



Effects of salinity level on growth performance, feed utilization, and chromatic deformity of the hybrid Red tilapia, *Oreochromis niloticus* x *O. mossambicus*

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ABSTRACT

Four different salinities (0, 13, 26, and 39 ppt) were tested to evaluate their effects on growth performance, feed utilization, carcass composition, and chromatic deformity of the hybrid red tilapia (*Oreochromis niloticus* and *O. mossambicus*). 204 samples of 97 days post-hatch fingerlings red tilapia were stocked in a glass aquarium filled with 80 liters of water at a stocking rate of 17 fingerlings/ aquarium (0.21 fingerlings/L), with an average initial weight of 12.48 ± 0.04 g/fish and three replicates for each treatment. Fingerlings were fed on the experimental diet containing 30.3% protein at a 5% feeding rate, and re-adjusted periodically every 10 days. The experimental period was 42 days. The results revealed that the final weight, weight gain, ADG, and SGR values increased significantly ($P \leq 0.05$) at the salinity level of 26 ppt, followed by 13 ppt salinity level. The survival % showed that all salinity levels had a high percentage without recording significant differences ($P > 0.05$) between treatments. The best FCR was recorded in a salinity level of 26 ppt with significant differences ($P \leq 0.05$) between the treatments. The highest values of PER, PPV, and energy gain were recorded at 26 ppt. Salinity levels significantly influenced dry matter, protein, ether extract, ash, and carcass energy under the investigated salinities. The lowest percent of the chromatic deformity was achieved at salinity 26 ppt. It could be concluded that cultivating hybrid red tilapia in salinity levels between 13 and 26 ppt would significantly improve growth performance and feed utilization and reduce the percentage of fish with chromatic deformity as well.

INTRODUCTION

Tilapia fish is a globally important aquaculture species because of its rapid growth rate, flavoursome flesh, and reproduction facility in captivity. Furthermore, its strong resistance to environmental stresses makes them widely proper in aquaculture (Thodesen *et al.*, 2013). In recent years, Red tilapia has been increasingly used for aquaculture production in many parts of the world, such as China, Malaysian and Thailand (Jayaprasad *et al.*, 2011). The Red tilapia genetics varieties are generally attributed to

the crossbreeding between mutant reddish-orange Mozambique tilapia (*O. mossambicus*) with other tilapia species like Nile tilapia (*O. niloticus*) and blue tilapia (*O. aureus*). Red tilapia has gained popularity due to its rapid growth rate, forfeit of the black membrane in the body cavity, salinity tolerance, and adaptability to most cultural systems (Pradeep *et al.*, 2014). However, in the main Red tilapia production area in the south of China, Red tilapia cultured in soil pond, and lack of heating supply in winter caused lots of deaths due to sudden cooling. Acclimation to low salinity 10–15 ppt reduces the lower water temperature limit of many tilapia fish species, which seems to be a good method (Zale & Gregory, 1989).

Though basically a freshwater fish, many tilapia species are euryhaline and can be cultured in freshwater, brackish water, or saltwater (Hassan *et al.*, 2013). Salinity may affect the growth, osmoregulatory, metabolic modifications, biochemical parameters, physiological, and the heat shock protein gene expressions (HSPs) of some species (Tine *et al.*, 2010; Lavery & Skadhauge, 2012; Vargas-Chacoff *et al.*, 2015).

Salinity impresses energy disbursement in fish; there is a significant energetic cost associated with fish's mechanisms to maintain osmotic balance (Bœuf & Payan, 2001). Osmoregulatory cost is generally lowest under isosmotic conditions (Likongwe *et al.*, 1996) and can increase when swimming from balanced to variable salinity environments (Hutchinson & Hawkins, 1990). However, an isosmotic environment is not preferential for all species where optimal salinity in terms of growth and condition can vary during ontogenetic development (Allen & Cech, 2007).

The present experiment was carried out to investigate the effect of salinity on fish performance, feed utilization, carcass composition, and chromatic deformity of hybrid Red tilapia under laboratory conditions and identify the optimal rearing salinity. Besides, the aim was to provide a theoretical and experimental basis to support the farming of hybrid Red tilapia and a method to improve fish coloration.

