

## Does the polyculture system of thinlip grey mullet, *Chelon ramada* (Risso, 1827) have positive impacts on water quality, fish performance, and hematological analyses of hybrid red tilapia, *Oreochromis mossambicus* × *O. urolepis* reared in concrete tanks with underground brackish water?

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**Abstract.** A field study was conducted to assess the effect of mono- and polyculture systems of Florida red tilapia (*Oreochromis* sp. and thinlip grey mullet (*Chelon ramada*) in concrete tanks. Growth performance, feed efficiency, body chemical composition, and health status of the experimental fish were evaluated after 42 days rearing period. Five experimental treatments were carried out as follow: T1= 100% red tilapia; T2 = 75% red tilapia and 25% mullet; T3 = 50% red tilapia and 50% mullet; T4 = 25% red tilapia and 75% mullet; and T5 = 100% mullet. Fish of T1 showed the highest values of growth performance, feed utilization, and productivity, while T5 showed the lowest levels. Mullet did not show any significant positive effect when co-cultured with red tilapia in a concrete tank. Rearing red tilapia under the monoculture system is much better than the polyculture system with mullet as the latter species have one-third growth performance, feed utilization, and productivity that of red tilapia. Contrary to what was expected, the co-culture of mullet did not significantly improve the parameters of water quality, fish performance, flesh quality, blood analyses, and heavy metals content in red tilapia.

**Key Words:** *Chelon ramada*, Florida red tilapia, growth performance, length-weight relationship, feed utilization, blood analyses, heavy metals content, concrete tanks.

**Introduction.** Due to the increase in the human population and the decline of wild fisheries, aquaculture becomes one of the fastest-growing food-producing sectors in the aquatic field. It plays a crucial role in meeting the rising demand for seafood products (Sing et al 2014). Recently, aquaculture represents 47% of the total global fish production, contributing to the most massive fish supply for human consumption (FAO 2018). The increase of fish production from aquaculture led to a rise in per capita consumption of fish to 20.3 kg in 2016 compared to 9 kg in 1961 (FAO 2018). The growth of the aquaculture sector in Egypt has gradually increased over the past three decades. Egypt occupies the 9th rank of global fish producers and the 3rd in global tilapia production after China and Indonesia (FAO 2018). In Egypt, aquaculture has become the main source of animal protein to cope with the country's growing population (GAFRD 2019).

The development of aquaculture systems and technology aims to increase productivity and minimize the environmental impacts of aquaculture to ensure food safety and sustainability (Bakeer et al 2008). The increase of aquaculture production can be achieved through the popular application of the polyculture system with different fish

species (Abdel-Hakim et al 2012). Polyculture is referred to as multi-trophic aquaculture, or simply integrated aquaculture, which consists of adding one or more fish species to the culture system of the main species (Bunting 2008). The use of integrated aquaculture systems may be an alternative way to improve nutrients retention in aquaculture. Additionally, polyculture is one approach to achieve aquaculture development. The mixture of fish species gives better utilization of available natural food produced in fish ponds (Griegel 2003). El-Sagheer et al (2008) indicated that the polyculture system showed the highest growth performance and survival rate compared with the monoculture system. Most of the aquaculture production in Egypt is pond-based using polyculture farming techniques (GAFRD 2010). Nile tilapia, *Oreochromis niloticus* is usually reared in polyculture systems with different species such as common carp, *Cyprinus carpio* (Abdel-Hakim et al 2012), striped mullet, *Mugil cephalus* (Tahoun et al 2013), African catfish, *Clarias gariepinus* (Shoko et al 2014) and European eel, *Anguilla anguilla* (Abdel-Hakim et al 2001).

The limited sources of freshwater in Egypt, together with the competition for agriculture and other urban activities, have increased the pressure to develop aquaculture in brackish and seawater (El-Sayed 2006). Though using salt water instead of freshwater in fish farming is a worldwide priority (El-Sayed 2006). Due to the increasing lack of freshwater globally, it would be beneficial to culture tilapia stocks in brackish or saline rearing environments to ensure a source of cheap and high-quality animal protein into the future (Mateen 2007). Groundwater is preferred as it maintains a constant temperature, free of parasites and larvae of predatory insects, and is usually less contaminated than surface water. However, it may contain high or reduced iron concentrations, high chemical hardness, and typically lacking oxygen (Lawson 1995).

*Oreochromis mossambicus* and its hybrids, including red tilapia, are the major representatives of these euryhaline cichlids in aquaculture (Tayamen et al 2002). Red hybrid tilapia are gaining popularity among fish farmers due to their resemblance to premium marine species. The ease of adaptation to culture conditions and confinement, salinity tolerance, and its attractive color that enhances the retail value (Nakphet et al 2017) are among the principal reasons for its popularity. Florida red tilapia strain grows well in high saline water (Watanabe & Fitzimmons 2006). It can tolerate salinity up to 36.2 ppt, and the optimum limit is 17.8 ppt (El-Sayed 2006), while Nile tilapia does not tolerate salinities above 20 ppt (Baroiller et al 2000). Red tilapia with *O. mossambicus* ancestors perform better than Nile tilapia in salinities over 10 g L<sup>-1</sup> because they are more salt-tolerant than Nile tilapia. Therefore, the world is moving towards expanding the cultivation of red tilapia, the most resistant strain to high saltwater (Sharaf et al 2013). Red tilapia is becoming more popular for aquaculture in certain markets of the world, such as China, Malaysia, and Thailand, primarily because of its uniform red color skin and the absence of black peritoneum (Pradeep et al 2014). Furthermore, red tilapia has several characters that make it an important farm fish, i.e., less off-flavor and pleasant taste (muddy off-flavor is more prevalent in tilapia raised in freshwater than in saltwater), ease to culture, high resistance to disease, omnivorous feeding behavior, short generation time as well as faster growth (Islam et al 2006). Red tilapia are grown to market size using various culture techniques similar to those used to culture pure-line tilapia species. Red tilapia are grown in fresh and saltwater in cages, concrete tanks, and earthen ponds using semi-intensive and intensive systems (Watanabe et al 1997).

