Short Communication



Growth performance and body composition of hybrid red tilapia (Oreochromis mossambicus × O. aureus) fed with different protein levels raised in saltwater

Daniel Correia¹, Brandon Escarcega-Miranda², Fernando Barreto-Curiel², José Mata-Sotres³ Oscar Del Rio-Zaragoza², María Teresa Viana² & Artur Nishioka Rombenso^{2,4}

 ¹Univerisidad Federal do Rio Grande, Rio Grande, RS, Brasil
²Universidad Autónoma de Baja California, México
³CONACYT – Universidad Autónoma de Baja California, México
⁴Commonwealth Scientific and Industrial Research Organization (CSIRO) Queensland, Australia
Corresponding author: Artur N. Rombenso (artur.rombenso@csiro.au)

ABSTRACT. The present study evaluated the growth performance and whole-body composition of the hybrid red tilapia (*Oreochromis mossambicus* \times *O. aureus*) raised in saltwater and fed three experimental diets containing different protein levels (25.6, 30.8 and 35.6% CP; crude protein). The hybrid red tilapia showed a high production performance across all dietary treatments: specific growth rate (SGR) = 3.12-3.51% body weight d^{-1} ; weight gain = 391-503%; feed conversion ratio (FCR) = 0.90-1.07; thermal growth coefficient (TGC) = 1.50-1.67; condition factor (CF) = 0.19-0.26. Dietary protein content significantly affected feed intake; fish fed the 30.8% CP diets displayed higher feed intake than those fed the 25.6% CP diet. Although the protein efficiency ratio (PER) was not significantly affected by dietary treatments, fish fed 25.6% CP diet demonstrated numerically higher values than those fed the other dietary treatments (4.34 vs. 3.06-3.35). The viscerosomatic index was significantly reduced in the 25.6% CP group compared to 30.8 and 35.6% CP groups. Regarding the whole-body composition, dietary protein affected all parameters, except lipid content. Fish fed the 30.8% CP diet exhibited significantly reduced relative protein content and elevated ash content in the whole-body in comparison to other dietary groups. The results of the present study indicated that juveniles of hybrid red tilapia accepted all dietary treatments. However, according to polynomial regression analyses based on PER and the high ash content in tilapias fed the 30.8% CP, it is suggested that the optimum dietary protein content for juvenile hybrid red tilapia was 25.6%.

Keywords: hybrid red tilapia; protein; nutrition; whole-body composition; saltwater; aquaculture

Tilapia (*Oreochromis* spp.) and its hybrids have been contributing to the global growth of aquaculture. It is expected by 2030 that tilapia culture will reach 7.3 million tonnes annually, mostly based on three species *O. niloticus, O. aureus* and *O. mossambicus* (Msangi *et al.*, 2013; FAO, 2016). The hybrid red tilapia is considered as the most important for commercial aquaculture. The ease adaptation to culture conditions and confinement, salinity tolerance and its attractive color that enhances the retail value (Brol *et al.*, 2017; He *et al.*, 2017; Nakphet *et al.*, 2017) are among the principal reasons for its popularity. The culture in the Americas began with the introduction of the *Oreochromis*

mossambicus species. However, the hybridization was a significant advance towards the growth in tilapia production in this continent (Watanabe *et al.*, 2002).

Research on the hybrid red tilapia (*O. mossambicus* \times *O. aureus*) is still scarce with few reports on nutritional demands and requirements, and even less under the marine environment (Barreto-Curiel *et al.*, 2015). For example, Botello-Leon *et al.* (2011) reported a successful partial fish meal replacement with sugar cane protein meal (14%) in this hybrid. Regarding the dietary protein content, it has been reported a requirement between 30-40% to optimize growth at the juvenile stage (Ma *et al.*, 2015; Liu *et al.*,

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2017). However, there is no published document on dietary protein demand with marine hybrid red tilapia. Currently, the availability of freshwater is limited, so in order to increase the sustainable tilapia culture, it is important to encourage their seawater culture.

When fish are fed protein-poor diets (*i.e.*, the dietary protein levels are lower than the fish physiological demand) growth and production performances are impaired and depending on the circumstances their health and welfare might be affected as well. On the other hand, when fish are fed protein-rich diets, in which dietary protein content exceeds their physiological demand, growth and production performances are not optimized due to catabolic processes with a concomitant increase in ammonia excretion (Kaushik & Seiliez, 2010; Carvalho *et al.*, 2017).

