

Research Article

Effect of Protein to Energy Ratio on Growth Performance, Body Composition and Enzymatic Digestive Activity of Juvenile Tilapia (*Oreochromis niloticus* x *O. mossambicus*) Reared in Seawater

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Abstract: The objective of this study was to evaluate the effect of digestible Protein to Energy ratio (P/E) on growth performance, body composition and enzymatic digestive activity of juvenile tilapia (*Oreochromis niloticus* x *O. mossambicus*) reared in seawater. Six diets were evaluated (30, 23, 20, 15, 10 and 8 P/E) on the growth, digestibility, body composition and enzymatic digestive activity of juvenile tilapia. The results indicated that the P/E ratios in diets, significantly affects the growth, survival, Feed Conversion Ratio (FCR), lipid content in the body and enzymatic digestive activity. Maximum body weight was recorded in the treatments with P/E ratios of 30, 23 and 20 (20.6, 23.7 and 25.7 g, respectively) and minimum body weight (5.9 g) was recorded with P/E ratio of 8. The FCR increased significantly in P/E levels of 15, 10 and 8 mg/kJ. In conclusion, the best P/E ratio to culture juvenile tilapia in seawater was estimated in 27 mg/kJ.

Keywords: Amylase, digestibility, lipid, trypsin

INTRODUCTION

An increasing interest for culturing tilapia in seawater exists in several countries. This fish has a high demand in the international markets. Tilapia resists wide ranges of temperature, dissolved oxygen and salinity, but to obtain its maximum growth rate, it is necessary to know the nutritional requirements for specific culture conditions. Nutritional requirements of tilapia cultured in freshwater are well known (Gunasekera *et al.*, 1997; Al-Hafedh, 1999; Al-Hafedh *et al.*, 1999; Sweilum *et al.*, 2005; El-Saidy and Gaber, 2005); since the fish physiology changes when they are cultured in an environment different to their normal, nutritional requirements need to be studied to provide information for the optimal diet elaboration (El-Sayed, 2006).

Protein retention efficiency in fish is affected by several endogenous and exogenous factors (Halver and Hardy, 2002). One of the main factors related with the diet efficiency is the Protein to Energy ratio (P/E). This parameter must be estimated for specific fish species and stages. When the diet has an excess of protein, it will be used as energy source, instead of body growth. In contrast, a diet with excess of energy and deficient in

protein can reduce the feed consumption and affect growth (NRC, 2011).

The objective of this study was to determine the effect of protein to energy ratio on growth performance, body composition and enzymatic digestive activity of juvenile tilapia (*O. niloticus* x *O. mossambicus*) reared in seawater.

MATERIALS AND METHODS

Formulation and elaboration of diets: Six diets with different levels of crude protein (150, 300 and 450 g/kg, respectively) and gross energy (15 and 20 kJ/g) were formulated (Table 1) using the Nutri^R 5 PRO software. Prior to elaboration the experimental diets, all ingredients were pulverized and sieved through a 250 µm mesh. The dry ingredients and oil mix (fish oil and soybean lecithin) were mixed in a food mixer. The resulting mixture was pressure pelleted using a meat grinder (2 mm diameter). The pellets were dried in a convection oven at 45°C for 12 h.

Tilapia acclimation to seawater: Males induced hybrids were acquired from a commercial laboratory in the state of Jalisco, Mexico. The individuals were maintained during 15 days in fresh water and fed with a

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Table 1: Diet composition and proximate analysis (g/kg)