*Chelon ramada* is native in the Eastern Atlantic from the coasts of Southern Norway to Morocco, including the Mediterranean and the Black Sea (Wonham et al 2000). It is a euryhaline and catadromous species that enters coastal lagoons during early life stages to fulfill growth previous to sexual maturation (Papa et al 2003). In addition to this, *C. ramada* is an important and attractive species for farming in the sea, brackish, and freshwater. The culture of mullet is closely dependent on the availability of its fry (Mousa 2010). The availability and abundance of the wild fry of this species as compared to those of *Mugil cephalus* make it the dominant aquaculture species (Sadek & Mires 2000). In comparison with other mullet species, these fish have a higher resistance to environmental changes; and, in turn, higher survival rates (Bardach et al 1972). Therefore, *C. ramada* is an excellent candidate for the intensive culture in Egypt. In

Egypt, mullet fish, especially *M. cephalus* and *C. ramada* are economically very important fish because they have high market value and have been cultivated successfully by fish farmers (GAFRD 2019).

Studies on the co-culture of hybrid red tilapia with thinlip grey mullet, *C. ramada* are lacking in Egypt. Therefore, the present work aimed to study the effect of monoculture and polyculture of hybrid red tilapia and *C. ramada* reared in concrete tanks using medium saline water on water quality, growth performance, feed utilization, body chemical composition, blood parameters, and heavy metals content under different culture systems.

## Material and Method

**Description of the study sites.** This experiment was carried out in Baltium experimental station, National Institute of Oceanography and Fisheries (NIOF), Ministry of Scientific Research, and lasted six weeks during 21 June - 1 August 2018 using cement tanks.

### Experimental fishes

**Red tilapia (*Oreochromis spp.*).** Apparent healthy hybrid red tilapia larvae "Florida strain" (*O. mossambicus* × *O. urolepis*) with a total number of 1500 individuals, with an average initial body weight of  $1.02 \pm 0.01$  g fish<sup>-1</sup>; initial total length =  $3.67 \pm 0.03$  cm fish<sup>-1</sup> obtained from a governmental marine fish hatchery located in Alexandria, K21, Egypt. The fish were transported in well-aerated oxygen bags. Larvae were acclimatized for one week to well water with salinity 20 ppt.

**Thinlip grey mullet (*Chelon ramada*).** Mullet larvae used in the present study were collected from El-Kassara fry collection station, Gamasa, Egypt with a total number of 1500 individuals, with an average initial body weight of  $2.5 \pm 0.04$  g fish<sup>-1</sup>; initial total length =  $5.49 \pm 0.06$  cm fish<sup>-1</sup>. The fish were transported in well-aerated oxygen bags, and acclimatized for one week to well water with salinity 20 ppt.

**Culture conditions.** Florida red tilapia and thinlip grey mullet were stocked into cement tanks (4.7 m \* 2.5 m \* 1.0 m) with a water volume of 11.75 m<sup>3</sup> at a stocking rate of 200 fish per tank (17 fry m<sup>-3</sup>). The fish fry was fed 3 times daily (at 8:00 AM, 12:00, 3:00 PM) with satiation on powder diets (40% protein + 5.3% lipids + 4.7% fibers) a product of Koudijs company in Egypt for three weeks then with pelleted diet (30% protein - 6% lipids - 4.5% fibers - 4100 Kcal GE) which was purchased from Nile Valley Company in Egypt for three weeks. This experiment continued for six weeks.

**Experimental design.** Five treatments in three replicates per each were performed using different culture systems. Fifteen concrete tanks were used for this. Treatments were tested as follow:

1. 100% red tilapia (200 fish RT);
  2. 75% red tilapia and 25% mullet (150 fish RT + 50 fish M);
  3. 50% red tilapia and 50% mullet (100 fish RT + 100 fish M);
  4. 25% red tilapia and 75% mullet (50 fish RT + 150 fish M);
  5. 100% mullet (200 fish M);
- RT = red tilapia; M = mullet.

The concrete tanks used in this study were filled with 11.75 cubic meters of underground brackish water with a salinity 20 ppt. Each tank was supported with an artificial aeration system through air blower. The aeration system was operated daily from 2 pm to 8 am throughout the experimental period. A volume of 20% of the rearing water was exchanged daily to maintain a safe limit of dissolved oxygen (DO). Every two weeks, the entire tank water was changed and the fish measurements (total length and weight) were measured. Water temperature, salinity, pH and DO were monitored twice a week while ammonia (NH<sub>3</sub>) and nitrite were monitored once a week throughout the experimental period.

**Physico chemical measurements.** Water temperature, pH, and DO were measured by SensoDirect 150 MultiMeter. The water salinity was measured by a refractometer (<http://www.atago.net>). Ammonia (NH<sub>3</sub>) and nitrite were measured by YSI 9300 photometer and HANNA HI83399 benchtop photometer.

### **Blood analysis**

**Blood sampling.** The blood samples were collected from the caudal vein according to Feldman et al (2000).

**Erythrocytic and leukocytic counts determination (RBC<sub>s</sub>–WBC<sub>s</sub>).** The erythrocytes and leukocytes were counted according to the method described by Stoskopf (1993) using hemocytometer and Natt-Herrick solution.

**Hemoglobin concentration determination (Hgb).** Hemoglobin concentration was determined using the cyanomet hemoglobin method Drabkin's solution, according to Stoskopf (1993). The cyanomet hemoglobin method converts all hemoglobin derivatives to methemoglobin using ferricyanide and cyanide ion. Methemoglobin is a stable red compound and can be measured color metrically.

**Packed cell volume determination (PCV).** According to Dacie & Lewis (1991), the microhematocrit method was used for the estimation of PCV%.