Optimizing dietary protein is essential for a sustainable and flourishing aquaculture operation. As fish exhibits a high protein requirement, this macronutrient influences directly on the feed cost, and consequently, the operational aquaculture costs (El-Saidy & Caber, 2005). Accordingly, the present study evaluated the performance, and whole-body composition of juvenile hybrid red tilapia fed three dietary protein levels raised in saltwater.

Dietary formulations were based on the known nutritional demands and requirements of the species (Barreto-Curiel *et al.*, 2015). Diets were then prepared with a combination of fish meal, poultry by-product meal, soybean meal and gelatin as primary protein sources (Table 1). The experimental diets were formulated to contain three different levels of protein (25.6, 30.8, 35.6% crude protein; CP) and approximately 8% of crude fat; CF.

The experimental diets were manufactured at Laboratorio de Nutrición y Fisiología Digestiva (UABC, B.C., Mexico) according to the in-house protocols. Briefly, macronutrients were pulverized using a 0.8 mm mesh (Pulvex 2000, Mexico), sifted (500 µm mesh; Kemutek-Gardner K300, USA) and mixed using a vertical cutter-mixer (Robot Coupe, USA) until a homogenized meal was achieved. Micronutrients were mixed for a minute (Robot Coupe R-60, USA), and then incorporated into the bulk mixture. Beef tallow was heated prior to addition to the dough and mixed thoroughly, and then water was added until the desired texture was achieved. The dough was pelleted 2-3 mm using a commercial-grade food grinder and dried at 60°C until >90% dry matter was obtained. All diets were maintained cooled at 4°C throughout feeding trial.

Additionally, the experimental diets were analyzed in triplicate to confirm proximate composition, according to AOAC (1995; Table 1). The dry matter content was determined by weight difference before and after drying ground samples at 60°C for 24 h. Ash was determined gravimetrically from the ground sample after using the muffle furnace at 550°C for six hours. Crude protein was calculated by nitrogen conversion (%N × 6.25), nitrogen content was measured by the micro-Kjeldahl method followed by distillation and titration. Crude fat was determined gravimetrically and extracted with Soxhlet method using petroleum ether as a solvent.

The study was carried out at UABC, where juvenile hybrid red tilapia (*O. mossambicus* × *O. aureus*) with an average of 4.17 ± 0.53 g (mean ± SE, standard error) were acclimated for two weeks in 500 L tanks under a recirculation aquaculture system. Twelve fish per tank were stocked into nine tanks of 200 L with natural marine seawater under a recirculation system. Each tank consisted of an experimental unit with an individual biological filter and settler, and the water was recirculated by an air-lift pump. The aeration was kept constant in all tanks using aeration stones.

Water salinity, temperature and dissolved oxygen (YSI, PRO 20), were monitored daily. The nitrogenous components and pH (API test; Aquarium Pharmaceutical, Inc., Canada) were monitored three times a week. Every two days the experimental tanks were siphoned to remove feces. Fish were hand-fed daily to apparent satiation three times a day (8:00, 12:00 and 16:00 h) for 52 days.

Water quality conditions were maintained within the optimum range for the hybrid red tilapia, based on the known of the species *O. mossambicus* × *O. aureus*: temperature 25.4 ± 0.7°C, salinity 34.8 ± 0.5, dissolved oxygen 5.1 ± 0.2 mg L⁻¹, total ammonia nitrogen 0.3 ± 0.2 mg L⁻¹, nitrite-nitrogen 0.2 ± 0.3 mg L⁻¹ and pH 7.8 ± 0.2 (mean ± SE).

After 52 days, fish from each experimental unit were counted and individually weighted to assess the following performance metrics: weight gain (%) = [(final mean weight - initial mean weight)×100 / initial mean weight]; feed intake (FI) = [(total food consumed per tank for the duration of the experiment / number of fish per tank]; feed conversion ratio (FCR) = (total amount of feed consumed / final body weight gain); specific growth rate (SGR) = (ln final weight - ln initial weight) / time (days) × 100; protein efficiency ratio (PER) = g body weight increase, wet weight / g protein ingested; thermal growth coefficient (TGC) = [(final weight^{1/3} - initial weight^{1/3}) / (T°C×D_{days})]×1000; condition factor (CF) = weight / length³.

