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EFFECT OF FEEDING DURATION OF SODIUM CHLORIDE CONTAINING DIETS ON GROWTH PERFORMANCE AND SOME OSMOREGULATORY PARAMETERS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) AFTER TRANSFER TO WATER OF DIFFERENT SALINITIES

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Final Report

Chhorn Lim, Mediha Yildirim-Aksoy, and Thomas Welker
Aquatic Animal Health Research Unit
Auburn, Alabama, USA

Karen Veverica
Fisheries and Allied Aquacultures
Auburn University
Auburn, Alabama, USA

ABSTRACT

Two feeding experiments were conducted to evaluate the effect of feeding duration of dietary salt (NaCl or S) on hematocrit, blood glucose, and serum osmolarity and cortisol of Nile tilapia acclimated for various time periods to saltwater (SW) of different salinities (three-factor experiment). Quadruplicate groups of fish averaging 5.52 ± 0.13 g (study I) and 10.04 ± 0.19 g (study II) were fed to apparent satiation twice daily with the following four feeding regimens: feeding the control diet (C) for six weeks (6-wk C), feeding the 6% NaCl (S) diet for 6 weeks (6-wk S), feeding the C diet for 2 weeks and the S diet for 4 weeks (2-wk C + 4-wk S), and feeding the C diet for 4 weeks and S diet for 2 weeks (4-wk C + 2-wk S). At the end of week six, fish in each aquarium were weighed for growth measurement. Fish from each replicate aquarium in Experiment I were transferred to SW at 0, 15, and 30 ppt whereas those from Experiment II were transferred to SW at 0, 10, and 20 ppt. Hematocrit (study II only), blood glucose, and serum osmolarity and cortisol were determined at 48 and 96 h, and 0, 6, 12, 24, and 48 h for studies I and II, respectively, after transfer to SW. In both experiments, weight gain after six weeks of feeding did not differ ($P > 0.05$) among treatments, although all fish in the treatment receiving the NaCl-containing diet had consistently higher weight gain than those fed the C diet. Dry matter feed intake and survival were similar in both studies. Feed efficiency, though significantly different only in Experiment I, was consistently better for the groups that were fed the NaCl-containing diet. All fish transferred to 30 ppt salinity died within 8 h. No mortality occurred in fish transferred to 0, 10, 15, or 20 ppt salinity. Feeding dietary salt had no effect on blood glucose and hematocrit levels in either study. Serum osmolarity of fish in Experiment I decreased in fish fed dietary salt, but the differences were not always significant. This value was similar among fish fed dietary salt in Experiment II. In both experiments, blood glucose and serum osmolarity significantly increased, whereas hematocrit decreased with increasing water salinity. Duration of exposure to saltwater also significantly increased blood glucose levels but decreased hematocrit values. Duration of saltwater exposure had no effect on serum osmolarity. The interaction between dietary salt and water salinity, water salinity and exposure time, and dietary salt and exposure time had no effect on hematological and serological values in both experiments, except blood glucose and plasma osmolarity and cortisol in study II were significantly affected by water salinity and exposure time. The interaction between the three main factors had no effect on measured hematological parameters.

INTRODUCTION

Tilapia, because of their incredible adaptability and ability to reproduce in a wide range of physical and environmental conditions, excellent growth rates on a variety of natural and artificial diets, resistance to handling and disease causing agents, and their wide acceptance as a food fish, are the most commonly cultured species worldwide. Although endemic to tropical freshwater in Africa, Jordan, and Israel, their distribution has widened by artificial introductions in the early part and after the middle of the 20th century. Tilapia are currently cultured in virtually all types of production systems, found in both fresh and saltwater in tropical, subtropical, and temperate climates. Tilapia continue to dominate both small- and large-scale aquaculture in many tropical and subtropical countries as a low-priced product for the masses or a high-price, upscale product for export mar-

kets. They are increasingly recognized as the species of choice for intensive aquaculture and are likely to become the most important among all cultured fish in the 21st century.