**Determination of differential leukocytic count (DLC).** Thin blood films were obtained, air-dried, fixed with methanol for 3-5 min and stained with Giemsa stain for 8-10 min, then rinsed with distilled water and left to dry. The white blood cells were counted among one hundred of a blood smear, according to Stoskopf (1993). The absolute DLC was calculated according to Thrall et al (2004), following the formula:

$$\text{Absolute DLC} = \text{no. of each white cell} \times \text{no. of total leukocytic count}/100$$

**Blood serum biochemical analysis.** Serum total proteins were determined according to Doumas et al (1981) at the wavelength of 540 nm, and serum albumin was estimated colorimetrically at wavelength 550 nm according to Doumas & Biggs (1972). Globulins' content was calculated mathematically by subtracting albumin from total protein. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically at the wavelength of 540 nm (Reitman & Frankel 1957). Glucose level (mg/100 mL) was determined using glucose enzymatic PAP (Trinder 1969) kits obtained from Bio-Merieux (France).

**Triacylglycerol (TG) and cholesterol (CHL) analysis.** They were determined with GPO-PAP and CHOD-PAP (commercial clinical kit) methods, respectively, according to Fynn-Aikins et al (1992).

### **Heavy metal analysis**

**Sample collection.** The collected fish were kept in clean plastic bags stored in the icebox and transported to the Laboratory of Zoology Department, Faculty of Science, Damietta University, where they were kept deeply frozen at -20°C until the samples were prepared for digestion and analysis.

**Sample preparation and digestion.** The preparation of fish muscle for heavy metal (Pb, Cd, Fe, Zn, Cu) measurements were carried out according to Bahnasawy et al (2011). In the Laboratory, before analysis, each fish was measured, weighed, dissected with sterilized stainless equipment. Parts of epi axial muscles were put in pre weighted clean, dry 25 mL beaker, dried in an oven for 48 hours at 80°C. The sample was then digested on a hot sand bath using concentrated nitric acid (HNO<sub>3</sub>) (69%) and concentrated perchloric acid (HClO<sub>4</sub>) (70%) with a 2:1 ratio.

Digestion was continued until the liquid becomes clear. When the digest becomes clear, it was allowed to cool, filtered through an acid-resistant filter paper and transferred to 25 mL volumetric flasks and diluted to the mark with distilled water and stored in clean, sterile plastic bottles until the determination of the selected heavy metals using an A Perkin Elmer Atomic Absorption Spectrometer (Pin AA cle 500). Metals concentrations in tissues were presented as  $\mu\text{g metal g}^{-1}$  dry weight. The values of the heavy metal concentrations in the tissues were calculated based on dry weights as this discount the variability due to inner parts differences in the moisture content of organisms.

### **Measured parameters for the experiment**

*Growth performance parameters.* Growth performance parameters of the tested fish were calculated according to Abdel-Rahim et al (2019). Weight gain (WG), average daily gain (ADG), specific growth rate (SGR), survival rate, length gain, and condition factor were conducted according to the following equations:

$$\text{Weight gain (g fish}^{-1}\text{): } WG = W_t - W_0$$

where:  $W_0$  = the initial mean weight of fish (g);

$W_t$  = the final mean weight of fish (g).

$$\text{Average daily gain (g fish}^{-1}\text{ day}^{-1}\text{): } ADG = (W_t - W_0)/n$$

where:  $n$  = duration period.

$$\text{Specific growth rate (\% day}^{-1}\text{): } SGR = 100 \times (\ln W_t - \ln W_0)/\text{days}$$

where:  $\ln$  = natural logarithm.

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

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

where:  $L_0$  = initial mean length of fish (cm);

$L_t$  = final mean length of fish (cm).

$$\text{Condition factor} = 100 \times (W_t \text{ (g)} / L_t^3 \text{ (cm)})$$

The length-weight relationship (LWR) for red tilapia and thinlip grey mullet was analyzed by measuring the length and weight of fish specimens collected from the study area. The statistical relationship between these parameters of fishes was figured and calculated using SPSS 22 software. The best equation was determined based on the highest value of R square and significance level.

### *Feed and nutrients utilization parameters*

Feed intake ( $\text{g fish}^{-1}$ ): this is the amount of feed given or supplied during the experimental period for each fish per gram.

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

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

$$\text{Protein productive value (PPV \%)} = 100 \times (\text{Pt} - \text{P}_0)/\text{protein intake (g)}$$

where:  $P_0$  = protein content in fish carcass at the start.

$P_t$  = protein content in fish carcass at the end.

$$\text{Energy gain, EG (Kcal): } EG = E_t - E_0$$

where:  $E_0$  = the energy content in the fish carcass (Kcal) at the start of the experiment;

$E_t$  = energy content in the fish carcass (Kcal) at the end of the experiment;

$$\text{Energy utilization (EU, \%)} = 100 [\text{Energy gain (Kcal/100g)} / \text{Energy intake (Kcal/100g)}]$$

**Fish and feed analytical methods.** At the beginning and the end of the experiment, fish and feed samples were taken to determine the proximate composition of diets and fish, including moisture, protein, lipid, and ash contents. One sample of fish larvae in 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 on dry bases as follows:

**Moisture content.** Water content was determined by drying a pre-weighed sample into an oven thermostatically regulated at 105°C overnight and then weighed until constant weight (complete dryness). The difference between the final and initial weights represents the water content of the sample:

$$\% \text{ Moisture} = 100 [\text{weight loss (g)}/\text{weight of sample (g)}]$$

**Crude protein content (CP).** Protein content (on a dry weight basis) was estimated as the total nitrogen content using the semi-automatic Kjeldahl (Model VELP Scientifica, UDK 127). Protein content was then calculated by multiplying total nitrogen by 6.25:

$$\% \text{ protein} = \% \text{ nitrogen content} \times 6.25.$$

**Ether extract (EE).** Lipid content was determined on a dry weight basis using a Soxhlet apparatus as described with petroleum ether as an extraction solvent:

$$\% \text{ lipids} = 100 [\text{weight of lipid (g)}/\text{weight of sample (g)}]$$

**Ash content.** Ash content was done by ignition of a pre-weighed dry sample in a porcelain crucible at 600°C, in a Muffle furnace, for about 2 hours. The difference between the final and initial weights equals the ash content:

$$\% \text{ ash content} = 100 [\text{weight of ash (g)}/\text{weight of dry sample (g)}]$$

**Energy content (Kcal/100g).** Gross energy (GE) was calculated as 5.64, 9.44, and 4.11 kcal/100g for protein, lipid, and NFE, respectively (NRC 1993):

$$\text{GE (kcal/100g)} = (\text{protein content} \times 5.64) + (\text{lipid content} \times 9.44) + (\text{carbohydrate content} \times 4.1)$$

**Statistical analysis.** Data were statistically analyzed with one-way ANOVA and Duncan's (1955) multiple range tests and expressed as mean values±SE. Effects with a probability of  $p \leq 0.05$  were considered significant. Statistical analyses were performed using SPSS 22 for Windows (Standard Version 22 SPSS Inc. Chicago, Illinois).