Ingredient	Diet					
	30	23	20	15	10	8
Fishmeal ^a	369.0	419.0	54.00	254.0	50.0	10.0
Wheat flour ^b	220.0	250.0	390.0	540.0	894.0	750.0
Soybean meal ^b	350.0	150.0	480.0	20.0	0.0	69.0
Casein ^c	0.0	45.0	0.0	20.0	0.0	0.0
Sodium alginate ^d	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin mix ^e	8.0	8.0	8.0	8.0	8.0	8.0
Mineral mix ^f	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride ^g	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin C ^h	1.0	1.0	1.0	1.0	1.0	1.0
Dibasic sodium phosphate ⁱ	5.0	5.0	5.0	5.0	5.0	5.0
Cholesterol ^j	5.0	5.0	5.0	5.0	5.0	5.0
Fish oil ^a	10.0	85.0	25.0	115.0	5.0	120.0
Soybean lecithin ^k	5.0	5.0	5.0	5.0	5.0	5.0
BHT ^l	0.01	0.01	0.01	0.01	0.01	0.01
Nutrient						
Crude protein	445±42	460±60	301±21	309±15	146±8	155±06
Lipids	56±01	150±11	39±03	146±14	55±01	160±18
Ash	25±01	28±02	26±01	19±01	30±02	20±01
Fiber	123±3	80±01	124±05	57±01	126±02	80±01
N.F.E.	220	282	402	469	550	589
Gross energy ^m (kJ)	15	20	15	20	15	20

^a: Industrias Barda, Yavaros, Sonora, Mexico; ^b: Alimentos Colpac S.A. de C.V., Navojoa, Sonora, Mexico; ^c: ICN Cat. Num. 901293. biomedical Inc; ^d: Química Meyer Cat. Num. 6780. México, D.F.; ^e: Composition of the vitamin premix (g/kg premix): Vit. A acetate (20,000 UI/g) 5, vitamin D₃ (850,000 UI/g) 0.001, dl- α -tocopheryl acetate (250 UI/g) 12, menadione 2.4, thiamin-HCl 2.5, riboflavin (B₂) 5, pyridoxine-HCl (B₆) 4, DL Ca-pantothenate 0.012, nicotinic acid 0.024, biotin 0.048, inositol 0.4, vitamin B₁₂ 4.8, folic acid 1.2, filler (alfa-cellulose) 962.62; ^f: Composition of the mineral premix (g/kg premix): Cobalt chloride 0.04, cupric sulfate pentahydrate 2.5, ferrous sulfate 40, magnesium sulfate heptahydrate 283.98, manganous sulfate monohydrate 6.5, potassium iodine 0.67, sodium selenite 0.1, zinc sulfate heptahydrate 131.93, filler (alfa-cellulose) 534.28; ^g: SIGMA Cat. Num. C1879. SIGMA-ALDRICH chemical company, St. louis, MO, USA; ^h: Stay C 35% active agent. Roche, México, D.F.; ⁱ: SIGMA Cat No. S-0876. SIGMA-ALDRICH Chemical Company, St. Louis, MO, USA.; ^j: SIGMA Cat. No. C-8503, SIGMA-ALDRICH chemical company, St. louis, MO, USA); ^k: ODONAJI, distribuidora de alimentos naturales y nutricionales S.A. de C.V. México, D.F.; ^l: Butylated hydroxytoluene, ICN Cat. No.101162. aurora, ohio, USA; ^m: protein = 23.9 kJ/g, lipid = 39.8 kJ/g, carbohydrates = 17.2 kJ/g (Tacon, 1989)

commercial diet (400 mg/kg of protein). Culture conditions were: 28±1.7°C and 4.8±0.3 mg/L oxygen. During the acclimation, the salinity was increased at a rate of 10 psu/day (Martínez, 2003).

Growth trial: The growth and digestibility trial were conducted in a recirculation seawater system (35 psu). The tilapia juvenile (4.2±0.5 g; mean±S.D.) were stocked in 50 L plastic tanks (5 fish/tank). Three replicated tanks were randomly assigned for each diet. The water temperature was maintained at 27°C (controlled with titanium heaters), the oxygen was maintained at saturation with supplementary aeration. Fish were fed two times daily (9:00 and 16:00 h). At the beginning of the experiment, food was provided at a rate of 10% of the biomass in each aquarium and it was corrected daily on the basis of the residual food. All individuals were weighed every 15 days, during 75 days. At the end of the experiment, the fishes were collected and frozen to -50°C until proximate composition analysis.