MATERIALS AND METHODS

1. Experimental Fish

Apparent healthy 204 Red tilapia 97 days post-hatch (dph), with an average initial body weight of 12.48 ± 0.04 g/fish, were used in this experiment. Fish were obtained from Fish Farming and Technology Institute, Suez Canal University, Ismailia, Egypt.

2. Rearing condition and experimental design

Red tilapia fingerlings were stocked in a glass aquarium with a water volume of 80 liters at a stocking rate of 17 fingerlings/aquarium (= 0.21 fingerlings/L). Fish were tested with four different salinities (0, 13, 26, and 39 ppt). The rate of water exchange is 20 % per day. Each aquarium was supported with continuous artificial aeration through an air blower. Each treatment was performed in three replicates, and the experiment lasted for 42 days (6 weeks).

3. Feeding protocol

Fingerlings were fed on the experimental pellet diet, size 1.5 mm containing 30.3% protein, 6.26% lipids, 3.47% fiber, 449.5 kcal/100g gross energy, and 67.41mg CP: Kcal purchased from Aller Company in Egypt. Fish were fed at a 5% feeding rate and re-adjusted periodically based on the average fish body weight every 10 days.

4. Measured parameters of the experiment:

Water quality measurements

Water temperature, salinity, pH, total ammonia-nitrogen (TAN) and un-ionized ammonia (NH₃) were monitored weekly throughout the experimental period. Temperature and pH were measured using a portable pH Meter (pH-8424) (HANNA Instrument). Dissolved oxygen was measured by HI-9142 (HANNA Instrument). Total ammonia nitrogen (TAN) was monitored by HANNA HI96715-11 Ammonia Medium Range photometer once a week. The concentration of un-ionized ammonia was calculated as a percentage of TAN according to U.S. Environmental Protection Agency.

Survival and growth performance parameters

Survival %

$$\text{Survival (\%)} = 100 \times (\text{final number of fish} / \text{initial number of fish})$$

Final body weight (FW), weight gain (WG), average daily gain (ADG), and specific growth rate (SGR), length, length gain, and condition factor were conducted according to **Brody (1954)** following equations:

$$\text{Weight gain (g/fish): } WG = W_t - W_0$$

Where: W_0 : the initial mean weight of fish in grams.

W_t : the final mean weight of fish in grams.

$$\text{Average daily gain (g/fish/day): } ADG = (W_t - W_0) / n$$

Where: n: duration period.

$$\text{Specific growth rate (\%/day): } SGR = 100 \times [(\ln W_t - \ln W_0) / \text{days}]$$

Where: ln: natural logarithm.

$$\text{Length gain (cm)} = L_t - L_0$$

Where: L_0 : initial mean length of fish in cm.

L_t : final mean length of fish in cm.

$$\text{Condition factor} = 100 \times (\text{BW (g)} / L^3 \text{ (cm)}).$$

Feed and nutrients utilization parameters

Feed Intake (g/fish) = the amount of feed given during the experimental period/fish (g).

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)} / \text{weight gain (g)}.$$

$$\text{Protein efficiency ratio (PER)} = \text{gain} / \text{protein intake}.$$

$$\text{Protein productive value (PPV \%)} = 100 \times \text{gained protein} / \text{protein fed}.$$

$$\text{Energy retention (ER \%)} = 100 \times \text{gained energy} / \text{Energy fed}.$$

The protein efficiency ratio, Protein productive value, and Energy retention were calculated according to **Weatherly and Gill (1989)**

5. Fish and Feed Analytical methods

At the beginning and the end of each experiment, fish and feed samples were taken to determine the proximate composition analyses of diets and fish, including moisture, protein, lipid, and ash contents. One sample of fish larvae on the day of stocking was taken randomly for body chemical analysis. Whole fish body moisture, crude protein, and crude fat contents, on a dry matter basis, were determined according to **AOAC (2000)** methodology.

Energy content (Kcal/100g)

Gross energy (GE) content of the diets was estimated according to the following equation:

Gross energy GE (kcal/100g) = (protein content x 5.64) + (Lipid content x 9.44) + (carbohydrate content x 4.1).

6. Chromatic deformity

The chromatic deformity was measured at the end of the experiment using **ImageJ 3 software** (National Institute of Mental Health, Bethesda, Maryland, USA). Photos for three fish samples of each aquarium were taken using a high-resolution camera. Each photo was analyzed (12 samples per treatment) regarding the chromatic deformity for each image's fixed area and obtained a reading number of chromatic deformities for each sample. Then, the obtained readings for 36 samples were statistically analyzed.