## Results

**Water quality.** Analysis of saline underground water is presented in Table 1. No significant differences ( $p > 0.05$ ) were observed in temperature, dissolved oxygen, salinity, and nitrite among treatments during the experimental period. However, total ammonia nitrogen (TAN) and pH indicate a significant difference ( $p \leq 0.05$ ) between treatments with the minimum values recorded for T1. TAN was at the lowest level for T1.

Table 1  
Analysis of water used in rearing red tilapia with mullet in different culture systems using medium saline underground water (data are means±SEM)

Treatments	Water quality parameters					
	Nitrite (ppb)	TAN (ppm)	Salinity (ppt)	DO (ppm)	Temp (°C)	pH
T1	2.10±0.26	0.24±0.05 <sup>b</sup>	20.31±0.46	6.68±0.24	29.95±0.29	8.20±0.09 <sup>b</sup>
T2	2.41±0.21	0.38±0.09 <sup>a</sup>	19.75±0.49	6.57±0.19	30.12±0.25	8.36±0.05 <sup>ab</sup>
T3	2.71±0.32	0.43±0.12 <sup>a</sup>	19.97±0.50	6.58±0.22	30.12±0.26	8.43±0.05 <sup>a</sup>
T4	2.74±0.22	0.41±0.12 <sup>a</sup>	19.94±0.49	6.57±0.23	30.17±0.26	8.43±0.05 <sup>a</sup>
T5	2.84±0.27	0.38±0.12 <sup>a</sup>	20.03±0.49	6.61±0.28	29.10±0.19	8.29±0.07 <sup>ab</sup>

Note: Means within the same column not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

### **Growth performance, condition factor, length-weight relationship, and survival.**

Growth performance of red tilapia integrated with mullet in different culture systems was presented in Table 2. The results indicated there were no significant differences ( $p > 0.05$ ) in the final body weight for mullet, but for red tilapia, there were significant

differences ( $p \leq 0.05$ ) in the final body weight, weight gain, ADG, and SGR parameters. The highest value of growth performance parameters was observed in T4. Condition factors for red tilapia were much higher than those of mullet with more than 2.2-folds. There were no significant differences ( $p > 0.05$ ) in the condition factor of mullet, while significant differences ( $p \leq 0.05$ ) were found in red tilapia under different culture systems. The statistical equation that summarizes the regression relationship between length and weight in red tilapia and mullet is exhibited in Figures 1 and 2, respectively.

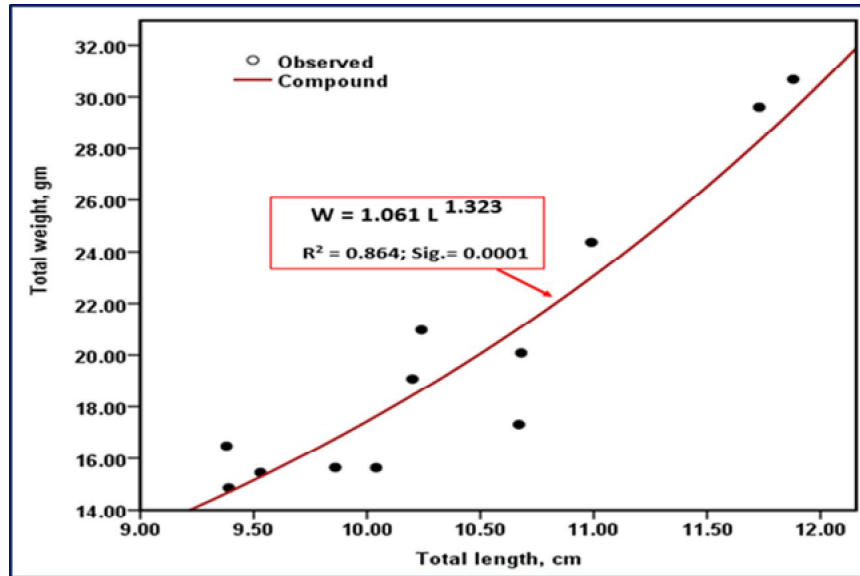


Figure 1. Length-weight relationship of red tilapia integrated with mullet in different culture systems in concrete tanks using medium saline underground water.

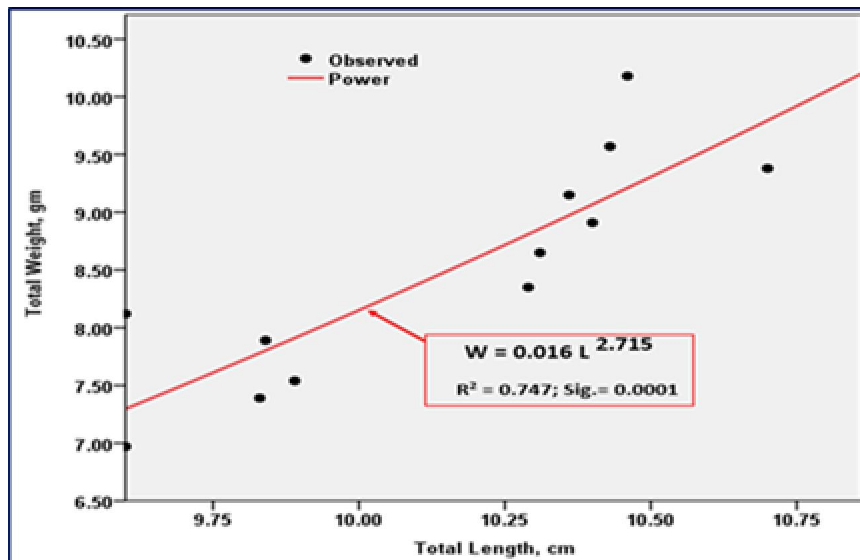


Figure 2. Length-weight relationship of mullet integrated with red tilapia in different culture systems in concrete tanks using medium saline underground water.