Tilapia are euryhaline fish (Evans et al., 1984), and some species can survive direct transfer from freshwater (FW) to saltwater (SW) (Foskett et al., 1981; Foskett et al., 1983; Hirano, 1986; Yada et al., 1994). Among tilapia species, the Nile tilapia, *Oreochromis niloticus*, is one of the most important species in aquaculture due to its rapid growth. However, it is less salt-tolerant than *Oreochromis aureus* (Watanabe et al., 1985a; Avella et al., 1993), *O. mossambicus*, or *Tilapia zillii* (Stickney, 1986). Payne and Collinson (1983) reported that salinities for optimum growth of Nile tilapia ranged from 5 to 10 ppt. Cataldi et al. (1988) reported that *O. niloticus* survived only for brief periods in full-strength SW. Pre-acclimation also has a crucial physiological significance for some euryhaline teleosts during SW adaptation. Early salinity exposure through spawning and hatching under elevated salinities enhances salinity tolerance of young tilapia fry and may facilitate acclimation to SW (Watanabe et al., 1985b). Acclimation with diets supplemented with NaCl is another acclimation method that has been used with some success for adapting rainbow trout (*Salmo gairdneri*) (Salman and Eddy, 1987) and chinook salmon (*Oncorhynchus tshawytscha*) (Zaugg et al., 1983) to SW. Thus, this study encompassing two separate feeding trials was conducted to determine the effect of feeding duration of a sodium chloride (NaCl)-supplemented diet on growth, hematocrit, blood glucose, and serum osmolarity and cortisol levels of Nile tilapia after exposure to waters of different salinities.

Methods and Materials

Experimental fish and rearing facilities

Two separate experiments were conducted using two batches of Nile tilapia (*Oreochromis niloticus*) fry produced and reared at our laboratory on a commercial fry and larval diet. Before initiation of the experiments, fish were acclimated in holding tanks for 2 weeks. During this period they were fed the control (basal) diet twice daily to apparent satiation. At the end of the acclimation period, fish with average weights of 5.52 ± 0.13 g (Experiment I) and 10.04 ± 0.19 g (Experiment II) were randomly stocked into 16, 55-L aquaria at a density of 50 fish per aquarium. Aquaria were supplied with flow-through (0.6–0.7 L min^{-1}) dechlorinated municipal water maintained at 27 ± 1°C by a centralized heater. Water was continuously aerated using air stones. Water temperature and dissolved oxygen in four randomly chosen aquaria were measured once every other day in the morning using a YSI model 58 Oxygen Meter (Yellow Spring Instrument Co., Inc., Yellow Spring, Ohio). During the trials, water temperature averaged 26.9 ± 0.10 °C and 27.2 ± 0.04 °C for study I and study II, respectively, and dissolved oxygen averaged 4.8 ± 0.12 mg L^{-1} and 4.7 ± 0.10 mg L^{-1} for study I and study II, respectively). Photoperiod was maintained at a 12:12 h light:dark schedule.

Feed and feeding

Two practical diets (control and NaCl-supplemented) were formulated to contain approximately 31.5% crude protein and 2,850 kcal kg^{-1} digestible energy on an as fed basis (Table 1). The NaCl-diet was formulated by replacing celufil in the control diet with 6% of NaCl. The diets were prepared and stored as described by Lim et al. (1996). Pellets were ground into small pieces, sieved to obtain appropriate sizes, and stored frozen in plastic bags at -8 °C until needed.

In both experiments, the following four feeding regimens were used: feeding the control diet (C) for 6 weeks (6-wk C), feeding the NaCl diet (S) for 6 weeks (6-wk S), feeding the control diet for 2 weeks and the S diet for 4 weeks (2-wk C + 4-wk S), and feeding the control diet for 4 weeks and the S diet for 2 weeks (4-wk C + 2-wk S). Fish in four aquaria were randomly assigned to each feeding regimen and fed twice daily (between 0730–0830 and 1500–1600 h) to apparent satiation for 6 weeks. The amount of diet consumed was recorded daily by calculating the differences in weight of diets prior to the first and after the last feeding. Aquaria were cleaned once a week and fish were fed only in the afternoon on the cleaning days.