Statistical analysis

The mean values and standard Error (mean \pm S.E) for each parameter were first calculated. The results were subjected to statistical analysis, one-way analysis of variance (ANOVA), using (**SPSS software program, Version 22**) to assess the effect of different salinity levels on survival, growth performance, feed utilization, carcass composition, and chromatic deformity. Differences between means were compared using LSD according to (**Calinski *et al.*, 1981**) using SPSS (version 22).

RESULTS

1. Water quality

Results of water quality parameters are illustrated in Table (1). Water quality was significantly ($P \leq 0.05$) affected by the increase in salinity concentration. Dissolved oxygen decreased significantly with increasing salinity. Total ammonia nitrogen (TAN) and un-ionized ammonia (NH_3) increased significantly with increasing salinity.

2. Growth performance

The growth efficiency of Red tilapia fish after 6 weeks under different levels of salinity is presented in Table (2). The final weight, weight gain, ADG, and SGR values w increased significantly ($P \leq 0.05$) at salinity level 26 ppt followed by 13 ppt salinity level. Still, no significant difference was located between control 0 ppt and 39 ppt. The survival % was high at all salinity levels ranged from 78.43 (at 39 ppt) to 84.31 (at 26 ppt) without significant differences ($P > 0.05$) between treatments.

Table 1: Effects of salinity level on water quality parameters of Red tilapia, *O. niloticus* x *O. mossambicus*.

Salinity (ppt)	Temperature, ($^{\circ}\text{C}$)	Oxygen (ppm)	pH	TAN (ppm)	NH_3 (ppm)
0	24.87 \pm 0.12	6.50 \pm 0.06 ^{ab}	7.78 \pm 0.015 ^{ab}	0.20 \pm 0.014 ^c	0.009 \pm 0.001 ^c
13	24.63 \pm 0.09	6.60 \pm 0.06 ^a	7.74 \pm 0.023 ^b	0.17 \pm 0.012 ^c	0.005 \pm 0.001 ^d
26	24.77 \pm 0.07	6.30 \pm 0.06 ^{bc}	7.72 \pm 0.012 ^b	0.53 \pm 0.040 ^b	0.014 \pm 0.001 ^b
39	24.57 \pm 0.12	6.17 \pm 0.09 ^c	7.87 \pm 0.050 ^a	0.67 \pm 0.052 ^a	0.022 \pm 0.002 ^a

Table 2: Effects of salinity level on fish performance and the survival of Red tilapia, *O. niloticus* x *O. mossambicus*.

Salinity (ppt)	Final weight (g/fish) *	Gain (g/fish)	ADG (g/fish/day)	Specific growth rate (%/day)	Survival (%)
0	29.83±0.22 ^c	17.40±0.26 ^c	0.41±0.01 ^c	2.08±0.03 ^c	80.39±1.96
13	31.13±0.07 ^b	18.59±0.11 ^b	0.44±0.00 ^b	2.16±0.01 ^b	82.35±3.40
26	33.15±0.25 ^a	20.75±0.19 ^a	0.49±0.00 ^a	2.34±0.01 ^a	84.31±1.96
39	29.47±0.31 ^c	16.92±0.21 ^c	0.40±0.00 ^c	2.03±0.01 ^c	78.43±1.96

*Initial weight = 12.48±0.04 g/fish

3. Feed utilization

The effects of salinity levels on feed utilization of red tilapia are given in Table (3). The best and lowest significant ($P \leq 0.05$) results of FCR were recorded in salinity level 26 ppt (1.46), comparing to other treatments. Significant highest values of PER, PPV, and energy gain were observed at 26 ppt (2.27, 63.88, and 33.50, respectively). Energy utilization values showed a significant ($P \leq 0.05$) decrease at different salinity levels compared to the highest value at 0 ppt (495.23). However, the lowest value was observed at 13 ppt.

Table 3: Effects of salinity level on the feed utilization of Red tilapia, *O. niloticus* x *O. mossambicus*.