The compound equation expresses well the LWR in red tilapia through the following equation:

$$W = 1.061 L^{1.323} \quad (R^2 = 0.864; \text{Sig.} = 0.0001)$$

where: W = weight (in grams); L = length (in centimeters).

For mullet, the compound equation expresses well the LWR as the following equation:

$$W = 0.016 L^{2.715} (R^2 = 0.747; \text{Sig.} = 0.0001).$$

Survival (%) was 100% for both red tilapia and mullet.

**Fish biomass.** Red tilapia, mullet, and total fish biomasses are presented in Table 3. The results indicated that there were significant variations ( $p \leq 0.05$ ) among treatments. Red tilapia biomass was higher in T1 than in any other treatment, whereas mullet biomass was the highest in T5. Total biomass was the highest in T1.

**Feed utilization.** Feed utilization efficiency indices of red tilapia are presented in Table 4. The results indicated that there were significant differences ( $p \leq 0.05$ ) among treatments. FCR was lower in T1, and PER was more elevated in T1. The highest PPV value was observed in T1. Energy gain showed the highest value in T1. Better utilization of energy was detected in T1, where the highest value in T1 and the lowest value was obtained in T5. Mullet showed the worst data for feed utilization indices.

**The proximate body chemical composition.** The chemical analyses of red tilapia and mullet under different culture systems using medium saline groundwater are presented in Table 5. The results indicated that there were significant variations ( $p \leq 0.05$ ) in moisture, crude protein, ether extract, and ash content levels. For red tilapia, ash was higher in T4, whereas moisture was lower in T2. Crude protein value and ether extract were higher in mullet than in red tilapia, while moisture and ash were lower in mullet than in red tilapia. For mullet, there were no significant differences ( $p > 0.05$ ) in ash; whereas moisture was higher in T2. Crude protein value was the highest in T3, and ether extract was the highest in T5.

**Blood and serum analysis.** Results of blood and serum analysis of red tilapia are presented in Table 6. The results indicated that there were significant differences ( $p \leq 0.05$ ) among treatments. Hemoglobin, red blood cell counts, white blood cells count, and haematocrit were higher in T1, T2 and lower at T3, T4. Platelet count was the highest in T1 and the lowest in T4. Glucose content was higher in T3 in comparison to other treatments. The results indicated that there were no significant differences ( $p > 0.05$ ) in total protein. Albumin increased significantly ( $p \leq 0.05$ ) in T3 while globulin increased in T1. Additionally, the albumin/globulin ratio was the highest in T4, T3, respectively. The content of cholesterol decreased significantly ( $p \leq 0.05$ ) in T2 compared to other treatments. Triglyceride was the highest in T1, and the lowest value was obtained in T3. Blood urea increased significantly in T3 compared to other treatments. Amylase level increased significantly ( $P \leq 0.05$ ) in T3, T4. Cortisol level was the highest in T3, and the lowest value was obtained in T1. ALT and AST content exhibited the highest significant value in T3. T1 was the best treatment, followed by T2 regarding the blood and serum analyses indices.



Table 2

The growth performance of red tilapia integrated with mullet in different culture systems using medium saline underground water (data are means±SEM)

Treatments	Final weight (g fish <sup>-1</sup> )		Weight gain (g fish <sup>-1</sup> )		ADG (g fish <sup>-1</sup> day <sup>-1</sup> )		SGR (% day <sup>-1</sup> fish <sup>-1</sup> )		Condition factor (K-value)	
	Red tilapia*	Mullet**	Red tilapia	Mullet	Red tilapia	Mullet	Red tilapia	Mullet	Red tilapia	Mullet
T1	18.64±0.28 <sup>b</sup>	--	17.64±0.28 <sup>b</sup>	--	0.42±0.01 <sup>b</sup>	--	6.96±0.04 <sup>b</sup>	0.0±0.0	1.84±0.04 <sup>a</sup>	--
T2	16.19±0.30 <sup>c</sup>	8.40±0.21	15.19±0.30 <sup>c</sup>	5.90±0.21	0.36±0.01 <sup>c</sup>	0.14±0.01	6.63±0.04 <sup>d</sup>	2.88±0.06	1.54±0.00 <sup>b</sup>	0.82±0.04
T3	17.00±0.28 <sup>c</sup>	8.73±0.32	16.00±0.28 <sup>c</sup>	6.23±0.32	0.38±0.01 <sup>c</sup>	0.15±0.01	6.75±0.04 <sup>c</sup>	2.97±0.09	1.88±0.04 <sup>a</sup>	0.88±0.04
T4	28.21±0.42 <sup>a</sup>	8.69±0.23	27.21±0.42 <sup>a</sup>	6.19±0.23	0.65±0.010 <sup>a</sup>	0.15±0.01	7.95±0.04 <sup>a</sup>	2.96±0.06	1.85±0.02 <sup>a</sup>	0.79±0.06
T5	--	8.22±0.15	--	5.72±0.15	--	0.14±0.00	--	2.83±0.04	--	0.78±0.05

\* Initial weight RT (g fish<sup>-1</sup>) = 1.02±0.01; Initial length (cm fish<sup>-1</sup>) = 3.67±0.03; \*\* Initial weight M (g fish<sup>-1</sup>) = 2.50±0.00; Initial length (cm fish<sup>-1</sup>) = 5.49±0.06.