Weight measurement and saltwater exposure

At the end of week six, fish in each aquarium were group-weighed and counted for measurement of weight gain and survival. The day after weighing, fish in four replicate aquaria within the same treatment were combined, and triplicate groups of 20 fish each were transferred to new aquaria filled with water of 0, 20, and 30 ppt salinity for Experiment I, whereas those from Experiment II were transferred to water at 0, 10, and 20 ppt. Water at different salinities was obtained by mixing synthetic sea salt with freshwater. After transferring to saltwater, fish in Experiment I were fed with the control diet. No feeding was done for fish in Experiment II which were held in saltwater for 48 hours.

Hematological parameters assays

Prior to transfer to saltwater, three fish from each aquarium of both experiments were randomly chosen, anesthetized with tricaine methanesulfonate (MS-222) at 150 mg L^{-1}, and blood samples collected from the caudal vasculature with heparinized (20 U L^{-1}) tuberculin syringes. After transfer to saltwater, three fish from each aquarium in Experiment I were bled at 48 and 96 h for measurement of blood glucose and plasma osmolarity. In Experiment II, blood samples from three fish per aquarium were collected at 0, 6, 12, 24, and 48 h after saltwater transfer for measurement of hematocrit, blood

glucose, plasma osmolarity, and cortisol levels.

Hematocrit (two determinations for each blood sample) was determined using the microhematocrit method of Brown (1988). Glucose concentrations in whole blood samples were determined using the Accu-Chek Active blood glucose monitoring meter and test strips (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions. The remaining whole blood was chilled in ice and centrifuged at 3000 x g for 10 min at 4 °C to collect plasma, which was immediately assayed for osmolarity using the 5500 Vapor Pressure Osmometer (Wescor Inc., Markham, ON, Canada) and the SS-033 sample discs. Distilled water and calibration solutions were used as references. The remaining plasma was stored at 80 ºC for subsequent cortisol analysis. Plasma cortisol concentrations were measured by time-resolved fluoroimmunoassay as described by Small and Davis (2002) using the DELFIA® cortisol kit (PerkinElmer Wallac, Inc., Gaithersburg, MD).

Statistical Analysis

Data were analyzed by two-way analysis of variance using the general linear model to test for diet, salinity, and time effects and their interactions. Duncan's multiple range test was used to compare treatment means. Differences were considered significant at the 0.05 probability level. All analysis was performed using the SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, 2001).

RESULTS

Average weight gain (WG), feed intake (FI), feed efficiency ratio (FER), and survival of fish in Experiments I and II after 6 weeks of feeding with various dietary NaCl regimens are presented in Tables 2 and 3, respectively. There were no significant differences ($P > 0.05$) among WG, FI, and survival of tilapia receiving different treatments. In Experiment I, fish that were fed the NaCl-supplemented diet for 2, 4, or 6 weeks had similar FER, but these values were significantly ($P < 0.05$) higher than that of the group fed the control diet. The values of this parameter for Experiment II, however, did not differ ($P > 0.05$) among treatments.

In Experiment I, regardless of the dietary regimen used, fish transferred to 30 ppt salinity water began to die 4 h after exposure and all fish died after 8 h. No mortality was observed during the 96-h period for fish transferred to 0 and 15 ppt salinity waters. Likewise, no mortality was observed during the 48-h period for fish in Experiment II which were transferred to saltwaters at salinity of 0, 10, and 20 ppt.

Mean blood glucose concentrations and plasma osmolarity of tilapia in Experiment I after exposure to waters at 0 and 15 ppt salinity for 48 and 96 h are presented in Table 4. Feeding regimen had no significant ($P > 0.05$) effect on blood glucose levels, but the values of this parameter significantly ($P < 0.05$) increased with increasing water salinity and duration of exposure. The interactions between feeding regimen and salinity, salinity and time, feeding regimen and time, and feeding regimen, salinity, and time on blood glucose levels were not significant ($P > 0.05$). Plasma osmolarity was highest for fish in the control treatment, but the differences were not always significant. Fish on the 2-wk C + 4-wk S feeding regimen had significantly lower plasma osmolarity than that of fed on the C diet throughout. These values, however, did not differ significantly from those of fish on the 4-wk C + 2-wk S and 6-wk S feeding regimens. Fish transferred to 15 ppt had significantly higher plasma osmolarity than fish transferred to freshwater. Time of exposure and interactions between feeding regimen and salinity, salinity and time, feeding regimen and time, and feeding regimen, salinity, and time did not have a significant effect on plasma osmolarity.