Salinity (ppt)	FCR	PER	PPV	Energy gain (Kcal)	Energy utilization (%)
0	1.93±0.04 ^a	1.71±0.04 ^b	38.10±0.95 ^c	23.63±0.41 ^c	495.23±0.41 ^a
13	1.77±0.09 ^a	1.87±0.09 ^b	41.56±1.95 ^{bc}	22.62±0.10 ^c	478.47±0.16 ^d
26	1.46±0.04 ^b	2.27±0.05 ^a	63.88±1.57 ^a	33.50±0.24 ^a	480.50±0.03 ^c
39	1.88±0.01 ^a	1.75±0.01 ^b	43.96±0.12 ^b	30.64±0.46 ^b	491.12±0.38 ^b

3. Carcass composition

Effects of salinity level on carcass composition of red tilapia are presented in Table (4) with significant differences ($P \leq 0.05$) among treatments. Salinity levels significantly influence dry matter, protein, ether extract, ash, and carcass energy under the investigated salinity. Fish at 39 ppt recorded significantly ($P \leq 0.05$) higher dry matter, ether extract, and ash content, but the highest protein content and carcass energy were observed at 13 ppt.

Table 4: Effects of salinity level on carcass composition of Red tilapia, *O. niloticus*, x *O. mossambicus*.

Salinity (ppt)	Dry matter (%)	Protein (%)	Ether Extract (%)	Ash (%)	Carcass energy (Kcal/100g)
0	24.50±0.08 ^c	75.51±0.06 ^c	7.35±0.02 ^b	17.15±0.03 ^c	146.22±0.92 ^c
13	23.70±0.10 ^d	78.48±0.01 ^a	3.80±0.01 ^d	20.68±0.03 ^a	178.46±0.96 ^a
26	28.90±0.06 ^b	78.20±0.03 ^b	4.18±0.01 ^c	17.65±0.02 ^b	156.85±1.52 ^d
39	29.93±0.09 ^a	66.89±0.02 ^d	12.06±0.03 ^a	20.74±0.04 ^a	172.03±0.36 ^b

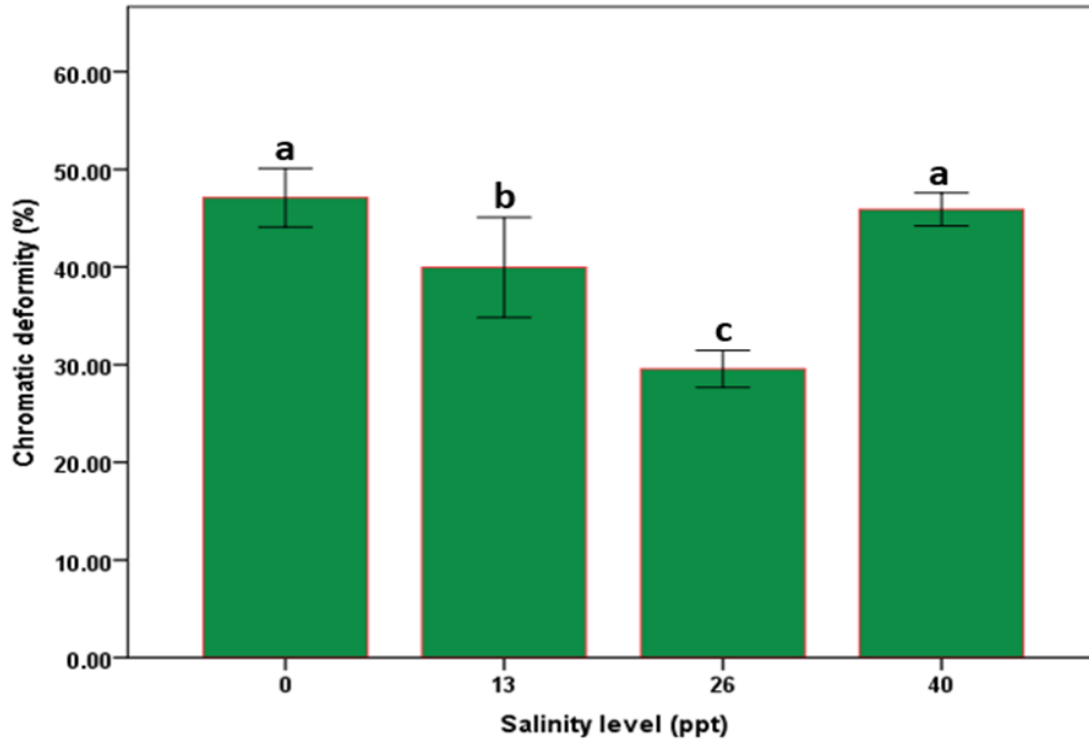


Figure 1. Chromatic deformity analysis of Red tilapia fish samples reared at four salinity levels (0, 13, 26, and 39 ppt). Data are average of 3 reading of each treatment.