Growth rate, expressed as Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and survival were determined using these equations:

$$FCR = \frac{\text{Total food intake}}{\text{Weight gain}}$$

$$PER = \frac{\text{Weight gain}}{\text{Protein intake}}$$

$$\text{Survival} = 100 \times \left(\frac{\text{final count}}{\text{Initial count}} \right)$$

Digestibility trial: At the end of the growth experiment a digestibility trial was conducted. Tilapias were fed *Ad libitum* three times daily during 7 days with the experimental diets before beginning faeces collection. Chromic oxide in the diets (1%) was used as an indirect marker. Each day before the initial feeding uneaten food and faeces were removed from tanks. Faeces were collected three times per day, one hour after each feeding. Faecal strands were collected by siphoning (including faeces from the first feeding). They were gently rinsed with distilled water and frozen at -80°C. At the end of the trial, collected faeces from each tank were pooled and freeze-dried.

Diets and fecal samples were analyzed for crude protein (AOAC International, 2002), carbohydrates (Dreywood, 1946), lipids (Folch-Lees and Sloane-Stanley, 1957) and chromic oxide (Olvera-Novoa, 1994). Apparent Digestibility Coefficients (ADC) for dry matter and nutrients in the diets were determined with the following equations (Cho *et al.*, 1982):

$$\begin{aligned} \text{ADC Dry Matter (\%)} \\ = 100 - \left[\left(\frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in faeces}} \right) * 100 \right] \end{aligned}$$

$$\begin{aligned} \text{ADC Nutrient (\%)} = 100 - 100 \\ \left[\left(\frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in faeces}} \right) * \left(\frac{\% \text{ Nutrient in faeces}}{\% \text{ Nutrient in feed}} \right) \right] \end{aligned}$$

Table 2: Final mean (\pm S.D.) weight, survival, Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) of juvenile tilapia (*O. mossambicus* x *O. niloticus*) fed diets with different P/E ratios

Diet (P/E)	Initial weight (g)	Final weight (g)	Survival (%)	FCR	PER
30	4.1 ^a \pm 0.6	20.6 ^{ab} \pm 4.2	80 ^b \pm 15	1.7 ^b \pm 0.3	1.11 ^a \pm 0.46
23	4.1 ^a \pm 0.2	23.7 ^a \pm 4.1	90 ^a \pm 12	1.6 ^b \pm 0.3	1.46 ^a \pm 0.33
20	4.2 ^a \pm 0.8	25.7 ^a \pm 5.1	87 ^a \pm 16	1.7 ^b \pm 0.2	1.81 ^a \pm 0.39
15	4.3 ^a \pm 0.7	12.2 ^{bc} \pm 2.3	80 ^b \pm 11	3.0 ^a \pm 1.9	1.41 ^a \pm 0.70
10	4.2 ^a \pm 0.7	9.90 ^c \pm 1.2	47 ^c \pm 12	3.5 ^a \pm 2.0	2.28 ^a \pm 0.97
8	4.2 ^a \pm 0.9	5.90 ^c \pm 0.8	20 ^d \pm 10	3.5 ^a \pm 1.8	2.75 ^a \pm 0.10

Means within the same column with different superscripts are significantly different ($p < 0.05$) (a>b>c>d>e)

Determination of enzymatic digestive activity: At the end of the digestibility trial, intestines from 10 fish per dietary treatment were extracted and frozen to -20°C , until analyzed. Samples from each treatment were homogenized in 3 volumes of distilled water. The homogenate was centrifuged at 11,300 x gravity (g) at 4°C for 20 min. The supernatant was used for the following determinations:

Trypsin activity: Benzoin-Arg-P-Nitroaniline (BAPNA) was used as substrate. BAPNA was dissolved in 1 mL of Dimethyl Sulfoxide (DMSO) and made up to 100 mL with 50 mM TRIS buffer pH 7.5, containing 20 mM CaCl_2 . Ten microlitres of the enzymatic extract were added to 1.25 mL of the substrate solution. After 10 min the reaction was stopped with 0.25 mL of 30% acetic acid and the absorbance was read at 410 nm. The trypsin-like activity was reported as activity units per mg protein (Erlanger and Cohen, 1961).