Mean blood glucose, hematocrit, and plasma osmolarity and cortisol of tilapia in Experiment II after transfer to saltwater at 0, 10, and 20 ppt for 0, 6, 12, 24, and 48 h are presented in Table 5. Feeding regimen had no significant effect on blood glucose, hematocrit, and plasma osmolarity and cortisol. Blood glucose and plasma cortisol levels were similar among fish exposed to fresh and 10 ppt SW, but significantly increased in fish exposed to 20 ppt. Hematocrit significantly decreased in fish transferred to SW, but the values were similar for the groups held at 10 or 20 ppt. Plasma osmolarity significantly increased at each incremental level of water salinity. However, exposure time had no effect on this parameter. Blood glucose concentration significantly increased with increasing exposure time, reached a maximum at 12 h and then significantly decreased thereafter. However, the blood glucose value at 48 h remained significantly higher than that at 0 h. Hematocrit significantly decreased with exposure time, and the value declined significantly after 48 h. Plasma cortisol peaked significantly at 6 h, gradually decreased thereafter, and at 48 h the value became significantly lower than those at 6 and 12 h. Interactions between feeding regimen and salinity, salinity and time, feeding regimen and time, and feeding regimen, salinity, and time had no effect on hematocrit. Blood glucose and plasma osmolarity and cortisol were significantly affected by the interaction between salinity and time but not by interactions between other factors. Blood glucose of fish in 20 ppt salinity significantly increased at 6 h, reaching a maximum value at 12 h, and then significantly decreased thereafter. Blood glucose levels of fish held at 0 and 10 ppt increased only slightly and were similar throughout the 48-h period. The trend of plasma osmolarity of fish held in 20 ppt SW was similar to that of blood glucose of fish held at the same salinity. For fish exposed to 0 and 10 ppt SW, plasma osmolarity gradually increased with increasing exposure time, but

the values of this parameter at 12, 24, and 48 h for fish held at 10 ppt were significantly higher than those of the control group. Plasma cortisol concentrations of fish held at 10 and 20 ppt peaked at 6 h and decreased thereafter. For fish held at 20 ppt, the values at 6, 12, and 24 h were significantly higher than those of fish held at 10 ppt. At 48 h, plasma cortisol of fish held at 20 ppt declined to a level that was significantly lower than those of fish held in freshwater (0 ppt) and 10 ppt SW. Cortisol of fish held in freshwater remained relatively constant for the first 12 h, significantly increased at 24 h to a value similar to that of fish held for 6 h at 10 ppt, and remained at that level after 48 h.

Discussion

In both experiments, weight gain of juvenile Nile tilapia did not significantly differ among dietary treatments. However, fish receiving the NaCl-supplemented diet for 2, 4, or 6 weeks was consistently higher (5.8 to 10.5%) than the group fed the control diet for 6 weeks. This growth improvement appeared to be related to better nutrient utilization since feed intake of fish in both experiments was essentially the same in all treatments but feed efficiency, although significantly better for that obtained in Experiment I only, was also consistently higher in fish receiving NaCl feeding regimens in Experiment II. The beneficial effect of feeding the NaCl-containing diet was probably related to a metabolic need of sodium and chloride ions in hypotonic freshwater environment where loss and environment uptake of these ions are the major problems (Brett, 1979; Gatlin, 2001). Gatlin et al. (1992) reported that inclusion of 2% NaCl and/or 2% potassium chloride in practical diets had positive effect on growth of red drum (*Sciaenops ocellatus*) in freshwater and brackish water (6 ppt) but no effect on fish reared in full-strength sea water.