4. Chromatic deformity analysis of the fish sample

Chromatic deformity analyses were performed by ImageJ 3 software. Data showed that deformity in Red tilapia's body color was significantly ($P \leq 0.05$) affected by water salinity (Figure 1). The lowest chromatic deformity percent value was achieved at salinity 26 ppt followed by 13 ppt, while at salinities 0 and 39ppt, fish exhibited a higher level of chromatic deformity. Histogram of count masks of Red tilapia fish samples reared at four levels of salinity are shown in Figure (2).

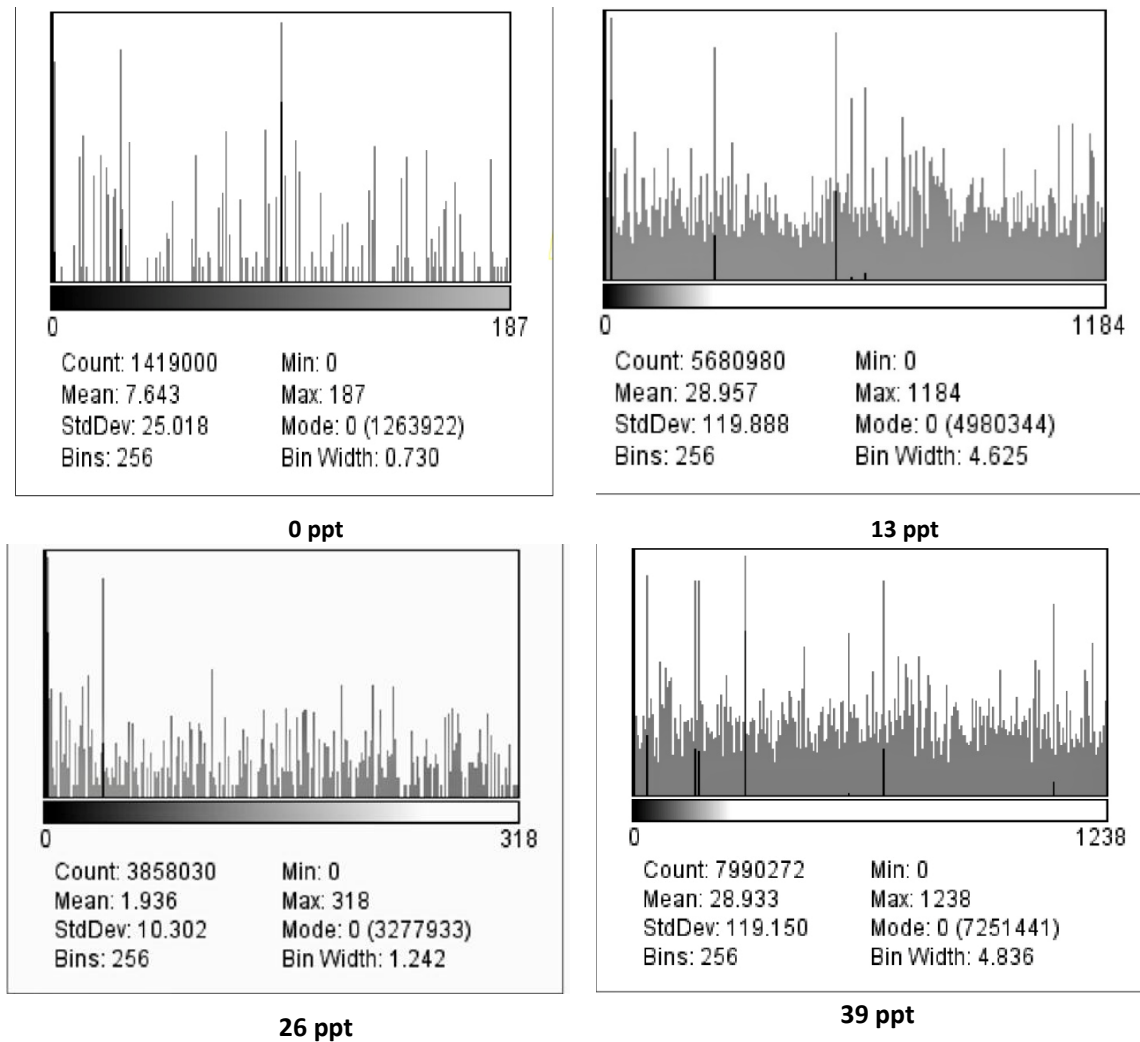


Figure 2. Histogram of count masks of Red tilapia fish samples reared at four salinity levels (0, 13, 26, and 39 ppt).

DISCUSSION

The present study results showed that salinity influenced the water quality, fish performance, feed utilization, carcass composition, and chromatic deformity of hybrid Red tilapia under experimental conditions. According to the current experimental data, water quality parameters were within the safe limits to tilapia culture (**Setiadi et al., 2018**). The optimal range of dissolved oxygen must be over 3 ppm (**Cavalcante et al., 2014**), and total ammonia nitrogen (TAN) should be in the range of 0.17-3.87 ppm (**Caldini et al., 2015**). The un-ionized ammonia (UIA) level with no long-term adverse effects on growth or survival was calculated based on the method of **Tomasso (1994)**; it was found to be 0.131, 0.120, and 0.088 ppm UIA at 24-48, 72, and 96 h exposure time, respectively.

The obtained results showed a significant ($P \leq 0.05$) increase in red tilapia's growth performance at 26 ppt salinity followed by 13 ppt's salinity comparing with 0 and 39 ppt.

There are insignificant differences between salinity levels on survival rate. The obtained results agree with **Liao and Chang (1983)** findings, who reported that hybrid Red tilapia in brackish water and seawater grew faster than in freshwater. **Sallam *et al.* (2017)** noted that the best final body weight, weight gain, average daily gain, specific growth rate, and survival rate of red tilapia were observed at water salinity 36 ppt and 24 ppt, respectively.