Chymotrypsin activity: The chymotrypsin-like activity was measured using succinyl-(Ala) 2-for-Phe-P-Nitroanilide (SAPNA) as substrate. Twenty microlitres of the enzymatic preparation was added to a solution containing 50 μL substrate (0.02 mM SAPNA) and 590 μL of TRIS-HCl buffer (pH 7.8) containing 0.01 M CaCl_2 . The reaction was stopped after 5 min by adding 0.25 mL of 30% acetic acid. Absorbance was read at 410 nm. Chymotrypsin-like activity is reported as activity units per mg protein (García-Carreño *et al.*, 1994).

Amylase activity: Corn starch was used as substrate. Five μL of the enzymatic extract, 500 μL 50 mM Tris-HCl buffer, pH 7.5 and 500 μL of 1% soluble starch were mixed. After 10 min of incubation at 37°C , 200 μL of 2 N sodium carbonate and 1.5 mL of 3, 5-Dinitric Salicylic Acid (DNS) were added. The mixture was agitated and warmed to boil in a water bath for 15 min, and 7.3 mL distilled water was added. The absorbance was read at 550 nm and one blank was run at the same time. The amylase units are reported as units per mg of protein (Vega-Villasante *et al.*, 1993).

Chemical analysis: Moisture, crude protein, ash, lipid and crude fiber were determined according to the AOAC International (2002) methods. The nitrogen free extract was calculated by difference. Digestible carbohydrates were determined using the anthrone method (Dreywood, 1946).

Chromic oxide was quantified by the method described by Olvera-Novoa (1994). Briefly, 50 mg of sample were digested in 5 mL nitric acid and later in perchloric acid at 300°C until a red ring in the surface of the solution appeared. After digestion, 25 mL of distilled water was added. Absorbance was read at 350 nm.

Statistical analysis: After data verification it was established that data was normal and homoscedastic, a parametric one-way analysis of variance ANOVA was used to determine significant differences between treatments, with a confidence level of 95%. When significant differences were found, Tukey's multiple comparison of means test was used. A regression analysis of digestible P/E ratio and final weight of juvenile tilapia was conducted. Procedures available in STATISTICA™ 5.0 (StatSoft, Inc., Tulsa, OK, USA) were used to conduct the statistical tests.

RESULTS AND DISCUSSION

Growth trial: Protein to Energy ratio (P/E) has been used to analyze the effect of nutrients in the diet. Optimal P/E ratio represents a balance of the energy needed for basal metabolism and growth. The balance of dietary energy and the requirements of protein for growth are considered as the key to obtain an efficient diet (Rosas *et al.*, 2001).

In this experiment, the reduction on the P/E produced a significant ($p < 0.05$) decrease in final weight and survival (Table 2). The lowest final weight (5.9 g) was obtained with the diet containing 150 g/kg of crude protein and 20 kJ of gross energy (8 P/E) and the highest final weight, moreover, the highest weight was obtained with the diet containing 300 g/kg crude protein and 15 kJ of gross energy (20 P/E). Survival was significantly lower ($p < 0.05$) in the diet with 8 P/E ratio and remained around 90% in fish fed diets containing a P/E ratio of 23 and 20. FCR was significantly improved when the P/E ratios were superior to 20.

The FCR increased significantly ($p < 0.05$) in diets with P/E levels of 15, 10 and 8 mg/kJ, probably because the essential amino acids and energy were limited and were compensated by an increase in food intake (Tacon, 1989). Shiau and Huang (1990) found an inverse behavior, with high P/E ratios (30 to 25 mg/kJ) a FCR of 2.56 was observed; in contrast, a P/E ratio of 12 resulted in an FCR of 1.58. In the present study, PER was not affected ($p > 0.05$) by the P/E ratio in the