Nile tilapia have been reported to be less salt-tolerant than blue tilapia, *Oreochromis aureus* (Watanabe et al., 1985b; Avella et al., 1993), Mozambique tilapia, *O. mossambicus*, and red belly tilapia, *Tilapia zillii* (Stickney, 1986). The natural distribution of Nile tilapia is dictated by salinity range. Payne and Collinson (1983) reported that upper estimates for salinities giving unimpeded growth of *O. niloticus* ranged from 5 to 10 ppt salinity. Cataldi et al. (1988) reported that *O. niloticus* survived only for brief periods in seawater. Results of Experiment I confirm these findings. Regardless of feeding regimens used, juvenile Nile tilapia transferred to 30 ppt SW began to die after 4 h and none of the fish survive after 8 h. Even for Mozambique tilapia which are know to tolerate relatively high level of water salinity, mortality occurred 6 h after direct transfer from freshwater to 30 ppt sea water (Hwang et al., 1989). Acclimation of Mozambique tilapia to full strength seawater (34 ppt) over a 30-h period also resulted in 92% mortality (Morgan et al., 1997). With juvenile fall chinook salmon, Zaugg et al. (1983) obtained greater survival of fish fed NaCl-supplemented diets than the group fed the control diet when transferred directly to seawater.

Being a typical euryhaline fish (Evans, 1984), tilapia have been widely used as a model in studies on endocrine aspects of stress and osmoregulation in teleost fish because they can survive direct transfer from freshwater to saltwater (Foskett et al., 1981; Fosket et al., 1983; Hirano, 1986; Yada et al., 1994). In tilapia, the transition from FW to SW is associated with temporary elevation in plasma osmolarity and sodium (Na^+) and chloride (Cl^-) ion concentrations (Assem and Hanke, 1979; Hwang et al., 1989), accompanied by a transient rise in plasma cortisol (Assem and Hanke, 1981) and growth hormone (GH) levels (Yada et al., 1994). There is an alteration in branchial chloride cell morphology (Hwang, 1987) and an increase in gill Na^+, K^+-ATPase activity (Dharmamba et al., 1975; Dange, 1985; Hwang, 1989) in tilapia transferred to SW. These adaptive changes are a coordinated set of responses meant to sustain physiological homeostasis subsequent to osmotic stress and to mediate the hyperosmoregulatory transition to maintain ionic balance in SW.

Fish moving from a FW hypoosmotic to a SW hyperosmotic environment are characterized by water loss and solute gain (Diouf et al., 2000). The electrolyte influx produces an osmotic pressure differential between the blood and cells. As water flows out of cells, they shrink, causing reductions in the red blood cell volume or hematocrit (Diouf et al., 2000). Assem and Hanke (1979) observed a decrease in intracellular volume of white epaxial muscle of Mozambique tilapia adapted to 3.5% sea water relative to those held in freshwater. In the present study (Experiment II), a significant decrease in hematocrit was observed in tilapia transferred to 10 or 20 ppt SW. The reduction of hematocrit persisted throughout the 48-h period.

An increase in plasma osmolarity of Mozambique tilapia (Assem and Hanke, 1979; 1981) and Nile tilapia (Fontaínhas-Fernandes et al., 2001) resulting from the increase in blood electrolytes caused by SW transfer has been documented. In the current study, plasma osmolarity increased significantly in tilapia exposed to SW in both experiments, and the rate of increase was directly related to salinity concentrations. Feeding regimen had no effect on plasma osmolarity in Experiment II, but in Experiment I marked reductions in plasma osmolarity were observed in fish on NaCl feeding regimens. Only tilapia receiving the 2-wk C + 4-wk S regimen had osmolarity values significantly lower than those of the control. Fontaínhas-Fernandes et al. (2001) reported slight reductions in serum osmolarity of Nile tilapia fed an 8% NaCl diet for 3 wk and then transferred to 15 ppt SW, but the values did not significantly differ from those of fish fed the control diet. However, they observed that,