Moreover, red tilapia's growth performance enhanced significantly with the increase of water salinity due to minimal metabolic energy diversion into osmoregulation (**Sallam *et al.*, 2017**). In Red tilapia culture, SGR increased with salinity (**Watanabe *et al.*, 1988a,b**). Concerning the comparison between Red tilapia and Nile tilapia on final weight and body weight gain under salinity levels, it was found that the final weight and body weight gain of Red tilapia was higher than Nile tilapia (**Sharaf *et al.*, 2013**).

According to **Watanabe *et al.* (1993)**, The growth rates of Florida Red tilapia, reared at different salinities, increased with increasing the temperature within the range 22°C to 32°C that supports the obtained results. The experimental period was conducted in a similar range of temperatures, which favored Florida Red tilapia's growth performance. The increase in the metabolic energy diverted into osmoregulation, with increasing water salinity, has also been reported in *O. niloticus* × *O. aureus* and common carp (**Payne, 1983**), *O. mossambicus*, and *O. spilurus* (**Payne *et al.*, 1988**). The high growth performance of hybrid Red tilapia in saline water might be attributed to higher osmoregulation energy costs in freshwater compared to brackish water or seawater (**Febry & Lutz, 1987**), repressed and restrained territorial aggressiveness by salinity (**Watanabe *et al.*, 1988**). Notably, the aggressive behavior was changed among different salinities (**Liao & Chang, 1983**).

On the other hand, **Hassan *et al.* (2013)** noted that the mortality rate of Red tilapia fingerlings increased with increasing the salinity level from 0 ppt to 35 ppt. **Sharaf *et al.* (2004)** revealed that the survival of the Red tilapia decreased from 98% to 79.2%, with increasing the salinity from 16 ppt to 40 ppt for 12 days. **El-Zaeem, SY *et al.* (2012)** stated that Florida Red tilapia fry's final body weight decreased significantly from 33.38 g to 18.68 g with increasing the salinity from 16 ppt to 32 ppt, respectively, after a gradual acclimation period.