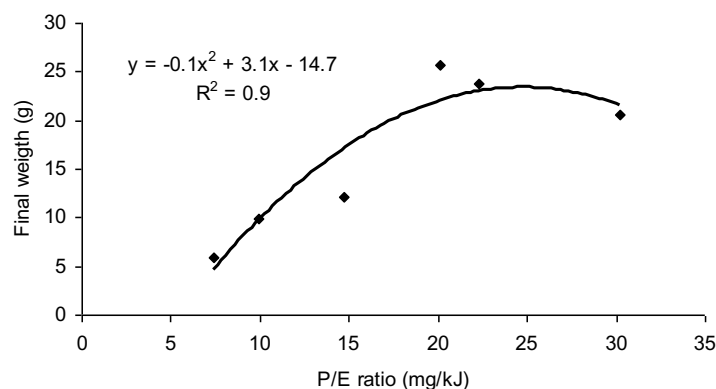


Fig. 1: Relation of final weight and digestible P/E ratio in the diet of juvenile tilapia (*O. niloticus* x *O. mossambicus*) in seawater

Table 3: Mean (\pm S.D.) digestibility of Dry Matter (DM), crude protein, lipids and carbohydrates of the experimental diets n = 3

Diet	DM	Crude protein	Lipid	Carbohydrates	Energy
30	83.1 ^a \pm 1.6	91.7 ^a \pm 0.8	91.3 ^a \pm 0.8	73.0 ^c \pm 2.5	90.9 ^a \pm 0.8
23	80.6 ^a \pm 1.3	87.8 ^{bc} \pm 0.8	90.5 ^a \pm 0.6	83.1 ^b \pm 1.2	88.4 ^{ab} \pm 0.8
20	82.3 ^a \pm 2.0	86.8 ^{bc} \pm 1.5	87.1 ^b \pm 1.5	73.2 ^c \pm 3.1	86.2 ^b \pm 1.6
15	85.2 ^a \pm 0.3	88.6 ^{ab} \pm 0.3	93.3 ^a \pm 0.2	86.6 ^{ab} \pm 0.3	90.3 ^a \pm 0.2
10	83.7 ^a \pm 0.2	85.3 ^c \pm 0.2	83.7 ^c \pm 0.2	91.9 ^a \pm 0.1	86.2 ^b \pm 0.1
8	83.6 ^a \pm 1.4	89.6 ^{ab} \pm 0.9	93.3 ^a \pm 0.6	89.6 ^a \pm 0.9	91.1 ^a \pm 0.8

Means within the same column with different superscripts are significantly different ($p < 0.05$) (a>b>c)

Table 4: Mean (\pm S.D.) proximate body composition (g/Kg dry matter, except moisture) of juvenile tilapia (*O. mossambicus* x *O. niloticus*) fed diets with different P/E ratios n = 3

Diet (P/E)	Moisture	Crude protein	Ash	Lipids
30	732 ^a \pm 2	726 ^a \pm 72	134 ^a \pm 3	275 ^a \pm 7
23	734 ^a \pm 10	747 ^a \pm 93	124 ^a \pm 6	256 ^{ab} \pm 8
20	727 ^a \pm 26	599 ^a \pm 77	127 ^a \pm 15	324 ^{bcd} \pm 20
15	705 ^a \pm 3	520 ^a \pm 40	111 ^a \pm 5	379 ^d \pm 20
10	705 ^a \pm 1	664 ^a \pm 63	108 ^a \pm 9	336 ^{cd} \pm 28
8	701 ^a \pm 15	630 ^a \pm 79	117 ^a \pm 11	316 ^{bcd} \pm 27

Means within the same column with different superscripts are significantly different ($p < 0.05$) (a>b>c>d)

Table 5: Mean (\pm S.D.) soluble protein and specific enzymatic activity in the intestine of juvenile tilapia (*O. niloticus* x *O. mossambicus*) fed diets containing different P/E ratios

Diet (P/E)	Soluble protein (mg/mL)	Like-trypsin (U/mg protein)	Like-chymotrypsin (U/mg protein)	Amylase (U/mg protein)
30	0.45 ^{abc} \pm 0.07	7.68 ^b \pm 0.79	0.44 ^c \pm 0.03	2.72 ^{bc} \pm 0.19
23	0.38 ^{cd} \pm 0.08	3.32 ^c \pm 0.40	1.42 ^b \pm 0.05	3.42 ^b \pm 0.41
20	0.45 ^{bc} \pm 0.06	15.31 ^a \pm 1.86	1.64 ^b \pm 0.21	2.83 ^{bc} \pm 0.27
15	0.51 ^{ab} \pm 0.06	6.69 ^b \pm 0.78	0.39 ^c \pm 0.02	2.13 ^c \pm 0.66
10	0.41 ^{bcd} \pm 0.09	1.49 ^c \pm 0.14	2.41 ^a \pm 0.27	3.184 ^{bc} \pm 0.57
8	0.29 ^d \pm 0.06	7.61 ^b \pm 0.89	0.31 ^c \pm 0.04	10.34 ^a \pm 1.90