for fish transferred to 20 ppt SW, plasma osmolarity values of the NaCl-fed group did not differ at 6 h but were significantly lower at 12 and 24 h than those of fish fed the control diet. In the present study, plasma osmolarity values, although significantly different only in Experiment I, remained elevated in both experiments over the course of the SW exposure period. Assem and Hanke (1979) observed that direct transfer of *O. mossambicus* to 2.7% sea water resulted in rapid increase of plasma osmolarity during the adaptation period, followed by a gradual increase reaching a peak after 9 h. Thereafter, the plasma osmolarity was reduced but was still higher compared to freshwater controls after 168 h. In another study, Hwang et al. (1989) observed a slow increase of plasma osmolarity of *O. mossambicus* following direct transfer from freshwater to 20 ppt SW which reached a peak at 12 h and began to decline at 24 h. Fontaínhas-Fernandes et al. (2001) found that plasma osmolarity values of Nile tilapia exposed to 15 and 20 ppt SW were significantly elevated after 24 h but began to decline after 48 h and returned to pre-exposure levels after 168 h (only for the 15 ppt SW group). A similar trend was also observed in Experiment II of this study for fish exposed to 20 ppt SW. The plasma osmolarity of this group of fish increased sharply at 6 h, gradually increased further reaching a peak at 12 h, and then gradually declined, but the value at 48 h remained significantly higher than those of fish exposed to freshwater or 10 ppt. For fish exposed to freshwater and 10 ppt SW, plasma osmolarity increased gradually but remained steady over the course of exposure period. This indicates that, in the current study, plasma osmolarity most likely needed to be monitored over a longer period to determine times at which the values of this parameter for fish exposed to different salinities return to pre-exposure levels. The data from this study and those of Fontaínhas-Fernandes et al. (2001) suggest that dietary salt may help SW-exposed tilapia to better hyperosmoregulate and that tilapia have an inherent ability to effectively maintain osmotic balance when exposed to moderately hyperosmotic conditions (10 ppt SW), but not when exposed to more concentrated SW environments (15 or 20 ppt SW). Although tilapia can survive when exposed to the highest physiologically tolerable SW concentrations, several days may be needed to attain hyperosmoregulatory competence and establish osmotic equilibrium, adding additional physiological stress over a protracted period of time.

The osmoregulatory response of fish to hyperosmotic stress is also characterized by an increase in metabolic rate, illustrated by a rise in oxygen consumption (Morgan et al., 1997) and blood glucose concentrations (Nakano et al., 1998). The initial increases in plasma cortisol and blood glucose in tilapia a after transfer to SW are most likely due to the activation of the glucocorticoid stress response by osmotic stress or by handling stress from transfer to the smaller exposure tanks (Morgan et al, 1997). However, plasma cortisol and blood glucose values were significantly greater in tilapia exposed to 20 ppt SW compared the control and 10 ppt SW groups (in Experiment II) indicating an increased osmotic stress effect at salinities greater than 10 ppt. Even though cortisol is a glucocorticoid hormone most often associated with stress in fish, it also plays a role in osmoregulation, especially in physiological adaptation from FW to SW environments (Sunny and Oommen et al., 2001; Dang et al., 2000). Cortisol levels began to decline over time in the present study; however, cortisol concentrations were still elevated even after 48 h post-transfer, which may in part have resulted from the osmoregulatory role of cortisol. In tilapia, cortisol increases gill chloride cell density and volume of the tubular membrane system and Na+, K+-ATPase density (Dange et al., 2000) and activity (Dharmamba et al., 1975; Dange, 1985; Hwang, 1989) within chloride cells. The trend for blood glucose was similar to that of cortisol. Nakano et al. (1998) report that after a transfer to SW, tilapia require higher blood glucose levels as an energy source for reorganization of osmoregulatory mechanisms. The hyperglycemic role of cortisol in providing energy for the increased physiological demands of tilapia transitioning to SW osmoregulation may be as important as its hormonal influences on gill microstructure. The glucocorticoid response to a SW transfer is probably two-fold: 1) as an initial glucocorticoid stress response to SW exposure and handling; and 2) to initiate physiological modifications and provide glucose for energy required for hyperosmoregulation.