Note: Means within the same column not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

Table 3

Red tilapia, mullet, and total fish biomasses reared under different culture systems using medium saline groundwater (data are means±SEM)

Treatments	Red tilapia biomass			Mullet biomass			Total biomass (RT+M)		
	Initial (g)	Final (g)	Increment (g)	Initial (g)	Final (g)	Increment (g)	Initial (g)	Final (g)	Increment (g)
T1	200.0±0.0	3727.3±56.0 <sup>a</sup>	3527.3±56.0 <sup>a</sup>	--	--	--	200.0±0.0	3727.3±56.0 <sup>a</sup>	3527.3±56.0 <sup>a</sup>
T2	150.0±0.0	2429.0±44.6 <sup>b</sup>	2279.0±44.6 <sup>b</sup>	125.0±0.0	419.8±10.7 <sup>d</sup>	294.8±10.7 <sup>d</sup>	275.0±0.0	2848.8±38.2 <sup>b</sup>	2573.8±38.18 <sup>b</sup>
T3	100.0±0.0	1700.3±28.3 <sup>c</sup>	1600.3±28.3 <sup>c</sup>	250.0±0.0	873.0±31.6 <sup>c</sup>	623.0±31.6 <sup>c</sup>	350.0±0.0	2573.3±27.0 <sup>d</sup>	2223.3±26.96 <sup>c</sup>
T4	50.00.0	1410.7±21.1 <sup>d</sup>	1360.7±21.1 <sup>d</sup>	375.0±0.0	1303.5±35.2 <sup>b</sup>	928.5±35.2 <sup>b</sup>	425.0±0.0	2714.2±52.1 <sup>c</sup>	2289.2±52.13 <sup>d</sup>
T5	--	--	--	500.0±0.0	1643.3±30.8 <sup>a</sup>	1143.3±30.8 <sup>a</sup>	500.0±0.0	1643.3±30.8 <sup>e</sup>	1143.3±30.75 <sup>e</sup>

Note: Means within the same column not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

Table 4

The feed utilization efficiency indices of red tilapia and mullet under different culture systems using medium saline groundwater (data are means±SEM)

Treatments	FCR	PER	PPV (%)	EG (Kcal/100 g)	EU (%)	CE (Kcal/100 g)
T1	1.13±0.02 <sup>d</sup>	2.52±0.04 <sup>a</sup>	16.00±0.26 <sup>a</sup>	9.72±0.13 <sup>a</sup>	10.78±0.15 <sup>a</sup>	176.79±0.40 <sup>c</sup>
T2	1.55±0.02 <sup>c</sup>	1.84±0.03 <sup>b</sup>	12.58±0.28 <sup>b</sup>	7.88±0.19 <sup>b</sup>	8.74±0.21 <sup>b</sup>	187.88±1.95 <sup>b</sup>
T3	1.80±0.02 <sup>b</sup>	1.59±0.02 <sup>c</sup>	10.37±0.20 <sup>d</sup>	6.16±0.18 <sup>d</sup>	6.82±0.20 <sup>d</sup>	177.16±1.83 <sup>c</sup>
T4	1.75±0.04 <sup>b</sup>	1.64±0.04 <sup>c</sup>	11.63±0.36 <sup>c</sup>	7.09±0.28 <sup>c</sup>	7.86±0.31 <sup>c</sup>	185.63±1.69 <sup>b</sup>
T5	3.50±0.10 <sup>a</sup>	0.82±0.02 <sup>d</sup>	5.49±0.28 <sup>e</sup>	3.67±0.18 <sup>e</sup>	4.07±0.20 <sup>e</sup>	199.38±1.55 <sup>a</sup>

Note: Means within the same column not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

Table 5

The proximate chemical analyses of red tilapia and mullet reared under different culture systems using medium saline groundwater (data are means±SEM)

Treatments	Moisture (%)		Crude protein (%)		Ether extract (%)		Ash (%)	
	Red tilapia	Mullet	Red tilapia	Mullet	Red tilapia	Mullet	Red tilapia	Mullet
T1	69.47±0.09 <sup>a</sup>	0.0±0.0	20.44±0.04 <sup>b</sup>	0.0±0.0	6.51±0.05 <sup>b</sup>	0.0±0.0	3.53±0.09 <sup>ab</sup>	0.0±0.0
T2	68.10±0.40 <sup>b</sup>	67.47±0.13 <sup>a</sup>	21.09±0.27 <sup>a</sup>	21.32±0.16 <sup>b</sup>	7.28±0.13 <sup>a</sup>	7.21±0.14 <sup>b</sup>	3.47±0.07 <sup>b</sup>	3.33±0.07
T3	69.97±0.12 <sup>a</sup>	66.50±0.32 <sup>b</sup>	20.61±0.08 <sup>b</sup>	22.12±0.19 <sup>a</sup>	5.82±0.15 <sup>c</sup>	7.44±0.28 <sup>b</sup>	3.57±0.09 <sup>ab</sup>	3.30±0.09
T4	70.00±0.31 <sup>a</sup>	66.80±0.21 <sup>ab</sup>	20.32±0.18 <sup>b</sup>	21.91±0.13 <sup>a</sup>	5.88±0.18 <sup>c</sup>	7.12±0.17 <sup>b</sup>	3.73±0.09 <sup>a</sup>	3.23±0.03
T5	0.0±0.0	66.73±0.32 <sup>ab</sup>	0.0±0.0	21.78±0.2 <sup>ab</sup>	0.0±0.0	8.11±0.08 <sup>a</sup>	0.0±0.0	3.31±0.09

Note: Means within the same column not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

Table 6

Blood biochemical parameters and serum analysis of red tilapia integrated with mullet in different culture systems using medium saline groundwater (data are means±SEM)