Three fish per tank were randomly collected and euthanized by ice-cold water. Firstly, fish were measured, weighed and dissected. Total viscera masses and liver samples were weighed to calculate the viscero**Table 1.** Dietary formulation (g kg⁻¹) and proximate composition. ^aSoybean meal 45-48% protein, Alimentos Integronaturales S.A. (COLPAC), Nuevo León, México, ^bPoultry meal 68% protein, National Renderers Association, Missouri, USA, ^cFish meal 65% protein. Scoular de México S. de R.L. de C.V., ^dIndustrializadora de Maíz S.A. de C.V., México, ^eBeef tallow Grasas y Derivados SA de CV, Tijuana, Baja California, México, ^fDSM Nutritional Products México, S.A. de C.V., contains (cholecalciferol, menadione, thiamine, riboflavine, pyridoxine, cyanocobalamin, pantothenic acid, folic acid, choline chloride, copper, zinc oxide, iron, manganese oxide, potassium iodide, selenium, phosphorus, potassium, sodium, calcium, organic acids, taurine, silicates, silicon oxide, silicon dioxide, aluminum oxide), ^gDSM Nutritional Products México, S.A. de C.V. contains (350 mg L-ascorbic acid per gram), ^hDHA Natur ADM Animal nutrition, Mexico contains (17.0% DHA).

Ingredient	25.6% CP	30.8% CP	35.6% CP			
Soybean meal ^a	47.6	65.4	82.6			
Poultry by-product meal ^b	180.8	217.8	252.3			
Fish meal ^c	71.4	88.8	108.3			
Corn meal ^d	171.3	168.2	165.1			
Beef tallow ^e	44.7	37.4	28.5			
Corn starch ^d	397.3	337.0	279.4			
Gelatin	57.0	56.1	55.0			
Rovimix ^f	19.0	18.7	18.3			
Stay-C ^g	3.8	3.7	3.7			
DHA ^h	4.8	4.7	4.6			
Sodium benzoate	2.2	2.1	2.1			
Tocopherol	0.1	0.1	0.1			
Proximate composition (% dry matter basis)						
Crute protein	25.6	30.8	35.6			
Crute fat	7.9	9.4	8.5			
Ash	6.0	6.0	9.9			
Dry matter	98.6	99.2	97.5			
Gross energy (kJ g ⁻¹)	17.4	17.7	16.8			

somatic index (VSI) = $100 \times$ (viscera weight / whole body weight) and hepatosomatic index (HIS) = $100 \times$ (liver weight / whole body weight). Then the same three fish per treatment were used for whole-body proximate composition analysis (same procedures as those previously described for dietary proximate composition analyses).

For statistical analysis, data homogeneity was tested by the Shapiro Wilk test, and the homoscedasticity was tested by Levene's test, followed by an analysis of variance (one-way ANOVA). When significant differences were detected, a Newman-Keuls' test was done. Significance was determined based on P < 0.05. Polynomial regression analyses were carried out to further investigate the relationship between analytically verified dietary protein levels and performance and morphometric parameters (weight gain, SGR, FCR, PER, Feed intake, TGC, HSI and VSI). Polynomial regression analyses were also chosen for resulting in stronger R² than other regression analyses, including logarithmic, exponential, among others. All the statistical analyses were performed using the software Statistica 7.0.

The dietary protein levels tested did not affect the survival rate and growth performance across different treatments. Overall, juvenile hybrid red tilapia (*O. mossambicus* × *O. aureus*) demonstrated high production performance in all treatments (weight gain = 391-503%, FCR = 0.90-1.07 and SGR = 3.12-3.51% body weight d⁻¹; Table 2).

Most production parameters were not statistically significant different (weight gain P = 0.789; FCR P = 0.582; SGR P = 0.604; TGC P = 0.705; CF P = 0.732). Only fish fed the 30.8% CP diet exhibited significantly higher feed intake (P < 0.05) compared to those fed 25.6% CP (4.29 *vs.* 3.11% body weight d⁻¹, respectively). (Fig. 1).

Although PER was not significantly affected by dietary protein level fish fed the 25.6% CP diets demonstrated numerically higher values (4.34) than those fed the other dietary treatments (3.06 and 3.35). The VSI was significantly reduced in 25.6% CP group (3.1) compared to 30.8% CP (7.9) and 35.6% CP groups (8.2), and no difference was observed for HSI (2.6-3.3).