Feeding regimens had no effect on plasma cortisol or blood glucose concentrations in tilapia transferred to SW. There were positive interactions between salinity and exposure time on plasma cortisol and glucose levels. For fish transferred to freshwater and 10 ppt SW, the values of plasma cortisol and blood glucose fluctuate only slightly during the course of exposure. In contrast, fish exposed to 20 ppt SW had a rapid increase in plasma cortisol, which peaked at 6 h then gradually declined to the pre-exposure value at 48 h. Blood glucose levels of fish exposed to 20 ppt SW increased rapidly during the initial adaptation period (6 h), then further increased to its peak after 12 h, and rapidly declined thereafter. However, at 48 h blood glucose values of 20 ppt SW exposed fish were still significantly higher than those of fish exposed to freshwater and 10 ppt SW. This suggests that juvenile Nile tilapia can be abruptly transferred from freshwater to 10 ppt SW with minimum osmotic stress. Fish directly transferred to higher salinity water (15 and 20 ppt), although all survived in both experiments, underwent a period of intense physiological stress indicated by sharp but temporary increases in plasma osmolarity and cortisol, and blood glucose levels followed by an adaptation period characterized by gradual decreases of these parameters as has been reported in earlier studies. At these salinities, the duration required to complete adaptation or to establish equilibrium with

the new medium was not met in the current study but has been reported to range from 7 to 10 days (Assem and Hanke, 1979; 1981; Bath and Eddy, 1983; Yada et al., 1994; Fontaínhas-Fernandes et al., 2001).

CONCLUSION

Juvenile Nile tilapia receiving dietary NaCl feeding regimens, even for a two-week period, exhibited consistently better growth and feed efficiency. Feeding NaCl-supplement may have a modest benefit to the osmoregulatory ability of Nile tilapia transferred sea water. Tilapia can be directly transferred from freshwater to 10 ppt SW with minimum stress. Transferring to higher salinity water may require gradual acclimation to reduce osmotic stress. Nile tilapia cannot be directly transferred from freshwater to 30 ppt SW, as death occurred after 4 h. It is also suggested that osmotic parameters evaluated should be monitored over a longer period (more than 96 h) to determine the duration at which osmotic stresses subside.

ANTICIPATED BENEFITS

These research results provide tilapia producers and researchers valuable information to improve growth performance, reduce osmotic stress, and improve survival of Nile tilapia through the use of NaCl-supplemented diets and proper acclimation water salinity.

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Table 1. Ingredient composition and estimated nutrient content of basal practical diet.

Ingredients	Percent in diet
Menhaden fish meal	8.0
Soybean meal	45.0
Corn meal	14.8
Wheat middlings	18.0
Dicalcium phosphate	1.0
CMC	3.0
Vitamin premix[1]	0.5
Trace mineral premix[2]	0.5
Corn oil	3.2
Celufil	6.0
Estimated Nutrients (%)	
Crude protein (%)	31.5
Crude fat (%)	6.0
D.E. (kcal/kg of diet)	2,850

[1]The vitamin mix, diluted in cellulose, provided the following in mg/kg diet: vitamin A (500,000 IU/g), 8; vitamin D_3 (1,000,000 IU/g), 2; vitamin K, 10; vitamin E, 200; thiamin, 10; riboflavin, 20; pyridoxine, 20; panthothenate, 200; nicotinic acid, 150; folic acid, 5; vitamin B_{12}, 0.02; biotin, 2; inositol, 400; choline chloride, 2,000; vitamin C, 100.

[2]Williams and Briggs mineral mix (U.S. Biochemical Co., Cleveland, Ohio) supplemented in mg/kg diet with aluminum potassium sulfate, 0.7; sodium selenite, 0.08; and cobalt chloride, 1.4.

Table 2. Mean final weight gain, dry matter feed intake, feed efficiency ratio (dry matter basis), and survival rate of Nile tilapia pre-acclimated by feeding practical diets with or without salt supplementation for 6 weeks before transferring into various salt levels of water (experiment I)[1].

Treatments	Weight Gain (*g*)	Feed Intake (*g*, DM-basis)	FER	Survival (%)
Control-C (6-wk C)	17.14	20.9	0.82[b]	98.5
4-wk C + 2-wk S	18.56	20.81	0.89[a]	98
2-wk C + 4-wk S	18.13	20.41	0.88[a]	100
Salt-S (6-wk S)	18.94	21.22	0.89[a]	98.5
Pooled SEM	0.46	0.31	0.01	0.98

[1]Means in the same column with different superscripts are significantly different ($P < 0.05$).