Blood and serum analysis	Treatments			
	T1	T2	T3	T4
Haemoglobin (g dL <sup>-1</sup> )	8.15±0.15 <sup>a</sup>	7.95±0.05 <sup>a</sup>	6.80±0.10 <sup>b</sup>	6.90±0.10 <sup>b</sup>
Red blood cells (cells mm <sup>-3</sup> ) *10 <sup>6</sup>	1.98±0.04 <sup>a</sup>	1.94±0.14 <sup>a</sup>	1.63±0.02 <sup>b</sup>	1.48±0.00 <sup>b</sup>
White blood cells (cells mm <sup>-3</sup> ) *10 <sup>3</sup>	152±0.0 <sup>a</sup>	137.5±4.5 <sup>b</sup>	112.5±1.5 <sup>c</sup>	113.5±0.5 <sup>c</sup>
Hematocrit (%)	30.45±0.6 <sup>a</sup>	27.90±0.1 <sup>b</sup>	23±0.1 <sup>c</sup>	21.35±1.2 <sup>c</sup>
Platelets count (mm <sup>3</sup> )	679±7 <sup>a</sup>	460±16 <sup>b</sup>	397.5±18.5 <sup>c</sup>	229±4 <sup>d</sup>
Glucose (mg dL <sup>-1</sup> )	125±20 <sup>b</sup>	141±16 <sup>b</sup>	244±40 <sup>a</sup>	149.5±9.5 <sup>ab</sup>
Total Protein (g dL <sup>-1</sup> )	2.20±0.30	2.0±0.30	1.75±0.55	1.50±0.00
Albumin (g dL <sup>-1</sup> )	1.30±0.1 <sup>ab</sup>	1.0±0.00 <sup>b</sup>	1.50±0.1 <sup>a</sup>	1.30±0.1 <sup>ab</sup>
Globulin (g dL <sup>-1</sup> )	0.90±0.1 <sup>a</sup>	0.75±0.05 <sup>ab</sup>	0.50±0.1 <sup>bc</sup>	0.25±0.05 <sup>c</sup>
Albumin/globulin ratio	5.95±0.05 <sup>b</sup>	2.90±0.1 <sup>c</sup>	8.80±0.2 <sup>a</sup>	9±0.2 <sup>a</sup>
Cholesterol (mg dL <sup>-1</sup> )	181.5±1.5 <sup>a</sup>	153.5±2.5 <sup>b</sup>	175.5±4.5 <sup>a</sup>	175.5±1.5 <sup>a</sup>
Triglyceride (mg dL <sup>-1</sup> )	449±9 <sup>a</sup>	267±1 <sup>c</sup>	238±4 <sup>d</sup>	306±4 <sup>b</sup>
Urea (mg dL <sup>-1</sup> )	2.45±0.05 <sup>c</sup>	3.90±0.1 <sup>b</sup>	5.55±0.15 <sup>a</sup>	4.1±0.1 <sup>b</sup>
Amylase (U L <sup>-1</sup> )	4±1 <sup>b</sup>	5.50±0.5 <sup>b</sup>	11±1 <sup>a</sup>	9.50±0.5 <sup>a</sup>
Cortisol (µg dL <sup>-1</sup> )	9.15±0.55 <sup>c</sup>	10±0.2 <sup>c</sup>	16.08±0.2 <sup>a</sup>	13.55±0.45 <sup>b</sup>
ALT (U L <sup>-1</sup> )	18±1 <sup>c</sup>	30±1 <sup>b</sup>	45.5±1.5 <sup>a</sup>	20.5±0.5 <sup>c</sup>
AST (U L <sup>-1</sup> )	98.5±7.5 <sup>c</sup>	131±3 <sup>b</sup>	260±9 <sup>a</sup>	101.5±1.5 <sup>c</sup>

Note: Means within the same row not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

**Heavy metal analysis.** Results of heavy metal concentration of fish flesh in red tilapia are presented in Table 7. The results indicated that there were no significant differences ( $p > 0.05$ ) in Cu, Cd, and Pb levels, but Fe and Zn levels were significantly different ( $p \leq 0.05$ ) among treatments. The lowest values of Fe and Zn were observed in T4, but the highest values were observed in T3. T4 was the best treatment, followed by T2. There are no significant differences in the concentration of toxic elements in favor of polyculture.

Table 7

The heavy metal concentration of flesh of Red tilapia integrated with mullet in different culture systems using medium saline groundwater (data are means $\pm$ SEM)

Treatments	Heavy metal analysis				
	Pb ( $\mu\text{g g}^{-1}$ )	Cd ( $\mu\text{g g}^{-1}$ )	Fe ( $\mu\text{g g}^{-1}$ )	Zn ( $\mu\text{g g}^{-1}$ )	Cu ( $\mu\text{g g}^{-1}$ )
T1	8 $\pm$ 1.0	1 $\pm$ 0.0	65 $\pm$ 1 <sup>b</sup>	94.5 $\pm$ 0.5 <sup>b</sup>	5.5 $\pm$ 0.5
T2	7.5 $\pm$ 0.5	1.5 $\pm$ 0.5	63 $\pm$ 3 <sup>b</sup>	98.5 $\pm$ 1.5 <sup>ab</sup>	5.5 $\pm$ 0.5
T3	7.5 $\pm$ 0.5	1.5 $\pm$ 0.5	74 $\pm$ 3 <sup>a</sup>	102.5 $\pm$ 0.5 <sup>a</sup>	5.5 $\pm$ 0.5
T4	7.5 $\pm$ 0.5	1 $\pm$ 0.0	50 $\pm$ 1 <sup>c</sup>	76.5 $\pm$ 3.5 <sup>c</sup>	4.5 $\pm$ 0.5

Note: Means within the same column not sharing a common superscript letter are significantly different ( $p \leq 0.05$ ).

**Discussion.** Polyculture is a production strategy that consists of co-culture one or more fish species to the main species to enhance fish production capacity by optimizing the use of available food/space resources (Ponce-Marbán et al 2006; Bunting 2008; Mehrim et al 2018), to reduce the negative environmental impacts of monoculture system and to increase production profit.

The better utilization of the offered feed by red tilapia has greatly affected the water quality and reduced TAN values obtained in T1 (FCR = 1.11) compared to the other treatments, especially T5. On the other hand, low feed utilization by mullet negatively affected water quality. Handajani et al (2018) stated that feed digestibility, the amount, quality and type of protein in the fish feed, and amino acid content affect feed utilization and, subsequently, ammonia excretion of fish. Improved feed utilization leads to a decrease in the proportion of ammonia in fish rearing water. Excess protein and amino acids in fish feed produced a higher rate of deamination and ammonia excretion and need more energy than the required energy for tissue growth, and protein retention in the fish body would be reduced (Guo et al 2012; Handajani et al 2018). Excess nitrogen excretion process and catabolism of amino acid nitrogen discharged a lot of ammonia into the water. About 80-90% of nitrogen metabolic wasted out by fish is in the form of ammonia (Bureau 2004). Phytoplankton blooming under integrated treatments (T2, T3, T4) lead to rising pH values in these treatments.