The obtained results of feed utilization of Red tilapia under the effects of salinity levels showed significant differences ($P \leq 0.05$) among treatments. The best results of FCR were found in 26 ppt salinity level and decreased in the salinity levels of 13 and 39 ppt than that of the control treatment. In the present study, the high values of PER, PPV, and energy gain were observed under salinity level 26 ppt. Energy utilization in the present study decreased significantly under salinity levels; the high value was recorded in 0 ppt while the low value was observed at 13 ppt. The obtained results are in agreement with the findings of **Sallam *et al.* (2017)**, who reported that when exposed to medium and high salinity levels, the offspring delivered from Red tilapia brood stock subjected to elevated salinity levels showed a better significance ($P \leq 0.05$) FCR and PER than those of the control with 0 ppt and salinity level 9 ppt. Therefore, it is crucial to culture Florida Red tilapia brood stocks in marine water that induces and enhances the fry growth performance and feed utilization in a marine environment without being acclimatized. **Suresh and Lin (1992)** reported that a better FCR was observed in some tilapia species reared in saline water when compared to those raised in freshwater. Where feeding was not restricted, Florida Red tilapia feed consumption increased, and FCR improved with

high salinity (**Watanabe *et al.*, 1988**). These results are incongruent with the findings of **El-Zaeem, SY *et al.* (2012)** who found that FCR elevated significantly Florida red tilapia fry with increasing the salinity from 16 ppt to 32 ppt.

In this study, a significant positive increase in crude protein content was recorded at salinity levels of 13 and 26 ppt compared to salinity levels of 0 and 39 ppt, with the lowest protein range at 39ppt. The obtained results agree with **Srouf (1999)** and **Sharaf *et al.* (2013)**. The previous authors reported that with increasing the salinity levels, the crude protein content increased, where the high value was gained at salinity level of 17 ppt followed by salinity 28 ppt. **Mazid *et al.* (1979)** and **Jauncey (1982)** reported that the whole-body protein content of fish, reared in seawater, was not significantly affected by changing dietary protein levels. The dry matter of the fish body was significantly affected by the salinity levels. The lowest value of dry matter was gained at salinity level of 13 ppt followed by 0 ppt, and dry matter values increased with increasing the salinity levels at 26 and 39 ppt, which is in agreement with the findings of **Sharaf *et al.* (2013)**. In the present study, the ether extract percentage of the fish body decreased under salinity levels, increasing at 13 and 26 ppt. Still, at the salinity level of 39 ppt, the EE increased recording a high percentage. The carcass energy of the fish body increased with increasing the salinity levels. The obtained results are in agreement with **Srouf (1999)** and **Sharaf *et al.* (2013)**, who reported that E.E of the fish body decreased with increasing salinity at 17 and 28 ppt and disagree with **El-Ebiary *et al.* (1997)**, who mentioned that the ether extract's highest value was observed in Red tilapia hybrid reared in brackish water. This may be attributed to the massive amount of energy that is absent during the osmoregulation process in freshwater.

The purity of carcass color in Red tilapia is one of the most vital features supporting the rise of the demand with profitable selling price (**Aly *et al.*, 2017**). The impacts of salinity on fish body color have not been studied before considering Red tilapia. However, the lowest value of chromatic deformity was recorded when Red tilapia was reared at 26ppt salinity might be attributed to the lowest value of stressful releasing hormones like cortisol (**Volpato & Barreto, 2001; Templonuevo & Vera Cruz, 2016**) and glucocorticoid levels (**Backström *et al.*, 2014**). Under healthy optimal rearing conditions with blue color, Red tilapia displayed a lower percentage of fish with chromatic deformity (12%)(**Aly *et al.*, 2017**). Among the ambient rearing conditions that affect fish coloration are: substrate color, light intensity (**Lin *et al.*, 2009; Yasir & Qin, 2009**), tank color (**Imanpoo & Abdollahi, 2011**), and light color (**Aly *et al.*, 2017**).

CONCLUSION

It could be concluded that cultivating hybrid Red tilapia in salinity levels between 13 and 26 ppt would significantly improve growth performance, feed utilization, the quality of fish in terms of dry matter, protein, lipids, and the energy utilization of the consumed feed and chromatic deformity. The positive effects of salinity levels of 13 and 26 ppt obtained in this study might be used in Red tilapia culture in a simple, economical, and sustainable way to improve fish growth, feed utilization, fish quality, fish coloration and the final production.

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