Means within the same column with different superscripts are significantly different ($p < 0.05$) (a>b>c>d)

diet. Twibell and Brown (1998) reported that an increment in the protein level in isocaloric diets for tilapia, the PER was not affected. In contrast, Shiau and Huang (1990) found that the PER was increased with a decrease of P/E ratio in the diet.

P/E ratio is not known for *O. niloticus* x *O. mossambicus*, it has been estimated that for *O. niloticus* x *O. aureus* reared in seawater, Shiau and Huang (1990) indicated that the optimal P/E ratio is around 15 to 25 mg/kJ, however, in their estimation several important factors intervene as: specie, salinity and digestibility of diet (Al-Hafedh, 1999).

Digestibility: In balanced diets, the nutrient digestibility is an important factor that must be

considered in the estimation of nutritional requirements of aquatic organisms, principally in experimental diets elaborated with commercial non-purified ingredients.

Dry matter digestibility was not significantly affected ($p > 0.05$) by the P/E ratios in the diet (Table 3). Moreover protein, lipid, carbohydrate and energy digestibility were affected by the P/E ratio in the diet, but not clear pattern was observed. The diet with 30 P/E ratios showed the highest digestibility of protein, lipids and energy (91.7, 91.3 and 90.9%, respectively).

After diet digestibility was estimated, P/E ratios were recalculated to obtain digestible P/E ratios of the diets. A quadratic model ($R^2 = 0.90$) showed that a P/E ratio of 27 mg digestible protein /kJ digestible energy provides the maximum growth of tilapia, (*O. niloticus* x

O. mossambicus) cultured in seawater (Fig. 1) This determination could be used in the elaboration of highly efficient diets for this tilapia hybrids.

Body composition: Lipid composition in the carcass increased significantly as P/E ratio diminished. Protein, ash and carbohydrate were unaffected (Table 4). On the contrary, Twibell and Brown (1998) found a significant effect of protein level in the diet over the muscle composition of *O. niloticus* using isocaloric diets. This difference is probably due to the effect of lower P/E ratios used in the present study. We observed a significant correlation ($p < 0.05$), between moisture and lipid contents in carcass of juvenile tilapia ($r = 0.77$), fed with different P/E ratios.

Enzymatic activity in the intestine of juvenile tilapia: There was a significant effect ($p < 0.05$) of the dietary treatments on soluble protein, trypsin, chymotrypsin and amylase activities (Table 5). Several studies suggest that the digestive enzymatic activity is modified by changes in food ingredients, food manipulations, starvation, feeding time and protein level (Sabapathy and Teo, 1993; Hakim *et al.*, 2006).

In mammals, there exists a positive correlation between trypsin and chymotrypsin activities as a result of the activation of chymotrypsinogens for the trypsin. Apparently in fish this pattern is different, since no correlation between the activity of these enzymes has been observed.

The soluble protein, trypsin like activity, chymotrypsin like activity and amylase activity in the intestine were significantly ($p < 0.05$) affected by the diet. A maximum trypsin like activity was observed in tilapia fed a diet with 20 P/E. Amylase activity increased in the intestine of tilapia fed with 8 P/E ratios in the diet.

Studies have demonstrated that herbivorous fish might compensate their natural low protein availability increasing their enzymatic activity. Some herbivorous fish exhibit trypsin activities similar to or even higher than carnivorous species, possibly to maximize protein digestive efficiency (Hidalgo *et al.*, 1999; Chan *et al.*, 2004).

As a result of this study we concluded that digestible P/E ratio of 27 mg/ kJ can be successfully utilized in juvenile tilapia diet to maximize growth performance.

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