Growth performance data for red tilapia were higher than those obtained by other researchers. SGR of red tilapia recorded in this study varied between 6.63 and 7.95 compared with 3.12-3.52 for hybrid red tilapia (*Oreochromis mossambicus*  $\times$  *O. aureus*) (Correia et al 2019). T4 group exhibited the highest growth performance due to low carrying capacity compared with the other treatments. Huang & Chiu (1997) stated a negative relationship between the logarithms of stocking density and the logarithms of fish size and growth performance. Red tilapia exhibits higher values of growth performance parameters in comparison with *Mugil capito*. Unfortunately, up-to-date, there are no available data on the growth rate of *M. capito* under tank farming conditions or even earthen ponds.

The low values of the condition factor obtained in this experiment for mullet compared to red tilapia reflect the significant difference in growth performance between the two species. El-Aiatt & Shalloof (2018) recorded that the condition factor of mullet varied between 0.8 and 0.90 in Bardawil Lagoon, North Sinai, Egypt. This result is consistent with the current results. The absence of any differences in the condition factor between the experimental and wild mullet in capture fisheries means that the tested fish exhibited proper utilization of the artificial feed. The mullet feed in nature on a wide

variety of prey types: diatoms (41.3%), polychaetes (18.8%), green algae (16.8 %), crustacea (11.0%), foraminifera (3.5%) and sediments (8.7%). The diatoms, polychaetes, green algae, and crustacea, were the major food item all year round (Rafalah & El-Mor 2014).

The statistical equation that summarizes the relationship between length and weight in mullet has not yet been studied in culture ponds. However, the equation obtained in red tilapia reflects an apparent positive effect of weight gain versus an increase in each centimeter of fish length compared to a much lower rate in *M. capito*. This reflects better utilization of artificial feed in red tilapia fish. Generally, there are several factors affecting condition factor (K) of fishes like sex, season, presence and utilization of food/feed, environmental conditions like temperature, dissolved oxygen, ammonia, pH, etc. (Olurin & Aderibigbe 2006; Migiro et al 2014). K is acting a key role in the assessment of the condition and comfort of fish (Ahmed et al 2011), monitoring of feeding availability, and growth performance (Ndimele et al 2010). Also, according to Lalrinsanga et al (2012), K-value is a powerful reflection of the interactions between abiotic and biotic factors affecting the physiological condition of fishes. K-values of greater than 1 is a good indication of the proper growth of fish species. Higher K-value shows that the fish has got a favorable condition (Nehemia et al 2012). These current findings of red tilapia are in contrast with previous studies of Malik et al (2017) who recorded 3.3-3.4 K-value for red tilapia broodstock and Ibrahim et al (2012) who gained a K-value of 1.98 and more than Correia et al (2019) for red tilapia (*Oreochromis mossambicus* × *O. aureus*) (0.2-0.26).

LWR can be used to expect weight from length sizes in the fisheries and aquaculture assessment (Pauly 1993) and can help in the estimation of condition factor of fishes (Malik et al 2017). In the present study, the regression equation of LWR of mullet was:  $W = 0.016 L^{2.715}$  presented values of a,b similar to those obtained by Mohamed (2016) 0.017 and 2.764, Salem et al (2010) 0.0177 and 2.7642, and El-Aiatt & Shalloof (2018) 0.0095 and 2.9505, for values of a, and b, respectively for *C. ramada* in Bardawil Lagoon. In the present study, the higher growth rate of red tilapia than mullet reflected positively on final total fish biomass for treatments with more density of red tilapia in the experimental ponds. A positive relationship between the logarithms of production and the logarithms of stocking density was documented (Huang & Chiu 1997).

In this study, increased ammonia levels in co-cultured treatments caused a significant decrease in hematological parameters. Kim et al (2017) found that increasing ammonia levels in fish ponds decreased hemoglobin levels, RBC count, and hematocrit of sablefish, *Anoplopoma fimbria*. Shin et al (2016) stated a decrease in hematological parameters affected by increased ammonia levels. Das et al (2004) reported that changes in hematological parameters indicate physiological effects by stress responses. In this study, the glucose level increased, and protein level decreased in co-culture treatments as a result of the ammonia increase. This result is congruent with Shin et al (2016). Oner et al (2007) stated that total protein and glucose could be reliable biomarkers to detect animal health. The rise in glucose in serum constituents may be a result of the glycogenolytic activity of catecholamines and gluconeogenic outcome of glucocorticoids via the stress response under stressful conditions (Dobsikova et al 2011). In this experiment, ALT and AST were increased notably in co-cultured treatments. Agrahari et al (2007) reported that the serum enzymatic components could be used to assess the tissue degeneration of the kidney and liver as a result of stress conditions. The GPT and GOT were significantly raised by the ammonia elevation in enzyme serum components of *Sebastes schlegelii* (Shin et al 2016) showed some degree of tissue necrosis in the rainbow trout, *Oncorhynchus mykiss* (Vedel et al 1998).

**Conclusions.** This study has successfully documented that *Chelon ramada* did not show any significant positive effect when co-cultured with red tilapia in a concrete tank. Monoculture of red tilapia showed much better results than the polyculture system with *C. ramada* as the latter species has only one-third growth performance and feed utilization indices compared to red tilapia. Also, contrary to what was expected, the polyculture of *C. ramada* did not result any significant improvement in water quality

parameters and heavy metals removal as result of lower feed utilization efficiency. Blood analysis parameters of red tilapia gave better results in monoculture treatment (T1), followed by the polyculture treatment (T2) that does not depend on storing many mullet fish with red tilapia. We recommend not storing mullet with red tilapia for the previous negative indices according to the present data.

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