Table 2. Production performance by dietary treatment. Values represent least-square means and standard error (SE) values and *P*-values resulting from one-way ANOVA tests are also provided; means with common letter labels are not significantly different (P > 0.05). FCR: feed conversion ratio, SGR: specific growth rate, FI: feed intake, PER: protein efficiency ratio, TGC: thermal growth coefficient, CF: condition factor, HSI: hepatosomatic index, VSI: viscerosomatic index.

Production variable	25.6% CP	30.8% CP	35.6% CP	P-value
Initial individual weight (g)	4.4 ± 0.4	3.8 ± 0.1	4.3 ± 0.7	0.309
Final individual weight (g)	21.6 ± 2.6	22.7 ± 2.1	23.6 ± 5.4	0.795
Weight gain (%)	391 ± 31	503 ± 64	472 ± 26	0.789
FCR	0.90 ± 0.02	1.07 ± 0.03	0.93 ± 0.19	0.582
SGR (% body weight d ⁻¹)	3.12 ± 0.12	3.51 ± 0.21	3.31 ± 0.76	0.604
FI (g fish ⁻¹)	$3.11\pm0.14^{\text{b}}$	4.29 ± 0.31^{a}	3.27 ± 0.25^{ab}	0.027
Survival (%)	100	100	100	
PER	4.34 ± 0.09	3.06 ± 0.26	3.35 ± 0.79	0.226
TGC	1.50 ± 0.05	1.67 ± 0.07	1.61 ± 0.23	0.705
CF	0.19 ± 0.06	0.26 ± 0.08	0.20 ± 0.04	0.732
HSI	3.3 ± 0.6	2.6 ± 1.7	3.2 ± 0.4	0.315
VSI	3.1 ± 0.7^{b}	$7.9 \pm 1.7^{\mathrm{a}}$	$8.2\pm0.2^{\rm a}$	0.002



Figure 1. Polynomial regression analyses between dietary protein content and protein efficiency ratio (PER) and feed intake (FI).

The polynomial regression analyses demonstrated a stronger relationship between dietary protein content and VSI and FI ($R^2 = 0.87$ and 0.70, respectively) than the rest of the parameters analyzed ($R^2 = 0.08-0.39$, Table 3). A positive correlation was noticed for PER and HSI, whereas the other parameters displayed a negative correlation. Regarding the relative wholebody composition (Table 4), the dietary protein significantly affected all parameters, except lipid content. The whole-body dry matter was reduced in the 25.6% CP group (980 g kg⁻¹) compared to the 30.8 and 35.6% CP groups (985 and 987 g kg⁻¹, respectively). Decreased protein content (563 g kg⁻¹) and high ash content (171 g kg⁻¹) in the whole-body of fish fed the 30.8% CP diets was revealed compared to those fed the other dietary treatments (586-590 g kg⁻¹ and 139-136 g kg⁻¹, respectively). Collectively, these findings suggest the optimum dietary protein content for hybrid red tilapia under the experimental conditions was 25.6%.

The tilapia has a low trophic level, and consequently, it demands reduced dietary protein when compared with other higher trophic level commercial aquaculture species (e.g., 30-40% CP vs. 40-55% CP). Protein is an essential nutrient for growth and other numerous physiological processes; in general, fish has an elevated dietary protein demand compared to terrestrial animals (Chou et al., 2001; Ai et al., 2004; Li et al., 2008). The knowledge on optimum dietary protein demand is paramount in aquaculture for the growth maximization and performance, productivity optimization, and production costs reduction (Salhi et al., 2004). In our study, juveniles of hybrid red tilapia (O. mossambicus \times O. aureus) exhibited higher growth and production performance than those reported by Li et al. (2008).

On the other hand, Barreto-Curiel *et al.* (2015) reported higher weight gain and TGC compared to the present findings. In that study, the authors investigated the hybrid red tilapia performance (25.00 ± 0.06 g, initial weight) reared in fresh *vs.* seawater for 90 days fed diets containing 40% CP and 8.5% crude fat. These contradictory differences are difficult to explain, considering that fish from one of our treatments were fed similar protein content to those reported by Barreto-Curiel (37 *vs.* 40% CP). Those differences could be a result of many factors: such as fish size, genetics, length of the feeding trial and culture system, among others.

The protein efficiency ratio (PER value) reflects the dietary protein quality to growth performance. As such, the higher PER value indicates greater efficiency on

Table 3. Regression analyses of dietary protein content and production performance parameters, the type of regression analyses used was polynomial. SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio, FI: feed intake, TGC: thermal growth coefficient, HSI: hepatosomatic index, VSI: viscerosomatic index.