Table 3. Mean final weight gain, dry matter feed intake, feed efficiency ratio (dry matter basis), and survival rate of Nile tilapia pre-acclimated by feeding practical diets with or without salt supplementation for 6 weeks before transferring into various salt levels of water (experiment II)[1].

Treatments	Weight Gain (*g*)	Feed Intake (*g*, DM basis)	FER	Survival (%)
Control-C (6-wk C)	22.94	29.13	0.79	98
4-wk C + 2-wk S	24.39	29.62	0.82	97.5
2-wk C + 4-wk S	24.41	29.27	0.83	97.5
Salt-S (6-wk S)	24.36	29.81	0.82	95
Pooled SEM	0.46	0.37	0.01	0.94

[1]No significant differences were observed among various treatment means ($P > 0.05$).

Table 4. Mean blood glucose level and serum osmolarity of Nile tilapia pre-acclimated by feeding practical diets with or without salt supplementation for 6 weeks before transferring into various salt levels of water (experiment I).

Treatment	Blood Glucose *mg dL^{-1}*	Serum Osmolarity *mmol kg^{-1}*
DIET EFFECT (*P* LEVEL)	ns	0.0304
Control-C (6-wk C)	40.00	439.38[a]
4-wk C + 2-wk S	40.89	414.59[ab]
2-wk C + 4-wk S	39.11	390.60[b]
Salt-S (6-wk S)	40.28	415.41[ab]
SALINITY EFFECT (*P* LEVEL)	<.0001	0.0011
0	34.87[a]	395.73[b]
15	45.27[b]	434.26[a]
TIME EFFECT (*P* LEVEL)	0.0184	ns
48	37.65[a]	422.00
96	42.49[b]	407.99
Diet X salinity (*P* level)	ns	ns
Salinity X time (*P* level)	ns	ns
Diet X time (*P* level)	ns	ns
Diet X salinity X time (*P* level)	ns	ns
Pooled SEM	3.97	21.59

ns = Not significant at $P > 0.05$.

Table 5. Mean blood glucose level, hematocrit, serum osmolarity, and serum cortisol levels of Nile tilapia pre-acclimated by feeding practical diets with or without salt supplementation for 6 weeks before transferring into various salt levels of water (experiment II).

Treatment	Blood Glucose *mg dL^{-1}*	Hematocrit *%*	Serum Osmolarity *mmol kg^{-1}*	Serum Cortisol *ng mL^{-1}*
DIET EFFECT (*P* LEVEL)	ns	ns	ns	ns
Control-C (6-wk C)	76.17	28.19	401.62	99.69
4-wk C + 2-wk S	80.7	28.15	399.63	124.88
2-wk C + 4-wk S	75.43	27.94	396.08	112.48
Salt-S (6-wk S)	83.59	28.47	400.38	95.19
SALINITY EFFECT (*P* LEVEL)	<.0001	<.0001	<.0001	<.0001
0	52.56^{b}	29.83^{a}	354.05^{c}	80.04^{b}
10	59.08^{b}	27.13^{b}	379.46^{b}	80.91^{b}
20	131.88^{a}	27.19^{b}	475.82^{a}	165.44^{a}
TIME EFFECT (*P* LEVEL)	<.0001	<.0001	ns	0.0172
0	37.65^{d}	31.10^{a}	343.09	51.74^{c}
6	89.33^{ab}	27.89^{c}	401.44	138.36^{a}
12	95.99^{a}	29.28^{b}	398.99	125.47^{a}
24	82.77^{b}	27.96^{c}	407.41	107.95^{ab}
48	61.58^{c}	26.64^{d}	408.65	79.32^{bc}
Diet X salinity (*P* level)	ns	ns	ns	ns
Salinity X time (*P* level)	<.0001	ns	<.0001	0.0003
Diet X time (*P* level)	ns	ns	ns	ns
Diet X salinity X time (*P* level)	ns	ns	ns	ns
Pooled SEM	9.81	1.09	17.4	43.4

ns = Not significant at $P > 0.05$.

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