energy production in fish

liver with some contribution from β -oxidation of fatty acids however, in white muscle, the conversion of glycogen to lactate would be a major source of energy (Walton and Coway, 1982). The increase reported in PFK activity in white muscle of fish in D2 and D3 at T1 and D3 at T2 might indicate high energy expenditure. The increase in PFK activity in liver may indicate one of two things or both, 1st liver may relay on glucose as a source of energy at these conditions and/or 2nd production of a higher quantities of α -ketoglutarate which are needed for biosynthetic processes especially for the nitrogenous waste products capture.

The increase in the activity of mitochondrial AST, ALT, and GDH in white muscle and liver of fish in D2 and D3 at T1 and D3 at T2 may indicate an increased rate of protein catabolism. Barcellos, *et al.* (1999) reported high levels of cortisol in high stocked Nile tilapia. The experimental studies on the effect of cortisol administration to fish have showed some metabolic insults. Cortisol administration to fish resulted in an increase in blood glucose and AST activity without any increase in ALT in Am eel liver (Foster and Moon, 1986). In the present experiment, in contrast the activity of the enzymes AST, ALT, and GDH increased. This may relate to that the chronic stress response could provoke more alterations than resulted in cortisol administration. The increase in the enzyme activities of Nile tilapia is consistent, and can be addressed under a case of an increased rate of protein catabolism. It is widely accepted that chronic stress affects growth and metabolism through the action of cortisol. Cortisol induces catabolic and aerobic, inhibits anabolic and anaerobic processes (Houlihan *et al.*, 1995), decreases RNA/DNA ratio and protein in brain (Tripathi, and Verma, 2003), reduction of growth performance (Barton and Iwama, 1991). Generally, the alterations caused in the activity of the enzymes AST, ALT and GDH are qualitatively similar in white muscle and liver. This may suggest that stress beyond a level may elicit response of equal magnitude.

Clear evidence for an association between salinity and growth control is not presented, but it cannot be expelled, because such manners has an energetic expenditure. Numerous studies have shown that 20 to > 50% of the total fish energy budget are devoted to osmoregulation. However, some ones showed that the osmotic cost is not as high (roughly 10%) as this (see Bœuf and Payan, 2001). In terms of the influence of salinity on the growing capacities in large fish, juvenile, or adult, data from numerous studies are available and these results are summarized in Bœuf and Payan (2001). In sea bream, *Sparus aurata* larvae growth was estimated at 15 to 40 psu and the best results, in terms of weight increase and swim-

bladder inflation, were recorded at 25 psu. In greenback flounder, *Rhombosolea tapirina*, egg fertilization, incubation, and yolk sac resorption are dependent on salinity, with 28 psu resulting in the best overall performance. In other flatfish, as the summer flounder, *Paralichthys dentatus* or Southern flounder, *P. lethostigma*, early development, and larval growth were also affected by salinity, optimal conditions being 8–14 and 5–30 psu, respectively. For mullet, *Argyrosomus japonicus* and milkfish, *Chanos chanos*, yolk resorption, early embryogenesis and larval growth were optimal at salinities of 5–12.5 and 20–35 psu. In striped bass, *Morone saxatilis* growth, and yolk sac utilization were optimal at salinity units (psu), compared with 0 and 10 psu (Peterson *et al.*, 1996). In the chum salmon, *Oncorhynchus keta*, increasing the salinity to 33.5 psu during rearing, following 7 weeks in fresh-water (7–10 g fish), allowed an increased growth. The metabolic rate of newly hatched steelhead trout, *O.*

mykiss was significantly lower in 8 psu water and higher at 12, in comparison to either 0 or 4 psu.

In conclusion, it can be said that enzymatic activities of AST, ALT, GDH, and PFK increased in Nile tilapia reared in freshwater at high levels (150 and 300 fish m^{-3}) of stocking density. This could be considered as a tool that can maintain higher source of energy for maintenance at these conditions. The salinity level (8 psu) acts to improve stocking density up to 150 fish m^{-3} , and did not show any increased metabolic expenditure. Stocking density more than 150 fish m^{-3} increased the response to an acute stressor, and did not respond to salinity.

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الملوحة تقلل من تأثير زيادة الكثافة العددية والكلفة الأيضية في العضلات البيضاء والكبد في صغار سمك البلطي النيلي

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امينوترانسفيريز، وكذلك انزيم جلوتاميت ديهيدروجينيز في السيتوبلازم و الميتوكوندريا في العضلات البيضاء والكبد لقياس معدل كلفة الأيض (زيادة معدل كلفة الأيض لمقاومة الظروف الغير ملائمة). لوحظ زيادة في نشاط هذه الإنزيمات و نقص معدل النمو في السمك عند الكثافة العددية 150, 300 لكل متر مكعب في محاولة الماء العذب. بالمقارنة لوحظ ارتفاع في نشاط الإنزيمات ونقص معدل النمو عند الكثافة العددية 300 لكل متر مكعب فقط في محاولة الملوحة. من ذلك نرى أنه من الممكن استزراع سمك البلطي بمعدل كثافة عددية أعلى عند هذا المستوى من الملوحة مقارنة بالمستوى المعروف في الماء العذب مما يؤدي إلى زيادة الإنتاج.

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في حالة الاستزراع السمكي المكثف من المعروف أن لكل نوع من السمك حد أقصى من الكثافة العددية للحصول على أعلى إنتاج اقتصادي و أي زيادة عن هذا الحد يسبب تدهور في الإنتاج. في هذا البحث تم دراسة التأثير الوافي لزيادة ملوحة الماء على زيادة الكثافة العددية لصغار سمك البلطي النيلي (15 ± 2.4 جم). والدراسة معنية بتأثير مستوى متوسط من الملوحة ($8 \text{ psu} = 8 \text{ g NaCl L}^{-1}$) على الكثافة العددية لسمك البلطي. ولدراسة ذلك تم عمل محاولتين للمقارنة بين تأثير الملوحة (T2) و الماء العذب (T1) عند ثلاث مستويات من الكثافة 90 (D1), 150 (D2) , 300 (D3) سمكة في المتر المكعب على التوالي في كل محاولة. و استمرت التجربة لمدة 42 يوم عند درجة حرارة في ماء الحوض 26°C وهي درجة الحرارة المثلي لنمو سمك البلطي لدراسة تأثير درجات الكثافة العددية المختلفة في محاولتين. وتم تسجيل معدل النمو و الأيض في العضلات البيضاء والكبد في محاولتين كدلالة على تأثير الملوحة. تم قياس نشاط الإنزيم فوسفوفركتوكينيز بالإضافة إلى الإنزيمات الناقلية لمجموعة الامين وهي الانين امينوترانسفيريز، اسيرتيت