	Equation	\mathbb{R}^2
Weight gain (%)	$y = -0.026x^2 + 1.8743x - 14.496$	0.076
SGR	$y = -0.012x^2 + 0.7856x - 9.3732$	0.154
FCR	$y = -0.0064x^2 + 0.4131x - 5.582$	0.164
PER	$y = 0.0313x^2 - 2.1055x + 38.347$	0.391
FI	$y = -0.044x^2 + 2.8351x - 41.325$	0.700
TGC	$y = -0.0044x^2 + 0.2944x - 3.23$	0.110
HSI	$y = 0.0256x^2 - 1.6539x + 29.338$	0.319
VSI	$y = -0.0888x^2 + 6.1942x - 99.37$	0.871

Table 4. Whole-body composition (g kg⁻¹) of juvenile hybrid red tilapia *Oreochromis mossambicus* × *O. aureus* fed diets with increasing dietary protein content. Values represent least-square means and standard error (SE) values and *P*-values resulting from one-way ANOVA tests are also provided; means with common letter labels are not significantly different (P > 0.05).

-	25.6% CP	30.8% CP	35.6% CP	P-value
Dry matter	980 ± 00^{b}	985 ± 00^{a}	987 ± 00^{a}	0.002
Protein	$586\pm60^{\mathrm{a}}$	563 ± 20^{b}	590 ± 11^{a}	0.005
Lipid	265 ± 02	270 ± 11	270 ± 00	0.565
Ash	139 ± 00^{b}	171 ± 20^{a}	136 ± 04^{b}	0.026

protein utilization by fish, while lower PER value shows a decrease in protein utilization, probably as a result of low protein digestibility and absorption, or that protein is being used as an energy source (Clark et al., 1990). The protein efficiency is affected by many factors, mainly fish size, age and protein source (Al-Hafedh, 1999). Carvalho et al. (2017) reported a reduction of PER value with an increase of dietary protein level increasing ammonia excretion. In the present study, the PER values were not statistically different among dietary treatments. However, the polynomial regression analysis indicated a negative relationship between PER value and dietary protein content, where fish fed the 25.6 and 30.8% CP diets exhibited the highest and lowest PER value numerically, respectively, and in accordance to the literature (Wing-Keong & Rosdiana, 2007; Carvalho et al., 2017). So, considering the polynomial regression analysis, it can be stated that 25.6% CP can be considered as the optimum dietary protein content. Also, no signs of ammonia excretion increased were observed regarding the water quality monitoring throughout the feeding trial; which demonstrates that fish were satisfactorily utilizing the dietary protein.

The salinity content appears to no interfere with the PER values in hybrid red tilapia, as no statistical difference was reported in PER values for juvenile (25 g) reared in fresh (2.08) and seawater (1.97) (Barreto-Curiel *et al.*, 2015). Freshwater species when exposited to an increasing water salinity use the osmoregulation process to keep the homeostasis of fluids due to the size of the intestine (Verdegem *et al.*, 1997; Chourasia *et al.*, 2018).

In the present study, feed intake was affected by dietary treatments with fish fed the 30.8% CP diet exhibiting significantly higher feed intake than those fed the 25.6% CP diet. Generally, energy is considered the primary driver in feed intake; however, many other factors (e.g., availability of key nutrients, palatability and attractants, among others) should be considered as well (De la Higuera, 2001). Nevertheless, the feed intake should be taken with care, since it is difficult to establish a high accuracy due to the feed stability and feed recovery from the experimental tanks. Moreover, since the gross energy of the dietary treatments was equivalent, it can be suggested that other factors including availability of nutrients (e.g., higher dietary lipid content in the 30.8% CP diet compared to the 25.6 and 35.6% CP diets) might have influenced this outcome. The higher visceral somatic index in fish fed diets containing elevated protein levels could be related to the increase in feed intake and the mobilization of fat (Duarte et al., 2019).

Our results indicate that juveniles of hybrid red tilapia (*O. mossambicus* \times *O. aureus*) accepted all dietary treatments. Growth performance can be increased with higher dietary protein content (30.8 and 35.6% CP) although not as efficient as diets containing 25.6% CP. Regression analyses based on PER value suggested that the optimum dietary protein content for juvenile hybrid red tilapia was 25.6% under the experimental conditions.

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